



## Lysine restricted diet for pyridoxine-dependent epilepsy: First evidence and future trials

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### ARTICLE INFO

#### Article history:

Received 13 July 2012

Received in revised form 2 September 2012

Accepted 2 September 2012

Available online 10 September 2012

#### Keywords:

Antiquitin deficiency

Metabolic epilepsy

Dietary lysine restriction

AASA

Pipecolic acid

Developmental outcome

### ABSTRACT

**Objective:** To evaluate the efficacy and safety of dietary lysine restriction as an adjunct to pyridoxine therapy on biochemical parameters, seizure control, and developmental/cognitive outcomes in children with pyridoxine-dependent epilepsy (PDE) caused by antiquitin (ATQ) deficiency.

**Methods:** In this observational study, seven children with confirmed ATQ deficiency were started on dietary lysine restriction with regular nutritional monitoring. Biochemical outcomes were evaluated using pipecolic acid and  $\alpha$ -aminoadipic semialdehyde (AASA) levels in body fluids; developmental/cognitive outcomes were evaluated using age-appropriate tests and parental observations.

**Results:** Lysine restriction was well tolerated with good compliance; no adverse events were reported. Reduction in biomarker levels (measurement of the last value before and first value after initiation of dietary lysine restriction) ranged from 20 to 67% for plasma pipecolic acid, 13 to 72% for urinary AASA, 45% for plasma AASA and 42% for plasma P6C. For the 1 patient in whom data were available and who showed clinical deterioration upon interruption of diet, cerebrospinal fluid levels decreased by 87.2% for pipecolic acid and 81.7% for AASA. Improvement in age-appropriate skills was observed in 4 out of 5 patients showing pre-diet delays, and seizure control was maintained or improved in 6 out of 7 children.

**Conclusions:** This observational study provides Level 4 evidence that lysine restriction is well tolerated with significant decrease of potentially neurotoxic biomarkers in different body compartments, and with the potential to improve developmental outcomes in children with PDE caused by ATQ deficiency. To generate a strong level of evidence before this potentially burdensome dietary therapy becomes the mainstay treatment, we have established: an international PDE consortium to conduct future studies with an all-inclusive integrated study design; a website containing up-to-date information on PDE; a methodological toolbox; and an online registry to facilitate the participation of interested physicians, scientists, and families in PDE research.

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**Abbreviations:** AASA,  $\alpha$ -aminoadipic semialdehyde; ATQ, ATQ; CSF, cerebrospinal fluid; EEG, electro-encephalogram; MRI/S brain, MRI scan of the brain with spectroscopy; P6C,  $\Delta$ 1-piperidine-6-carboxylate; PLP, pyridoxal-5'-phosphate.

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## 1. Introduction

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive epileptic encephalopathy characterized by a therapeutic response to pharmacological dosages of pyridoxine and resistance to conventional antiepileptic treatment [1]. Recently, the underlying genetic defect was identified as deficiency of antiquitin (ATQ) ( $\alpha$ -aminoacidic semialdehyde dehydrogenase, *ALDH7A1*), an enzyme that facilitates cerebral lysine catabolism (MIM# 266100) [2]. Folinic acid-responsive seizures are also caused by mutations in this gene [3]. ATQ deficiency results in accumulation of chemical substrates arising from lysine degradation proximal to the deficient enzyme activity including  $\alpha$ -aminoacidic semialdehyde (AASA), its cyclic equivalent P6C, and pipercolic acid. Inactivation of 5' pyridoxal phosphate (PLP) via chemical reaction with P6C is a pathophysiological mechanism of pyridoxine dependency. While treatment with pyridoxine compensates chemical PLP inactivation, the accumulation of substrates from lysine degradation is not sufficiently reduced (Fig. 1). These potentially neurotoxic compounds could explain for the limited efficacy of pyridoxine, as 75–80% of patients suffer developmental delay or intellectual disability (IQ < 70) despite seizure control [4,5].

Standard treatment for inborn errors of metabolism affecting catabolic pathways of essential amino acids consists of substrate reduction to the deficient enzyme activity by dietary modification. Thus, for ATQ deficiency, dietary lysine restriction can reduce the accumulation of lysine-derived substrates and improve cerebral function (neurodevelopment, cognition, behavior). Based on this rationale, we initiated an open-label observational study to test the effectiveness and safety of dietary lysine restriction as an adjunct to pyridoxine therapy on chemical biomarkers, seizure control, and developmental or cognitive outcomes in 7 children with confirmed ATQ deficiency.

## 2. Patients and methods

### 2.1. Study setting and inclusion criteria

The patients in this report were cared for at three academic medical centers (Hannover, Germany; Vancouver, Canada; and Denver, USA) between 2008 and 2012. Patients were screened according to the following eligibility criteria: age 0 to 18 years, genetically and biochemically confirmed ATQ deficiency, clinically followed at one of the three study sites, and parents or family willing to provide informed consent. Of the 9 consecutive patients with confirmed ATQ deficiency at the 2 sites during the recruitment period [2008–2011], 7 were enrolled in the study. Reasons for exclusion were: age > 18 years ( $n = 1$ ), parents declined ( $n = 1$ ). All participating clinicians had been actively involved in international workshops and meetings held in Vancouver, Canada and Geneva, Switzerland during the period 2009–2011, to develop consensus on diagnosis and treatment of ATQ deficiency [5], and a protocol to study the effects of dietary lysine restriction.

### 2.2. Standard treatment

All patients were treated according to existing recommendations summarized by Stockler et al. [6]. During dietary treatment, patients received pyridoxine dosages between 1 and 30 mg/kg/day PO. In patient #2, higher doses of pyridoxine were prescribed prior to starting the diet, due to inadequate seizure control. Folinic acid, clobazam, and/or phenobarbital were prescribed to individual patients if the pyridoxine alone was not effective in controlling seizures.

### 2.3. Lysine-restricted diet

Diet prescriptions were based on the Kölker and Ross guidelines for Glutaric Aciduria type I, another inborn error of metabolism involving compromised degradation of lysine [7]: Lysine was prescribed at 70–100 mg/kg/day, 45–80 mg/kg/day and 20–45 mg/kg/day to children

aged respectively less than 1 year, between 1 and 7 years, and above 7 years. In patients #6 and #7, lysine was restricted in early infancy. In order to meet the recommended daily protein intake (DRIs), lysine-free amino acid formulas were provided additionally: Glutarex (Abbott) (patients #1, 2, 7) and Lys2-prima (Milupa) (patients 3–6). Glutarex is free of lysine and tryptophan, whereas Lys2-prima contains 1.3 g of tryptophan per 100 g. Tryptophan supplements (20 mg/kg/day) were provided to patients receiving Glutarex to meet the DRIs for this amino-acid [8,9].

