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Consensus guidelines for the diagnosis and management of pyridoxine-dependent epilepsy due to α-aminoadipic semialdehyde dehydrogenase deficiency

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Abstract: Pyridoxine-dependent epilepsy (PDE-ALDH7A1) is an autosomal recessive condition due to a deficiency of α-aminoadipic semialdehyde dehydrogenase, which is a key enzyme in lysine oxidation. PDE-ALDH7A1 is a developmental and epileptic encephalopathy that was historically and empirically treated with pharmacologic doses of pyridoxine. Despite adequate seizure control, most patients with PDE-ALDH7A1 were reported to have developmental delay and intellectual disability. To improve outcome, a lysine-restricted diet and competitive inhibition of lysine transport through the use of pharmacologic doses of arginine have been recommended as an adjunct therapy. These lysine-reduction therapies have resulted in improved biochemical parameters and cognitive development in many but not all patients. The goal of these consensus guidelines is to re-evaluate and update the two previously published recommendations for diagnosis, treatment, and follow-up of patients with PDE-ALDH7A1. Members of the International PDE Consortium initiated evidence and consensus-based process to review previous recommendations, new research findings, and relevant clinical aspects of PDE-ALDH7A1. The guideline development group included pediatric neurologists, biochemical geneticists, clinical geneticists, laboratory scientists, and metabolic dieticians representing 29 institutions from 16 countries. Consensus guidelines for the diagnosis and management of patients with PDE-ALDH7A1 are provided.

Synopsis: This manuscript details the consensus guidelines for the diagnosis and management of patients with PDE-ALDH7A1

Details of the contribution of individual authors: CRC, LAT, CDMvK lead the systematic review, development of the initial statements, reviewed comments, and drafted the manuscript. JEA, CA, FB, LAB, MB, DB, PTC, AD, AE, FF, EJF, SMG, HH, MK, EK, JL, RL, NL, PM, MTP, PLP, FP, BP, AGS, SS, DRS, SSI, PS, JLKVH, NMVD, SMZ participated in the consensus guideline survey or in person Delphi meeting. HD, RJL, FAW aided in consensus guideline development. All authors critically reviewed and contributed to the final manuscript and agreed to be accountable for the final version of the manuscript.

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INTRODUCTION

Pyridoxine-dependent epilepsy (PDE, OMIM 266100) is a developmental and epileptic encephalopathy historically defined by clinical or electroencephalogram (EEG) response to pyridoxine.[1] Pharmacologic dose of pyridoxine remains central to the treatment of seizures, although some patients may require additional antiseizure medications for optimal seizure control.[2] However, not all patients respond immediately to a trial of pyridoxine.[3] Furthermore, patients may present with concomitant findings such as hypoglycemia and lactic acidosis,[4] and other affected patients may present with seizures after the neonatal period.[5–8] These atypical presentations may further confound and delay the diagnosis, as such patients may not be considered for a therapeutic trial of pyridoxine early in the course of the disease.

The term PDE was initially used to describe the clinical symptoms of neonatal epilepsy in combination with clinical response to pyridoxine.[9] The clinical description itself has been referred to by numerous terms including pyridoxine dependency, pyridoxine-dependent epilepsy and pyridoxine-responsive seizure disorders. While pyridoxine responsiveness reflects the interruption or prevention of seizures when on pyridoxine, a dependency is defined by the recurrence of seizures following withdrawal. Although pyridoxine withdrawal is not common due to the availability of biochemical and genetic testing. The clinical phenotype of pyridoxine dependent or responsive seizures can be caused by many genetic disorders with unique disease mechanisms and treatment implications.[10] This guideline is focused on PDE due to deficiency of α-aminoadipic semialdehyde (α-AASA) dehydrogenase (E.C. 1.2.1.3), which is encoded by the gene *ALDH7A1*.[11] To standardize the nomenclature in this manuscript, we refer to this disorder as PDE-ALDH7A1.