### 2.4. Monitoring and outcome assessment during the lysine-restricted diet

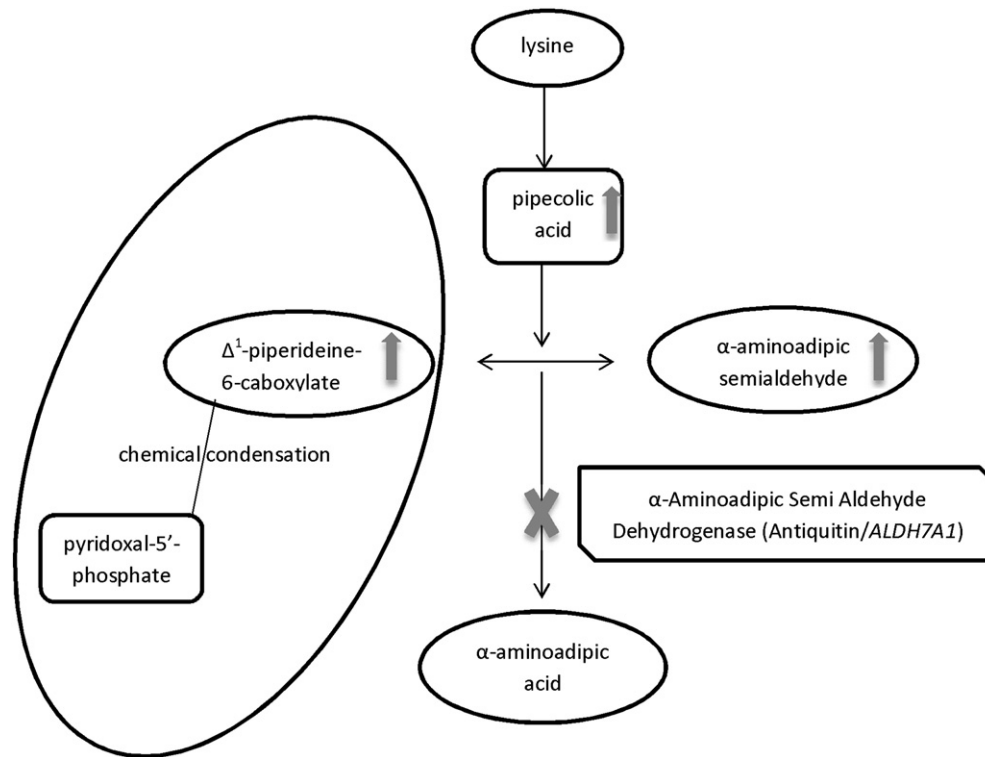
All patients underwent vision and hearing tests prior to starting dietary treatment to rule out sensory deficits as cause of developmental delay. Samples for plasma amino-acids were collected in a fasting state (minimum 3 h) every 3 months. Samples for plasma pipercolic acid were collected in a 4–6 h fasting state for patients #1 and #2 and less than 4 h for patients #3–7. Urinary AASA was measured in samples collected after overnight sleep. For patients #1 and #2, a monitoring protocol was implemented that included the following investigations every 3 months: clinical and neurological assessments (somatic growth parameters) as well laboratory testing for nutritional parameters, including pre-albumin, albumin, complete blood cell count and iron status.

Electroencephalogram (EEG) was performed during clinic visits (every 3–6 months). Cerebrospinal fluid (CSF) samples for measuring amino acids and AASA were collected for patients 1 and 2 while they were under sedation for MRI/spectroscopy brain scan. Nerve conduction studies were performed in patient #2 while on high pyridoxine dosage (40 mg/kg/day).

Developmental assessments were performed with tests routinely used at the collaborating centers. These included the Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III); Gesell Developmental Assessment; the Denver Developmental Screening Test; the Peabody Motor Scales II; the Wechsler Intelligence Scale For Children (WISC-IV); Pre-School Language Scale—4th ED; Clinical Evaluation of Language Fundamentals (CELF-IV); the Kaufman Assessment Battery for Children, 7th German Ed (K-ABC); and the Snijders-Oomen Non-Verbal Intelligence Test (Revised) 5.5–17 years (SON-R 5.5). Parental observations regarding their children's behavior, well-being, and developmental progress were also included as non-objective outcome parameters.

### 2.5. Chemical biomarkers

To determine the effects of treatment, plasma pipercolic acid was measured in all patients, urine AASA (frozen shipments) in patients #1–6, and plasma AASA in patient #7. For patients #1 and #2, plasma pipercolic acid was measured at Kennedy Krieger Institute in Baltimore, USA (method: Kok et al. [10]). For patients #3, #4, and #5, plasma pipercolic acid was initially measured at University Hospital in Graz, Austria (method: Kelley et al. [11]) and along with patient #6 from September 2009 onwards at the VU Medical Center, Amsterdam (Kok et al. [10]). Urine AASA was measured for patients #1–6 at the VU Medical Center, Amsterdam (methods: Struys et al. [12]) For patient #7, plasma AASA and P6C was measured at Seattle Children's Research Institute, Seattle (method: Sadiilkova et al. [13]) and plasma pipercolic acid at Mayo Medical Laboratories, Rochester (Kok et al. [10]). For patients #1, #2 and #7, biomarkers were measured in CSF samples at the VU Medical Centre, Amsterdam (method: Mills et al. [14]). The reduction of metabolites was calculated using the last value prior to and first value after initiation of dietary lysine restriction (values expressed as X-fold increase of the upper limit of normal value age related controls).



**Fig. 1.** Outline of the metabolism of L-lysine via pipercolic acid pathways and biochemical pathophysiology of antiquitin deficiency. In ATQ deficiency (indicated by the cross symbol), P6C and  $\alpha$ -AASA accumulate due to a block in  $\alpha$ -aminoadipic semialdehyde dehydrogenase. P6C undergoes chemical condensation with pyridoxal phosphate (PLP) resulting in PLP deficiency. Pipercolic acid (PA) accumulates due to backpressure from the enzymatic block.

## 2.6. Ethics approval

For patients #1 and #2, this study was approved as an “innovative treatment protocol” by the British Columbia Children’s Hospital Review Board. For patients #3, #4, #5, and #6 approval was obtained from the Ethics Review Board at Hannover Medical School. For patient #7, the treatment was performed as part of clinical care and the patient was enrolled in a Colorado Multiple Institutional Review Board approved research protocol. Parents provided written consent for publication of the study results.

## 3. Results

### 3.1. Patient characteristics

Patient characteristics are summarized in Table 1. Diagnosis was established at a median age of 6 weeks (range 0.5–5 months) during the period 1999 and 2011, based on clinical presentation of neonatal or infantile onset of epileptic seizures and response to pyridoxine therapy, with subsequent confirmation through demonstration of elevated urinary AASA and/or plasma pipercolic acid levels and at least one disease-causing sequence change in the *ALDH7A1* gene. The clinical follow-up varied in duration between 4.0 months and 12.1 years (mean 7.1 years) from age of clinical presentation to the most recent clinic visit in 2012. Adjunctive dietary lysine restriction was initiated at a median age of 3.6 years (range 1 month–11.8 years) for a period varying from 6 months to 4.2 years. The total duration of treatment with dietary lysine restriction was 140.8 months.

### 3.2. Chemical biomarker results

Lysine restriction resulted in a reduction of all measured chemical biomarkers, including plasma lysine (9.5–58.9%), plasma pipercolic acid (26.0–67.0%) in patients #1–7; urinary AASA levels (13.0–72.0%) in

patients #1, 2, 3, 5, and 6; CSF AASA (81.7%) and CSF pipercolic acid (87.2%) in patient #2; plasma AASA level (45%) and plasma P6C (42%) in patient #7. Figs. 2 to 8 show this trend for the individual patients as profile plots for the entire study duration.

In the CSF of patient #1, obtained at age 2.3 years, after 17 months of dietary therapy, the AASA measured 1.9  $\mu\text{mol/l}$  (reference range 0–0.1); pipercolic acid 0.57  $\mu\text{mol/l}$  (reference range 0.009–0.12); lysine 8.1  $\mu\text{mol/l}$  (reference range 5.9–37.1). For patient #2 (described in more detail by Gallagher et al. [3], prior to lysine restriction at age 4.5 months, CSF AASA measured 8.2  $\mu\text{mol/l}$ , CSF pipercolic acid 3.0  $\mu\text{mol/l}$ , CSF lysine 16.8  $\mu\text{mol/l}$ . At age 4.6 years after 13 months of diet, CSF AASA measured 1.5  $\mu\text{mol/l}$ , CSF pipercolic acid 0.39  $\mu\text{mol/l}$ , CSF lysine 11.2  $\mu\text{mol/l}$  (same reference ranges as noted for patient 1). For patient #7, the CSF AASA obtained at age 3 months (after 2 months of dietary therapy), AASA measured 0.5  $\mu\text{mol/l}$  (reference range 0–0.1). For the remaining patients, CSF biomarker measurements were not performed.

### 3.3. Safety

Nutritional status and somatic growth remained within normal limits for all patients throughout the dietary period; no adverse effects were reported. Plasma lysine levels remained within the lower age-adjusted normal range. Tryptophan levels remained within normal limits (data not shown). Further nutritional parameters in blood remained normal, as did somatic growth and well-being (data not shown).

### 3.4. Clinical outcomes

Patient #1 presented with neonatal epileptic encephalopathy, hypoglycemia, lactic acidosis, and MRI abnormalities [15]. She became clinically seizure free on pyridoxine and folinic acid therapy at age 6.5 weeks with abnormal EEG which normalized at age 6 months.

**Table 1**  
Patient characteristics.

Patient #	1*	2†	3	4	5	6	7
<b>Age at diagnosis</b>	5 weeks	2 months	5 months	2 weeks	6 weeks	6 weeks	2 weeks
<b>Presenting symptoms</b>	Neonatal epileptic encephalopathy, hypoglycemia, lactic acidosis, intra-cerebral hemorrhages	Therapy resistant focal clonic seizures at age 2.5 mos	Neonatal seizures and status epilepticus at age 4 mos	Prematurity (33 3/7w), neonatal epileptic encephalopathy, initially presenting with apnea, lactic acidosis, distended abdomen	Neonatal epileptic encephalopathy, distended abdomen	Neonatal epileptic encephalopathy	Neonatal seizures, vomiting, lethargy, hypoglycemia
<b>ALDH7A1 gene mutations</b>	c.834G>A (V278V) <sup>2</sup> /c.1192G>C (G398A)	c.750G>A (V250V) <sup>2</sup> /c.1195G>C (E399Q)	c.872G>A (G291E)/WT	c.1279G>C (E427Q)/c.1279G>C (E427Q)	c.1279G>C (E427Q)/c.902A>T (N301I)	c.427G>C (N488K)/c.1344T>A (G143R)	c.448_458del11 (ntfsX45)/c.1195G>C (E399Q)
<b>Pyridoxine dosage<sup>1</sup> (mg/kg/day)</b>	15–30	15–20	1	15–30	20–30	25–30	30
<b>Concomitant medication</b>	Folinic acid 5 mg/day	Clobazam 5 mg/day				Phenobarbital until age 8 mos, folinic acid 5 mg/day	
<b>MRI brain</b>	<i>At age 6 days:</i> bilateral hemorrhages temporal lobe and brain parenchyma; restricted diffusion ventral thalamic nuclei at age 2 years 4 mos: evolution of intracranial hemorrhage and delayed myelination adjacent to occipital horns	<i>At age 2 mos:</i> unremarkable at age 4 years 7 mos: incomplete myelination adjacent to trigones bilaterally	<i>At age 5 mos:</i> thinned corpus callosum, arachnoid cyst in the posterior fossa, wide extracerebral CSF spaces, myelination age-appropriate	<i>At 2 weeks:</i> tiny periventricular and cerebellar hemorrhagic white matter injuries, subdural bleed, wide posterior fossa	<i>At age 4 weeks:</i> thinned splenium of corpus callosum, wide posterior fossa at age 11 mos: thinned splenium of corpus callosum, normal MRS	<i>At age 4 weeks:</i> thin corpus callosum	<i>At age 1 week:</i> normal structure, but small areas of restricted diffusion in frontal white matter and thalami (obtained during seizures)
<b>Pre-diet clinical seizures and EEG</b>	No EEG: mild background suppression and multifocal spikes at age 1.5 mos; normal at age 6 mos	Focal clonic seizures. EEG: background slowing, temporal and frontal spike dipoles	No EEG: normal	No EEG: normal	No EEG: multifocal sharp wave, normal background activity	No EEG: increased beta activity (probably due to phenobarbital)	No EEG: normal
<b>Pre-diet development (test<sup>5</sup> and outcome)</b>	<i>Bayley-III at 4 and 8 mos:</i> truncal hypotonia, moderate delay in fine and gross motor skills with age adequate communication and language skills	<i>Peabody Motor Scale at age 3 years:</i> poor gross and fine motor skills, <i>Pre-School Language Scale (4th edition at age 3 years):</i> significant expressive and receptive language delay (1st percentile) <i>Clinical exam:</i> repetitive behaviors, encopresis	<i>Clinical exam at 11 years 3 mos:</i> mild speech delay (attends special needs school)	<i>Snijders Oomen at age 61 mos:</i> global IQ 70	<i>Denver Scales at 49 mos:</i> social contact 36 mos, fine motor skills 33 mos, speech 30 mos, gross motor skills 33 mos (moderate delay in all domains) <i>K-ABC attempted at 52 mos:</i> not interpretable due to lack of cooperation	<i>Clinical exam at 4 mos:</i> severe global developmental delay, severe global hypotonia	<i>Clinical exam at 28 days:</i> developmentally appropriate
<b>Treatment period (age at start-current) and duration</b>	11.5 mos–3.3 years (total 2.3 years)	3.6 years–6.5 years (total 2.9 years)	11.9 years–12.3 years (total 6 mos)	5.5 years–6.5 years (total 1 year)	4.4 years–8.6 years (total 4.2 years)	4 mos–10 mos (total 6 mos)	28 days–5 mos (total 4 mos)
<b>Lysine intake<sup>1</sup> (mg/kg/day)</b>	50–60	50–60	50	50	50	100	80–100