The enzyme α-AASA dehydrogenase oxidizes α-AASA to α-aminoadipic acid; a deficiency of this enzyme results in accumulation of pipecolic acid,[12–14] α-AASA,[11,15–17] and its cyclic equilibrium partner Δ^1-piperideine-6-carboxylate (Δ^1-P6C) (Figure 1).[18,19] The accumulated Δ^1-P6C is postulated to bind the active vitamer of pyridoxine (pyridoxal 5'-phosphate) through a Knoevenagel condensation, and pharmacologic doses of pyridoxine are used to overcome the secondary pyridoxal 5'-phosphate deficiency. α-AASA dehydrogenase is encoded by the gene *ALDH7A1* (NM_001182), which is also referred to as antiquitin.[11] *ALDH7A1* is located at chromosome 5q32.2, containing a transcript of 4,964 base pairs and 539 amino acids divided among 18 exons. Thus far, over 165 pathogenic variants in *ALDH7A1* have been published[20] or are listed in public databases such as The Human Gene Mutation Database (HGMD) and ClinVar. The vast majority of patients have had biallelic pathogenic variants identified in *ALDH7A1* consistent with the autosomal recessive inheritance of the disease.[6,15,21] When interpreting genetic testing results, it is important to note that a common synonymous variant p.Val278Val (historical nomenclature: c.750G>A, r.748_787del) results in a cryptic splice site[22] and that intragenic deletions are relatively common.[20,23]

Approximately 75% of all patients have intellectual or developmental delay (IDD), and IDD was observed despite early diagnosis and adequate seizure control.[24] The accumulating α-AASA and related compounds were suggested to be neurotoxic organic acids, which may contribute to the poor cognitive outcome described in PDE-ALDH7A1.[25] Shortly thereafter, an observational study was completed in seven children who were treated with a lysine-restricted diet (substrate reduction therapy) and pharmacologic doses of pyridoxine. The addition of a lysine-restricted diet resulted in decreased accumulation of pipecolic acid, α-AASA, and Δ^1-P6C as well as noting improvement in both age-appropriate skills and seizure management.[26] Subsequently, several observational studies have reported improved clinical outcomes following adjunct treatment with a lysine-restricted diet,[27] pharmacologic doses of arginine, which acts as a competitive inhibitor of lysine transport,[28–30] and a combination of pyridoxine, a lysine-restricted diet, and arginine referred to as triple therapy.[28,30–34] To standardize the nomenclature in this manuscript, we refer to these additional dietary treatment strategies as lysine reduction therapies.

The initial set of recommendations for diagnosis, treatment and follow up of PDE-ALDH7A1 was published in 2011.[25] Treatment recommendations were updated in 2014 to include the use of a lysine-restricted diet.[35] Over the last six years, several observational studies have reported improved clinical outcomes following treatment with various lysine-reduction therapies. The members of the PDE Consortium reviewed the current state and new developments and elected to revise the recommendations due to the increased number of treatment modalities and observational data focused on improved clinical outcomes. This article summarizes the consensus guidelines for the diagnosis and management of PDE due to the deficiency of α-AASA dehydrogenase.

METHODS

Guideline development

Guidelines for PDE-ALDH7A1 were developed by members of the Pyridoxine-Dependent Epilepsy Consortium (www.pdeonline.org), which was established in 2010 to facilitate international collaboration between clinicians and scientists with expertise in PDE-ALDH7A1. The guideline development group consisted of pediatricians specialized in inborn errors of metabolism, adult and pediatric neurologists, biochemical geneticists, clinical geneticists, laboratory scientists, metabolic dieticians, and patient advocates. The guideline development group included representation from 29 institutions across Africa, Asia, Australia, Europe, North America, and South America. The guideline committee members (CRC, LAT, CDMvK) selected a list of topics and transformed them into statements based on previous guidelines, available literature, and expertise of the committee. Two surveys based on these statements were distributed to members of the PDE Consortium. An in-person meeting was moderated on September 2nd, 2019 at the Amsterdam University Medical Centers in Amsterdam, the Netherlands, in conjunction with the 5th international PDE workshop. Communication occurred through e-mails and conference calls throughout the development of the guidelines.

Conflicts of interest

All members of the guideline development group were required to report potential conflicts of interest. Most group members (84%) reported no conflict of interest relating to this guideline. Two committee members (5.3%) reported serving on scientific advisory boards, receiving honoraria from pharmaceutical companies, and receiving research support for studies unrelated to PDE-ALDH7A1. Two committee members (5.3%) reported a patent pending for a diagnostic method for PDE-ALDH7A1 that was not discussed in this guideline. Two committee members (5.3%) reported research funding for clinical studies focused on PDE-ALDH7A1.