<b>Plasma lysine: mean level<sup>1</sup> and reduction between last value before diet and first value on diet</b>	85.9 μmol/l and 9.5%	85.1 μmol/l	78.0 μmol/l and 56.0%	88 μmol/l and 33.4%	65.0 μmol/l and 58.9%	129.5 μmol/l and 38.7%	71.7 μmol/l and 56.3%
<b>Urine AASA: reduction between last value before diet and first value on diet</b>	22 to 6 fold of upper limit normal 72% decrease	32 to 16 fold upper limit normal 50% decrease	7.6 to 4 fold upper limit normal 13% decrease	14 to 4.4 fold upper limit normal 68.5% decrease	NA <sup>3</sup>	23 to 8.5 fold upper limit normal 63% decrease	NA <sup>3</sup>
<b>Plasma pipercolic acid: reduction between last value before diet and first value after diet<sup>2</sup></b>	2 to 0.9 fold upper limit normal 5% decrease	1.8 to 1.3 fold upper limit normal 27% decrease	2 to 0.8 fold upper limit normal 60% decrease	3 to 1.3 fold upper limit normal 56% decrease	3.4 to 1.1 fold upper limit normal 67% decrease	5 to 4 fold upper limit normal 20% decrease	6 to 3 fold upper limit normal 50% decrease
<b>Post-diet clinical seizures and EEG</b>	No <i>EEG</i> : frequent spike wave activity with paroxysmal theta activity in bilateral anterior quadrants, no clinical correlates (likely due to neonatal hypoglycemia and cerebral; hemorrhages)	Relapse clinical seizures when diet stopped <i>EEG</i> : initial improvement (normalization background, few spikes) at age 4.9 years; deterioration when folinic acid stopped at age 6.5 years (background slowing, temporal spikes, multiple waves)	No <i>EEG</i> : normal	No <i>EEG</i> : normal background activity, focal sharp waves (worsening)	No <i>EEG</i> : normal background activity, multifocal sharp waves (unchanged)	Seizure during febrile illness at age 9 mos <i>EEG</i> : normal	No <i>EEG</i> : normal
<b>Post diet development (test<sup>4</sup> and outcome)</b>	<i>Gesell Test at ages 12, 19, 26, and 31 mos</i> : improvements in age-appropriate skills, except for mild fine motor delay	<i>Peabody Motor Scale at age 4.7 years</i> : normalization fine and gross motor skills <i>Clinical Evaluation of Language Fundamentals</i> mild expressive/receptive speech and language skills <i>Parental &amp; Clinical Impression</i> : 'dramatic' ongoing improvement of behavior, communication	<i>Parents' impression</i> (no formal testing): no significant change after 6 months	<i>Clinical exam at age 6.5 mos</i> : 'impressive progress', especially in speech. Formal assessment pending	<i>WISC-IV at age 7.5 years</i> : global IQ 74 <i>Clinical</i> : impressive progress in motor skills and speech.	<i>Denver Scales at age 10 mos</i> : Progress in motor skills and communication (social contact 7 mos, fine motor skills 8 mos, speech 6 mos, gross motor skills 7 mos).	<i>Clinical exam at age 5 mos</i> : developmentally appropriate
<b>Clinical response to diet (summary)</b>	Developmental progress, maintained seizure control	Improved development, behavior, seizure control (relapse when diet interrupted)	Unchanged	Developmental progress, maintained seizure control	Developmental catch-up, maintained seizure control	Developmental progress, one breakthrough seizure	Developmental progress, maintained seizure control
<b>Side-effects<sup>1</sup></b>	None	None	None Diet stopped due to non-acceptance	None	None	None	None

1. During dietary lysine restriction.

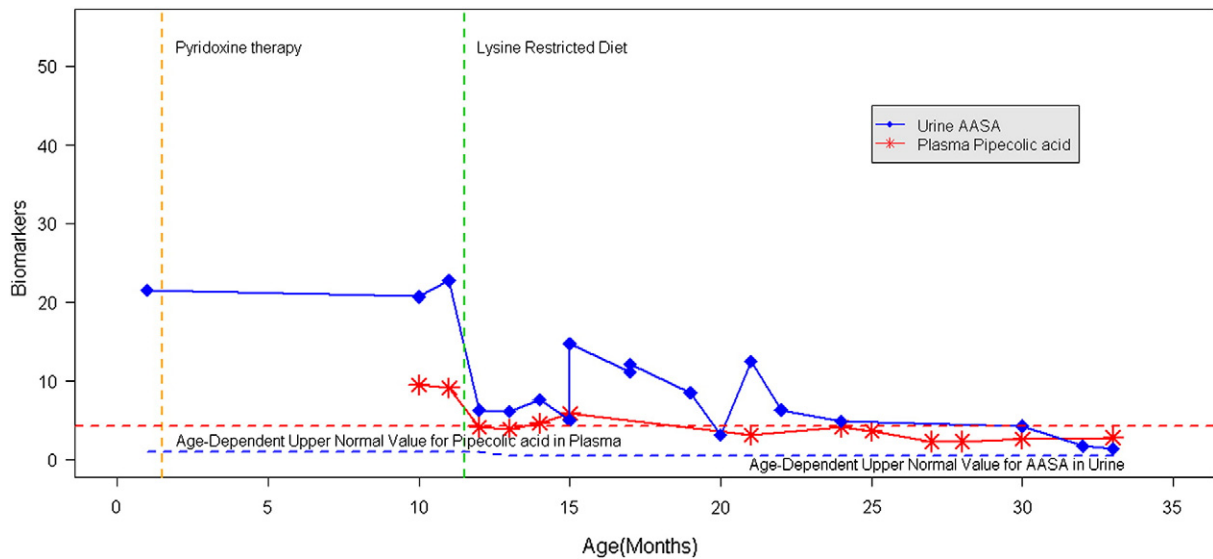
\* Patient 1: For a detailed report of her clinical presentation, see *Pediatrics* 2012;129:e1368-72.

† Patient 2: For a detailed report of his clinical presentation, see *Ann Neurol* 2009; 65: 550–556.

2. splice-site mutation.