Systematic literature review

A systematic literature search of MEDLINE and Cochrane Library was performed for manuscripts published between January 2005 and December 2019. Key search terms included pyridoxine-dependent epilepsy, pyridoxine-dependent seizures, pyridoxine-responsive epilepsy, pyridoxine-responsive seizures, antiquitin deficiency, α-AASA dehydrogenase, and *ALDH7A1*. All data was collected and analyzed using the revtools package[36] written for the statistical software R. The initial search identified 742 peer-reviewed publications. After a manual review by the guideline committee, 5 articles were added for a total of 747 abstracts. After duplicates were removed, a total of 336 abstracts were reviewed. Only those abstracts published in the English language and those that included clinical findings in patients with PDE due to a deficiency of α-AASA dehydrogenase were included. This was defined by the elevation of α-AASA/Δ^1-P6C in plasma, urine, or cerebral spinal fluid (CSF) or the presence of biallelic mutations in *ALDH7A1*. A total of 174 abstracted were accepted as relevant and their full-text articles were reviewed resulting in the final synthesis of 109 full-text articles (Supplemental Figure 1). The literature review was initially performed by committee members (CRC, LAT, CDMvK) and shared with the guideline development group.

Grading and strength of recommendations

The guideline committee members used the Grading of Recommendation Assessment, Development and Evaluation (GRADE)[37] approach to assessing evidence for each statement. The quality of evidence was graded as high, moderate, low, or very low as described in the GRADE consensus documents (Table 1). The quality assessment of the evidence was influenced by study design (i.e. randomized trial compared to observational study), risk of bias, and size of effect reported in the literature.

The GRADE system offers two categories of recommendations often referred to as “strong” and “weak.” These categories are typically based on the quality of evidence or the impact of a treatment.[37] To date, there have been no prospective randomized controlled trials to assess diagnostic approaches or management of PDE-ALDH7A1. This results in relatively low levels of evidence as most published studies are based on observational study designs and expert opinion. As a result, this guideline is highly dependent on expert opinion, which is not unique for clinical guidelines for inborn errors of metabolism.[38] The strength of recommendation provided herein is based on whether the recommendation can apply to most patients in most circumstances and the high degree of agreement (Table 1). A statement received a strong recommendation when there was at least 90% agreement (completely agree or mostly agree) among the PDE experts.

Consensus procedure

The consensus procedure consisted of two online surveys and one in-person meeting (Supplemental Figure 2). The surveys were created and distributed using Survey Monkey Inc. (San Mateo, California, United States). An initial survey was developed by the guideline committee and included 29 statements focused on definition and epidemiology, clinical findings, diagnostic investigations, chronic treatment, chronic treatment monitoring, and emergency management. Each statement was evaluated using a 5-point Likert scale as follows: completely agree, mostly agree, partially agree, mostly disagree, completely disagree. The consensus was defined as at least 66% agreement (completely agree or mostly agree) or disagreement (mostly disagree or completely disagree). The initial survey was sent to 49 members of the PDE Consortium, was available for six weeks, and 25 individual responses were received. Consensus was reached for 27 of 29 statements (>85% agreement N = 14; 67-84% agreement N = 13; <67% N = 2).

The results of the survey were discussed at the in-person meeting. A second survey was developed based upon the initial survey results and discussion of the nominal group at the in-person meeting. The Appraisal of Guidelines for Research and Evaluation (AGREE II) criteria was used as a guide.[39] One additional statement was added regarding patient outcome collection and future guideline updates. The revised statements were sent to the 49 members of the PDE Consortium, was available for ten weeks, and 29 responses were received. Consensus for the updated statements was reached for 29 of 30 statements (>85% agreement N = 22; 67-84% agreement N = 7; <67% agreement N = 1) (Supplemental Table 1). The proposed clinical guidelines were also reviewed by two patient advocacy groups based in Europe and North America, respectively.

RECOMMENDATIONS:

Definition and epidemiology

Statement #1: PDE-ALDH7A1 is caused by bi-allelic mutations in the ALDH7A1 gene. Mutations in this gene are associated with decreased activity of α-aminoadipic semialdehyde dehydrogenase (α-AASA) and result in the accumulation of pipecolic acid, Δ^1-piperideine-6-carboxylate (Δ^1-P6C), and α-AASA

- *Level of evidence: B*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (96.6%), mostly agree (3.4%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

The genetic etiology of PDE-ALDH7A1 was initially identified in patients with the clinical phenotype of pyridoxine responsive and dependent epilepsy.[11] There have been multiple larger case series that have consistent data suggesting that the clinical phenotype of PDE-ALDH7A1 is associated with mutations in *ALDH7A1*.[6,15,20,21,40] Increased α-AASA and Δ^1-P6C were reported in patients with PDE compared to a control group including those with epilepsy and normal controls.[16,18] There are no patients described in the literature with biallelic mutations in

ALDH7A1 and normal AASA/P6C. Increased pipecolic acid has been described in patients with PDE-ALDH7A1, however, normal pipecolic acid levels have also been found when patients were tested while treated with pyridoxine.[12,41]

Statement #2: PDE-ALDH7A1 is [a] rare disease with an estimated incidence of 1:65,000 to 1:250,000 live births. PDE-ALDH7A1 is pan-ethnic and > 98% of all patients have bi-allelic mutations in ALDH7A1.

- *Level of evidence: C*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (82.1%), mostly agree (17.9%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

A recent study estimated the incidence of PDE-ALDH7A1 at 1:65,000 live births based on publicly available genomic data.[20] This study has multiple limitations including the assumption that all fetuses with PDE-ALDH7A1 are viable. Previous estimates of the incidence of PDE have varied widely from 1:20,000 based on neonates referred to a single center in Germany[42] to 1:783,000 in the United Kingdom.[43] These studies were performed before the identification of genetic or biochemical diagnostic markers. Applying strict clinical criteria, Been *et al* estimated an incidence of 1:250,000 newborns.[44]

Clinical presentation

Statement #3: PDE-ALDH7A1 is characterized by an epileptic encephalopathy with varying degree of intellectual disability or developmental delay.

- *Level of evidence: C*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (65.5%), mostly agree (31.0%), partially agree (3.4%), mostly disagree (0%), completely disagree (0%)*

PDE-ALDH7A1 was initially defined by refractory neonatal seizures, although several case series have reported patients with associated IDD. In a review of pyridoxine-responsive and dependent seizures registry, 75% of all subjects were reported to have IDD by parental or physician report,[2] and 70% of patients with genetically confirmed PDE-ALDH7A1 had IDD with an intelligence quotient (IQ) below 85.[24] In these studies, there was no correlation between IDD and seizure control. At this time, there are no known patients who have presented with isolated IDD. Some patients who have remained seizure-free due to prenatal and neonatal pyridoxine supplementation have also been reported with significant IDD.[11,45]

Statement #4: Patients with PDE-ALDH7A1 may present with seizures outside of the newborn period

- *Level of evidence: B*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (96.6%), mostly agree (3.4%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

There are over 20 descriptive studies that report the onset of seizures outside of the newborn period. In a review of the pyridoxine-responsive and dependent seizures registry, 30% of subjects had onset of seizures outside of the newborn period,[2] and a retrospective study of patients confirmed to have PDE-ALDH7A1 noted that 25% of patients presented outside of the newborn period.[46] Recently, some patients have been reported with seizure onset after one year of life with the latest presentation of onset seizures at 17 years of age.[7,8,47,48] Late seizure onset has been postulated as beneficial for the developmental outcome with several factors hypothesized to contribute to this outcome including genetic and functional variation, variation in the treatment regimen, absence of neonatal seizures, and unknown protective factors.[27,49]

Diagnostic investigations

Statement #5: All individuals with an unexplained seizure disorder should be tested for PDE-ALDH7A1

- *Level of evidence: C*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (60.7%), mostly agree (21.4%), partially agree (14.3%), mostly disagree (3.6%), completely disagree (0%)*

The initial guideline recommendations stated that PDE-ALDH7A1 should be considered in some clinical scenarios including seizures of unknown etiology, infants and children with seizures which are partially responsive to antiseizure medications, and children under one year of age without an apparent causal brain malformation.[25] Since those initial guidelines were published, some patients have presented after one year of age and into late adolescence. There is not a well-defined electrographic signature that is pathognomonic for either neonatal-onset or late-onset PDE-ALDH7A1,[3] which emphasizes the importance of testing for this disease in the absence of an established etiology. Prospective genetic evaluations of patients with epilepsy have identified patients with PDE-ALDH7A1 that were otherwise not diagnosed.[50] Recent recommendations have included testing all children with seizures of unknown etiology.[51]

Statement #6: Diagnostic biomarkers include α-AASA and Δ^1-P6C, which can be measured in urine, blood, or cerebral spinal fluid. Pipecolic acid is elevated in urine, plasma, and cerebral spinal fluid in most patients with PDE-ALDH7A1. The biomarkers α-AASA/Δ^1-P6C can be used alone or in combination with other biomarkers to increase sensitivity and specificity.