3. NA = not available.

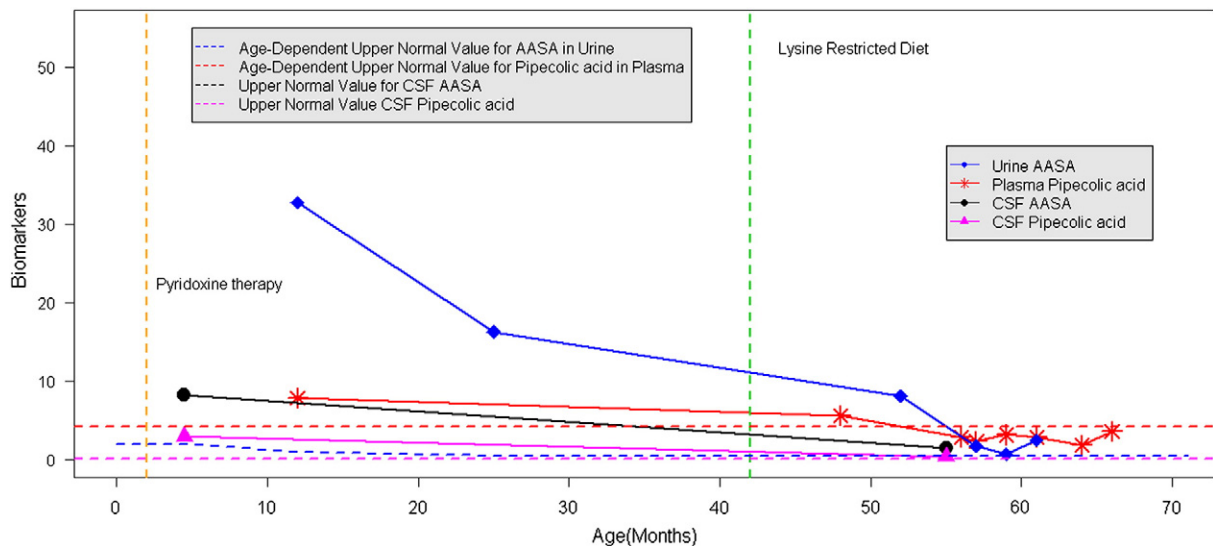
4. Abbreviations of Developmental Assessment Tools are listed in full in the Methods Section 2.4.



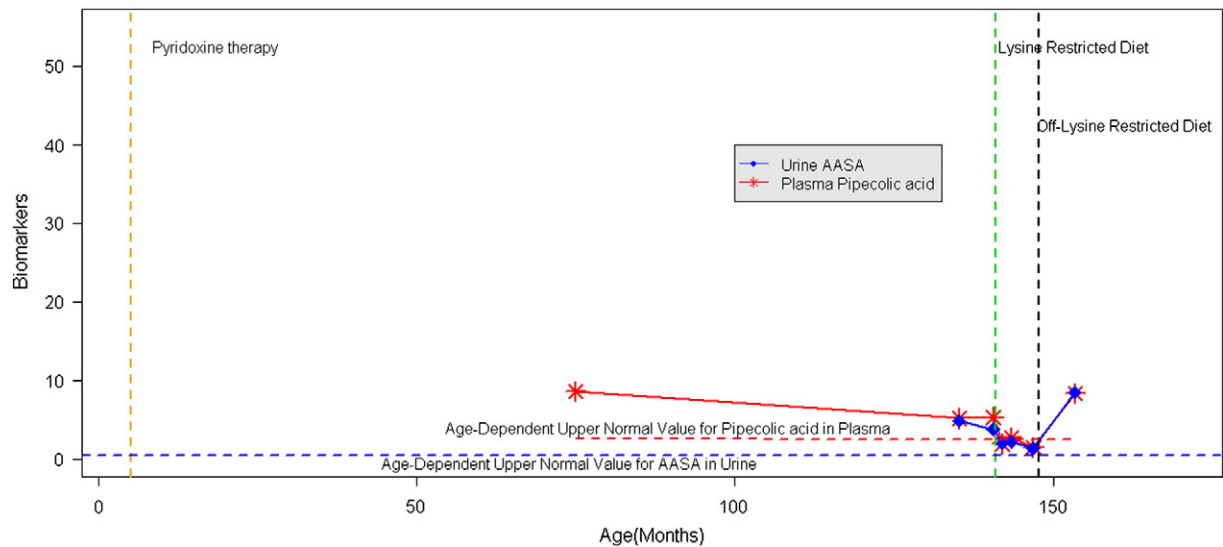
**Fig. 2.** Line graph depicting change in biochemical markers (y-axis) before and after dietary therapy was initiation as function of age (x-axis). Urine AASA (mmol/mol creatinine) and plasma pipecolic acid ( $\mu\text{mol/L}$ ) are expressed as absolute values. Patient #1 showed a 72% reduction of urine AASA (22 to 6 fold decrease from upper limit normal) and a 55% reduction of plasma pipecolic acid (2 to 0.9 fold decrease from upper limit normal), both defined as the difference between last value before and first value after initiation of lysine restriction.

After 2 years of dietary treatment neurodevelopmental testing showed improvement of fine and gross motor skills. Frequent spike waves without clinical correlate on EEG at age 3 years were attributed to neonatal hypoglycemia and cerebral hemorrhages. Pyridoxine dose was increased from 15 to 30 mg/kg/day, with pending EEG follow-up. *Patient #2* presented at age 2 months with focal clonic seizures treated with a loading dose of phenytoine and maintenance pyridoxine, folinic acid and phenobarbital (latter stopped at age 1.5 years). Triggered by fever or missed medication, seizures with abnormal EEG continued despite high dose pyridoxine (40 mg/kg/day). At age 3 years, he showed electrophysiological evidence of sensorimotor neuropathy and moderate global developmental delay. Dietary lysine restriction was initiated at age 3.5 years, with subsequent cessation of clinical seizures and normalization of the EEG, allowing a decrease of

pyridoxine dosage to 20 mg/kg/day. Also, developmental and behavioral improvement was observed with age-appropriate test results in all domains except for mild expressive speech delay. When the diet was shortly interrupted due to adherence problems, he experienced a relapse of seizures with Todd's paresis and a behavioral deterioration. Most recently at age 6.5 years, with clobazam started and folinic acid stopped in the meantime, he experienced rare unprovoked focal seizure with EEG deterioration, upon which folinic acid was restarted. *Patient #3* suffered neonatal seizures, poorly controlled seizures on and off Phenobarbital. She became seizure free when pyridoxine (10 mg/kg/day) was started at 4 months of age. She attended a special school for children with speech delay. Six months after the lysine restriction was started at age 11.9 years, the diet was stopped as parents did not note significant improvement of her speech and she



**Fig. 3.** Patient #2 showed an 50% reduction of urine AASA (32 to 16 fold decrease from upper limit normal), a 27% reduction and normalization of plasma pipecolic acid (1.8 to 1.3 fold decrease from upper limit normal), a 81.7% reduction of CSF AASA (82 to 15 fold decrease from upper limit normal), and a 87.2% reduction of CSF pipecolic acid (25 to 3.2 fold decrease from upper limit normal), all defined as the difference between last value before and first value after initiation of lysine restriction.



**Fig. 4.** Patient #3 showed a 13% reduction of urine AASA (7.6 to 4 fold decrease from upper limit normal) and a 60% reduction and normalization of plasma pipecolic acid later (2 to 0.8 fold decrease from upper limit normal), both defined as the difference between last value before and first value after initiation of lysine restriction.

developed a strong desire for animal protein. Biomarkers returned to pre-treatment levels.

*Patient #4* was born at 33<sup>+3</sup> gestational weeks, and presented with neonatal seizures and lactic acidosis. She has remained seizure-free on pyridoxine mono-therapy. Dietary lysine restriction was started at age 5.5 years and impressive clinical developmental progress (pre-diet IQ 70) was observed, especially regarding speech and language; formal testing is pending.