- *Level of evidence: B*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (65.5%), mostly agree (34.5%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

Statement #7: Genetic testing of ALDH7A1 should be performed as α-AASA/Δ^1-P6C elevations have been reported in disorders of sulfite oxidase

- *Level of evidence: D*
- *Strength of recommendation: 1*

- *Expert opinion: completely agree (69%), mostly agree (27.6%), partially agree (0%), mostly disagree (0%), completely disagree (3.4%)*

Statement #8: Biallelic pathogenic variants in the ALDH7A1 gene is consistent with the diagnosis of PDE-ALDH7A1. Genetic testing is the only reliable method for carrier screening of family members and prenatal diagnosis. Biochemical testing should be performed when a single pathogenic variant or a variant of uncertain significance is identified.

- *Level of evidence: B*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (82.8%), mostly agree (13.8%), partially agree (0%), mostly disagree (3.4%), completely disagree (0%)*

Historically, the diagnosis of PDE-ALDH7A1 was based upon the positive clinical response to pyridoxine. Unfortunately, patients may not have an immediate clinical or EEG response to a trial of pyridoxine and a positive clinical response is not unique to PDE-ALDH7A1.[3,10] All affected patients with PDE-ALDH7A1 have been reported to have increased plasma or urine α-AASA/Δ^1-P6C emphasizing the sensitivity of these biomarkers.[16,18] Patients with molybdenum cofactor deficiency and isolated sulfite oxidase deficiency have been reported with mild elevations of α-AASA/Δ^1-P6C due to secondary inhibition of α-AASA dehydrogenase.[52,53] The diagnosis of PDE-ALDH7A1, molybdenum cofactor deficiency, or isolated sulfite oxidase deficiency can be confirmed by further biochemical testing or molecular genetic testing. Timely confirmation of molybdenum cofactor deficiency is paramount as disease-specific treatment is available.[54]

Pipecolic acid was the first biomarker identified in patients with PDE-ALDH7A1, although patients who are tested after pyridoxine treatment may have normal levels of pipecolic acid.[12,41] Other biomarkers have been reported including peak X (an unidentified compound)[55,56] and 6-oxo-pipecolate[57,58] although the role of these biomarkers in diagnosis or treatment monitoring has yet to be established. Many clinicians have adopted the use of gene panels or genomic sequencing when patients are suspected to have a genetic disorder. In these cases, biochemical testing can confirm the diagnosis of PDE-ALDH7A1 when genetic testing is uninformative.

General principles of treatment

Statement #9: All patients with PDE-ALDH7A1 should be treated with pyridoxine supplementation.

- *Level of evidence: B*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (93.1%), mostly agree (6.9%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

Pharmacologic doses of pyridoxine for pyridoxine-dependent seizures has been the mainstay of treatment since the identification of these seizures by Hunt et al 1954, hence the name.[9] Descriptive studies have reported patients with PDE-ALDH7A1 who died before pyridoxine was provided.[34,59,60] Furthermore, the withdrawal of pyridoxine has been associated with recurrence of seizures,[61–65] underscoring the necessity of pyridoxine in the treatment of PDE-ALDH7A1.

Statement #10: Lysine reduction therapies have been associated with improved long-term neurologic outcomes. Therapies include pyridoxine supplementation in combination with a lysine-restricted diet or arginine supplementation and a combination of all three (i.e. triple therapy)

- *Level of evidence: C*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (65.5%), mostly agree (20.7%), partially agree (10.3%), mostly disagree (3.4%), completely disagree (0%)*

The association between lysine reduction therapies and neurologic outcomes has been reported in a total of 10 observational studies describing 27 individual patients with PDE-ALDH7A1.[26,28–34,66,67] These studies have reported improvement in seizure control and development in many but not all subjects. Unfortunately, several outcome measures were used in these studies ranging from subjective parental reports to formal neurocognitive testing, which limits the use of a meta-analysis. Other factors have been postulated to confound the impact of lysine reduction therapies including the timing of diagnosis and treatment.[28]

Treatment of newborns and infants

Statement #11: All newborns with PDE-ALDH7A1 should be treated with 100 mg per day of pyridoxine supplementation. Infants should be treated with 30 mg/kg/day of pyridoxine supplementation with a maximum dose of 300 mg per day.

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (50%), mostly agree (38.5%), partially agree (11.5%), mostly disagree (0%), completely disagree (0%)*

Although the use of pharmacologic doses of pyridoxine has been well supported, the dose of pyridoxine for newborns, infants, children, and adults is based on observational data and expert opinions. In the initial guidelines, a diagnostic trial of pyridoxine was recommended at 100 mg given intravenously and repeated up to four times (maximal dose 500 mg). It is important to note that intravenous pyridoxine is not without risk as apnea and comatose state have been reported after the initial intravenous dose of pyridoxine.[68,69] Although lower doses of pyridoxine have been reported, the recommended dose of pyridoxine for long term management was 15 – 30 mg/kg/day in infants and up to 200 mg/day in neonates.[25]

Statement #12: All newborns and infants with PDE-ALDH7A1 should be treated with lysine reduction therapies

- *Level of evidence: D*
- *Strength of recommendation: 1*

- *Expert opinion: completely agree (65.5%), mostly agree (27.6%), partially agree (6.9%), mostly disagree (0%), completely disagree (0%)*

As noted above, the benefit of lysine reduction therapies has been limited to case reports and case series. Of note, two studies focused on the benefit of lysine reduction therapies in patients under the age of one year. Even in patients who were treated in the first months of life, pre-treatment sequelae were noted on brain MRI,[31] and in a larger retrospective review, add-on treatment after seven months of age was associated with poor neurologic outcome.[28]

Statement #13: In newborns and infants, a lysine-restricted diet should include a lysine-free amino acid formula in order to maintain adequate total protein and micronutrient intake and low-normal plasma lysine level.

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (69%), mostly agree (27.6%), partially agree (3.4%), mostly disagree (0%), completely disagree (0%)*

The first revision to the guidelines was focused on the use of a lysine-restricted diet, and encouraged breastfeeding as the average protein content of breast milk is considerably lower than that of formula milk.[35] The use of a lysine-free formula with amino acid supplements was also recommended to ensure both the limitation of lysine and appropriate intake of nutritional supplementation.[35] It is important to note that the majority of lysine-free formulas are designed for glutaric aciduria type I, which is a disorder of both lysine and tryptophan metabolism and therefore is also low in tryptophan.[70]

Statement #14: In newborns and infants, arginine supplementation should be started at a dose of 200 mg/kg/day whether arginine is provided alone or in combination with a lysine-restricted diet

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (50%), mostly agree (25%), partially agree (14.3%), mostly disagree (3.6%), completely disagree (7.1%)*

Pharmacologic doses of arginine have been used to compete with lysine for intestinal absorption as well as the transport over the blood-brain barrier and entry into the mitochondria. Arginine has been used with both pyridoxine alone[29] and in combination with a lysine-restricted diet and pyridoxine.[30–32] The dose of arginine for newborn, children and adults are based on observational data and expert opinions. Previous recommendations for arginine dosing recommended 150 mg/kg/day if administered in addition to a lysine-restricted diet and 400 mg/kg/day if administered with pyridoxine alone.[71] A recent study using stable isotopes to measure lysine oxidation in healthy adult volunteers suggested a higher dose of arginine may be required to impact lysine transport.[72] Pharmacologic doses of arginine are also used in other disorders. In randomized controlled trials evaluating the use of arginine in preterm infants at risk for necrotizing enterocolitis, the mean dose of arginine was 261 mg/kg/day with no adverse events reported.[73–75] Arginine is recommended for the long-term treatment of most urea cycle disorders other than arginase deficiency. The dose of arginine in neonates and infants with urea cycle disorders ranges from 100 – 300 mg/kg/day and a dose of 2.5-6 g/m^2/day is recommended in patients who weigh more than 20 kg.[76]

Treatment of children and adolescents

Statement #15: Children and adolescents with PDE-ALDH7A1 should be treated with an average of 20 mg/kg/day (range 5-30 mg/kg/day) of pyridoxine with a maximum dose of 500 mg per day.

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (59.3%), mostly agree (33.3%), partially agree (3.7%), mostly disagree (3.7%), completely disagree (0%)*

Statement #16: All children and adolescents with PDE-ALDH7A1 should be offered treatment with lysine reduction therapies. Children and adolescents with cognitive delay, behavioral difficulties, or poor seizure control should be treated with lysine reduction therapies.