*Patient #5* presented with neonatal seizures, and remains seizure free upon pyridoxine mono-therapy. Dietary lysine restriction was started at 4.5 years, and she showed impressive progress of grossly delayed motor and language skills.

*Patient #6* was seizure free on pyridoxine, started at age 6 weeks. At 4 months, dietary lysine restriction and folinic acid were initiated when she was weaned off breast milk and phenobarbital was stopped. At age 9 months, she suffered a generalized tonic-clonic seizure during an infection, but EEG was normal. At age 10 months,

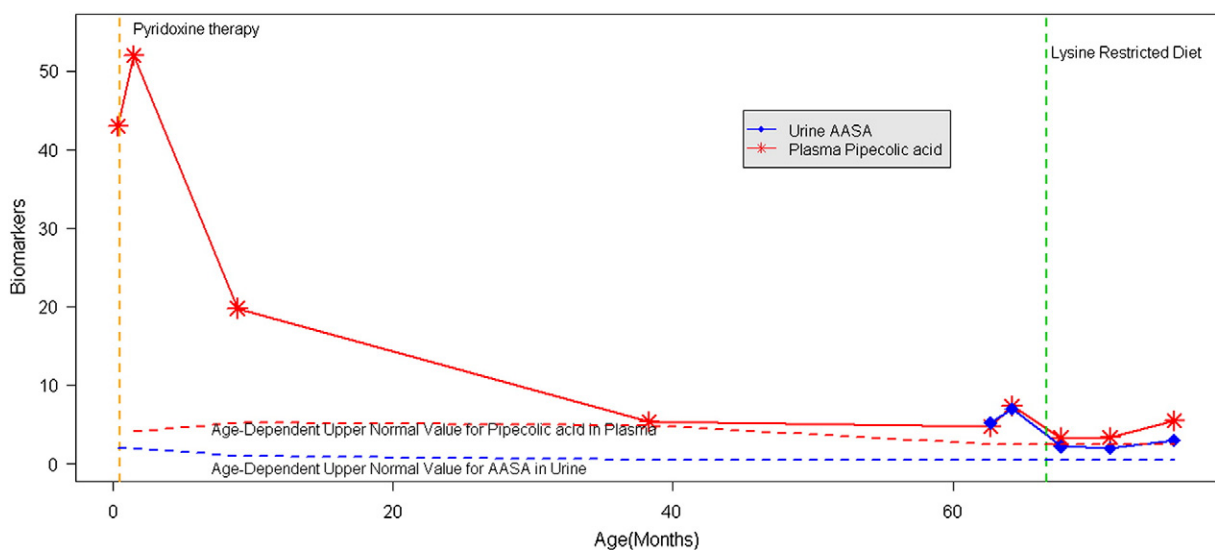
developmental testing showed an overall improvement of motor skills and communication, from moderately to mildly delayed.

*Patient #7* presented on the 9th day of life with vomiting, lethargy, hypoglycemia and therapy-resistant seizures with a 'near burst suppression' pattern on EEG. After 2 days, she responded after 2 days to pyridoxine with resolution of seizures and normalization of the EEG. Since initiation of diet at 4 weeks (reducing the calculated cerebral lysine influx from 8.6 to 4.9 nmol/min/g brain [16]), she has remained seizure free and is developmentally age-appropriate on clinical exam.

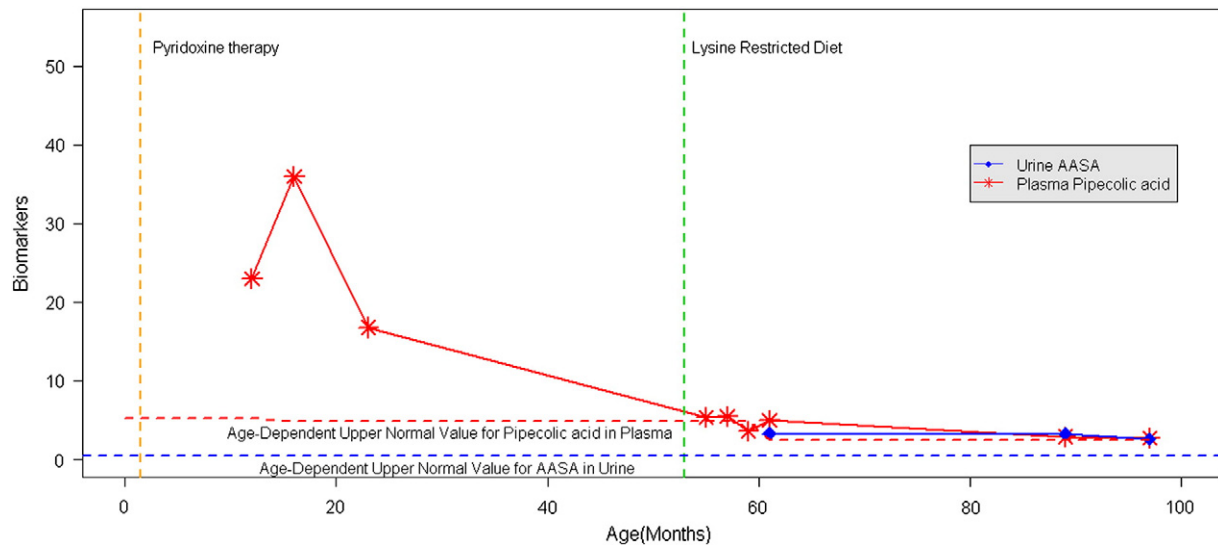
## 4. Discussion

### 4.1. First proof of principle

This article is the first to report on the biochemical and clinical results of dietary lysine restriction as adjuvant therapy for PDE due to ATQ deficiency. The fact that 75–80% of patients suffer global



**Fig. 5.** Patient #4 showed a 68.5% reduction of urine AASA (14 to 4.4 fold decrease from upper limit normal) and a 56% reduction of plasma pipecolic acid (3 to 1.3 fold decrease from upper limit normal), both defined as the difference between last value before and first value after initiation of lysine restriction.



**Fig. 6.** Patient #5 showed a 67% reduction of plasma pipecolic acid (3.4 to 1.1 fold decrease from upper limit normal), defined as the difference between last value before and first value after initiation of lysine restriction. Urine AASA values are only available during the period after dietary restriction was started.

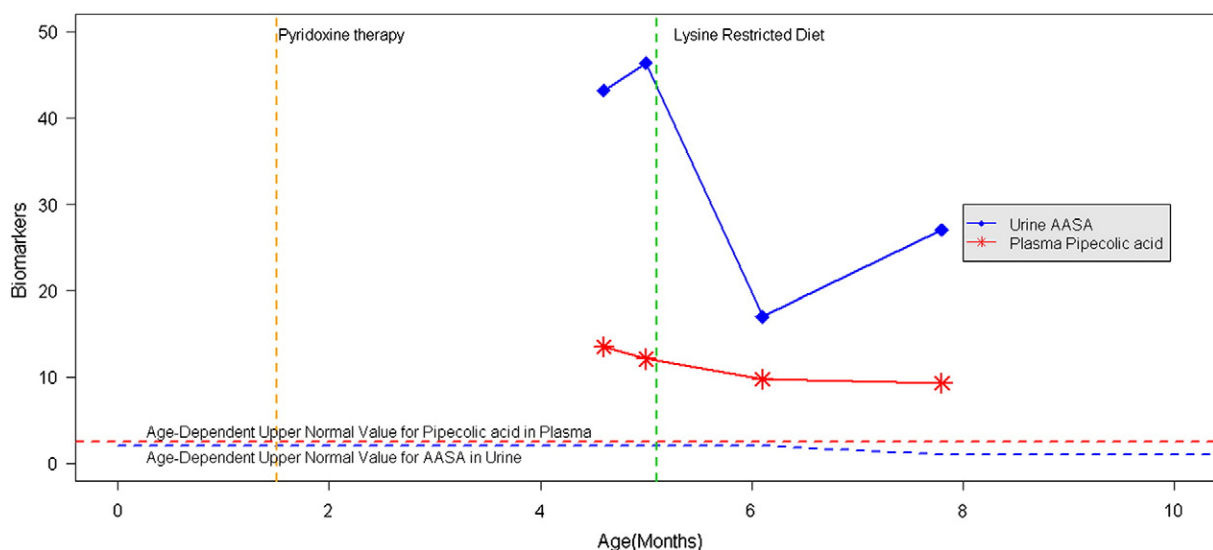
developmental delay or intellectual disability ( $IQ < 70$ ) despite adequate seizure control by pyridoxine treatment [4,5,16] illustrates the need for additional therapeutic strategies in PDE. Extrapolating experience from other neurotoxic inborn errors of metabolism, early intervention with dietary therapy is a beneficial approach to prevent brain damage and optimize neurodevelopment.

In our cohort, dietary lysine restriction effectively reduced chemical biomarkers. Patient #2 showed a significant reduction of CSF AASA as well as pipecolic acid. This observation is important, as the brain is the main pathophysiological target in ATQ deficiency. However, we cannot exclude that a proportion of this decrease may result from the folic acid treatment itself, in view of the case reported by Hyland et al., showing a 75% reduction of the 'unknown peak' on monoamine analysis (likely related to AASA metabolism) with age and on folic acid therapy [17]. The observed reduction of urinary AASA is in agreement with the published correlation of protein intake and urinary AASA excretion in neonates [12]. Urinary AASA and plasma pipecolic acid concentrations decline with age in healthy controls [10], and upon pyridoxine treatment in PDE [18,19]. With dietary restriction, we observed a

further decline of these biomarkers in all but one patient (#5 in whom pre-diet samples were lost), and most notably normalization of urinary AASA in patient #1.

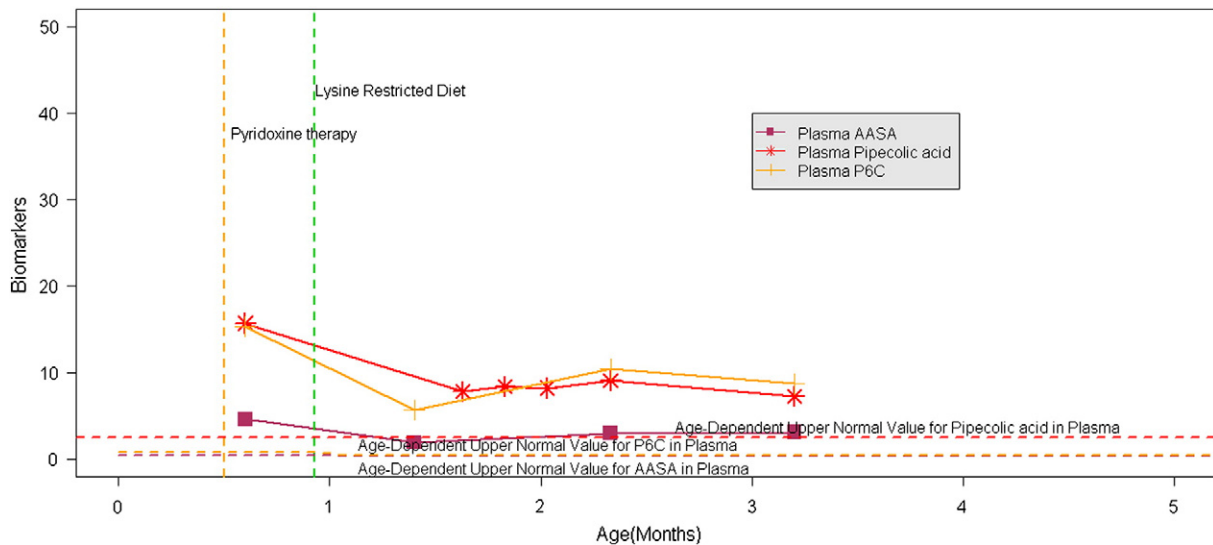
The data on patients #6 and #7 demonstrate that concomitant dietary lysine restriction with introduction of solid food in early infancy is well-tolerated and also results in a remarkable decrease of plasma and urinary biomarkers. For patient #7 the calculated cerebral lysine flux after institution of dietary therapy is similar to that reported in Glutaric Aciduria I patients [20]. These data provide proof of principle that reducing the flux through the lysine degradation pathway will lower the accumulation of potentially neurotoxic substrates arising from the enzymatic block.

The clinical impact of this treatment intervention is more difficult to quantify but seems promising: Up to 10% of patients with PDE have incomplete seizure control with pyridoxine mono-therapy, as in patient #2, seizures persisted despite high dosages of pyridoxine. Dietary lysine restriction resulted in seizure control allowing reduction of pyridoxine dosage; relapse of seizures during brief interruption of the diet illustrates a relation of cause and effect. His recent seizure at age 6.5 years is most likely due to cessation of folic acid by mother. Patient #6, having been



**Fig. 7.** Patient #6 showed a 63% reduction of urine AASA (23 to 8.5 fold decrease from upper limit normal) and a 20% reduction of plasma pipecolic acid (5 to 4 fold decrease from upper limit normal), both defined as the difference between last value before and first value after initiation of lysine restriction.





**Fig. 8.** Patient #7 showed an 45% reduction of plasma AASA (11 to 6 fold decrease from upper limit normal), a 50% reduction of plasma pipecolic acid (6 to 3 fold decrease from upper limit normal), and a 42% reduction of plasma P6C (19 to 11 fold decrease from upper limit normal), all defined as the difference between last value before and first value after initiation of lysine restriction.

seizure free for 6 months on pyridoxine and Phenobarbital, suffered a convulsion during a febrile illness 5 months following lysine restriction and 1 month after Phenobarbital was discontinued. The remaining patients remained seizure-free upon diet and vitamin B6.

Developmental progress was reported in 4 out of the 5 patients showing delayed development before initiation of dietary therapy; confirmation with formal assessment was possible in 3 patients. The most pronounced improvements were observed in patients #2 and #5.

As in patients #6 and #7, lysine restriction was introduced in early infancy; it will not be possible to delineate the influence of this adjuvant treatment on their development.

#### 4.2. Limitations

This is an observational study about the feasibility and safety of dietary lysine restriction as adjuvant treatment in PDE. As in all rare diseases,

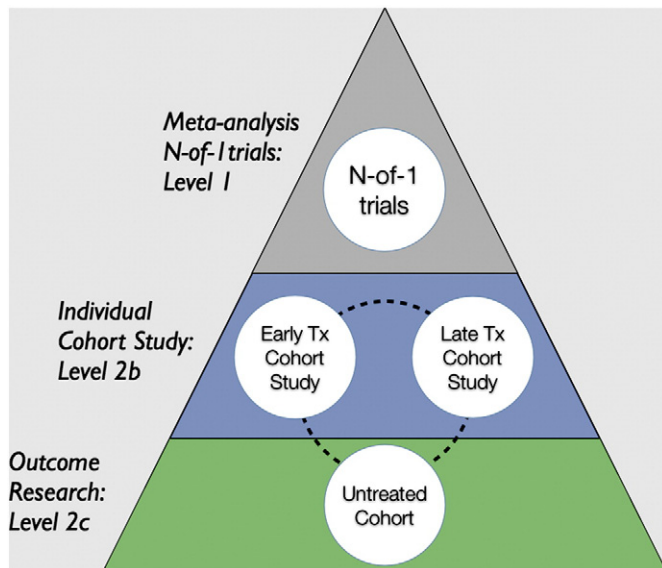
we have to accept certain limitations that need to be taken into account with regards to the interpretation of these study results:

- (1) Chemical markers: The patients cover a wide age range. It is well known that during the first year of life, metabolites of lysine degradation decrease significantly in PDE patients [18,19]. We tried to control for this by expressing the data as X-fold increase of the upper limit of normal value of age related controls. Biochemical analyses were performed by different laboratories applying slightly different methodology. However, in view of the large changes we observed with lysine restriction, the small differences between the respective normal values are insignificant.
- (2) Seizure control: In PDE, the optimal pyridoxine dosage required to achieve seizure control is not known and different therapeutic strategies are followed at different institutions. Consequently, the vitamin B6 dosage varied between our patients, but remained unchanged during dietary treatment, as is true for the additional folic acid therapy in 2 patients. Patients #2 and #6 received anticonvulsive treatment, which was weaned after initiation of lysine restriction.
- (3) Developmental outcome evaluation: Due to the wide age range of our patients and to the varying potential of the institutions involved, a large number and wide range of developmental tests ( $n=8$ ) were applied for assessment of neurodevelopmental outcome in 7 patients. The judgment of parents and investigators on children's developmental progress is prone to bias, but all families except for the family of patient #3 opted to continue the diet in spite of the well-known burden of protein limitation.

To address these limitations, a standard protocol and guidelines have been developed to evaluate the effect of lysine restricted diet for all future studies.

#### 4.3. Need for more evidence

Due to these limitations and the nature of the study, the evidence level for the effects of this treatment is 4 according to the Centre for Evidence-based Medicine criteria [21]. This situation is inherent for rare inborn errors of metabolism. In a systematic literature review [22], we identified 81 treatable inborn errors of metabolism causing intellectual disability, of which ATQ deficiency is one. For the majority



**Fig. 9.** 'All-Inclusive Approach' with adaptive study designs generating different levels of evidence: Meta-analysis of N-of-1 Trials = Level 1; Individual Prospective Cohort Study = Level 2b; Outcome Research (long-term observational (controlled) study) = Level 2c.

(79%) of the 91 treatments identified, the evidence level ranks 4 or lower. Still, 60% of these therapeutic modalities are considered as “standard of care”.

Dietary lysine restriction for ATQ deficiency places a burden on patients and their families, and they often conflict with social and cultural traditions [23]. Also, this diet requires monitoring by a specialist and metabolic dietician or nutritionist with regular laboratory testing and clinical follow-up. Finally, more data are needed to predict which patients with ATQ deficiency will benefit from additional dietary lysine restriction, as natural history data indicate that up to 20% of patients with PDE may show normal psychomotor development on pyridoxine therapy. Thus, before this potentially burdensome dietary intervention becomes mainstay treatment, to further document potential benefits a multicenter prospective study on short- and long-term outcomes of a lysine restricted diet as adjunctive therapy in ATQ deficiency is warranted. Additionally one could consider addition of arginine to the lysine-restricted formula, to exploit the transport competition between lysine and arginine over the blood–brain barrier and further reduce cerebral lysine flux. A similar treatment strategy has shown promising preliminary results in glutaric aciduria type I [24].

#### 4.4. International PDE consortium and research framework

As a first approach to improve evidence, an international consortium of leading clinicians and scientists with experience in PDE and ATQ deficiency developed a protocol for dietary lysine restriction and subsequent monitoring of safety and efficacy.

Randomized controlled trials are the gold standard to create high levels of evidence for treatments. However in rare diseases there are considerable methodological, logistic, and financial limitations to establish such trials. In order to overcome these roadblocks, we are suggesting the simultaneous use of different study designs including N-of-1 trials as well as observational controlled cohort designs, allowing inclusion of all patients, irrespective of age, severity, symptoms, and interventions. Fig. 9 shows how such an integrated approach allows generation of high evidence levels. The website [www.pdeonline.org](http://www.pdeonline.org) comprises an easy to use ‘methodological toolbox’ with different study designs & protocols. Also, it provides access to a registry for physicians, scientists, and families who wish to participate in any research on PDE. This novel research framework will enable physicians to provide the highest quality of care while using new media to connect people around the world and help generate a strong level of evidence for treatment of ATQ deficiency. We will use this study to build a model framework to study therapeutic interventions in this and other rare genetic conditions, aimed to improve evidence for treatments and outcomes of affected patients.

#### Acknowledgments

Funding for research activities in Vancouver (Canada) included the Bluma Tischler Fellowship provided by the University of British Columbia and the Treatable Intellectual Disability Endeavour in B.C. ([www.tidebc.org](http://www.tidebc.org)) as the 1st Collaborative Area of Innovation by BC Children’s Hospital Foundation, Vancouver, Canada. We gratefully acknowledge the contributions of the members of the PDE Consortium (established in Geneva, 2011) and the following individuals: Dr. Eduard Paschke, Dr. Karl Paul and Dr. Gunter Scharer (mutation analysis); Dr. Gabriella Horvath, Dr. Yolanda Lillquist, and Dr. Ramona Salvarinova (clinical care); Mrs. Carol Hartnett, Mrs. Alette Giezen and Mrs. Casey Burns (diet); Mrs. Claire Sowerbutt and Mrs. Lindsay Lynch (manuscript drafts & edits), Mrs. Ruth Giesbrecht and Mrs. Mirafe Lafek (organization of data and graphs); Mr. Richard Jones (contribution data on

biomarker measurement); and Mr. Roderick Houben (Health2Media) for the design of the figures and website [www.pdeonline.org](http://www.pdeonline.org).

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