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (58.6%), mostly agree (27.6%), partially agree (10.3%), mostly disagree (3.4%), completely disagree (0%)*

Statement #17: In children and adolescents, a lysine-restricted diet may include a lysine-free amino acid formula. If a lysine-free formula is not well tolerated, lysine reduction may be achieved by reducing total natural protein to the low end of age-appropriate needs

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (44.8%), mostly agree (37.9%), partially agree (10.3%), mostly disagree (6.9%), completely disagree (0%)*

In the previous update to the PDE guidelines, a protein-restricted diet was recommended when a lysine-free formula was unavailable or if a patient did not tolerate the formula.[35] Patients treated with protein-restricted diets, as well as pharmacologic doses of arginine and pyridoxine, had similar reductions in biochemical parameters and improved clinical outcomes. It is important to balance natural protein levels to both meet age-appropriate protein needs and reduce lysine oxidation. This emphasizes the importance of ametabolic dietitian as part of the clinical team.

Statement #18 focused on the dose of arginine in children and adolescents with a suggested dose of 200 mg/kg/day and a maximum dose of 600 mg/kg/day. Despite being included in both surveys and discussion at the in-person meeting, a consensus was not reached. Therefore, the statement is not provided in this manuscript although the proposed statement, expert opinion, and comments from guidelines members are available in the appendix.

Treatment of adults

Statement #19: All adults with PDE-ALDH7A1 should be treated with 200-500 mg per day of pyridoxine.

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (66.7%), mostly agree (18.5%), partially agree (11.1%), mostly disagree (3.7%), completely disagree (0%)*

As noted above, the dose of pyridoxine for adults is based on observational data and expert opinions. In the initial guidelines, the maximum recommended dose of pyridoxine was 500 mg/day in adults.[25]

Statement #20: All adults with PDE-ALDH7A1 should be offered treatment with lysine reduction therapy. Adults with cognitive delay, behavioral difficulties, or poor seizure control should be treated with lysine reduction therapies

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (55.2%), mostly agree (13.8%), partially agree (27.6%), mostly disagree (3.4%), completely disagree (0%)*

Statement #21: In adults, lysine reduction may be achieved by reducing total natural protein to the low end of age-appropriate needs

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (51.9%), mostly agree (40.7%), partially agree (7.4%), mostly disagree (0%), completely disagree (0%)*

Statement #22: In adults, arginine supplementation should be started at 4 g/m^2/day with a maximum dose of 5.5 g/m^2/day

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (29.2%), mostly agree (45.8%), partially agree (12.5%), mostly disagree (12.5%), completely disagree (0%)*

As noted above, the benefit of lysine reduction therapies has been limited to case reports and case series and the impact of treatment in adults is still unclear. Thus, these recommendations are dependent on expert opinion. As noted above, arginine is recommended for long-term treatment of most urea cycle disorders, and the dose of 2.5-6 g/m^2/day is recommended in patients who weigh more than 20 kg.[76] And in adults with mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS) a dose of 150 – 500 mg/kg/day is recommended.[77,78]

Treatment monitoring

Statement #23: All patients treated with pyridoxine supplementation should have clinical screening for peripheral neuropathy including assessment of deep tendon reflexes. Patients who are treated with >500 mg per day of pyridoxine may be at higher risk of peripheral neuropathy and further evaluation with electrodiagnostic testing may be warranted

- *Level of evidence: C*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (82.8%), mostly agree (6.9%), partially agree (6.9%), mostly disagree (0%), completely disagree (3.4%)*

In the initial guidelines, patients treated with >500 mg per day of pyridoxine were recommended to have screening for peripheral neuropathy.[25] Case reports of patients with PDE who had documented neuropathy were treated with relatively high doses of pyridoxine, although one of these patients did not have genetic or biochemical confirmation of PDE-ALDH7A1.[45,79] Adult patients with homocystinuria have also been reported with a sensory neuropathy at doses > 1000 mg per day.[80] In a systematic review of peripheral neuropathy associated with pyridoxine, authors suggested that even doses as low as 50 mg per day of pyridoxine used for greater than 6 months may increase the risk of neuropathy.[81] A clinician skilled in the performance of electrodiagnostic testing (specifically, measurements of sensory and motor nerve conduction velocities) in young patients may not practice in all centers caring for patients with PDE-ALDH7A1. If this resource is available, screening electrodiagnostic testing every 1-2 years should be considered in cases of a higher dose of pyridoxine or based upon clinical suspicion of neuropathy. In patients with symptomatic peripheral neuropathy or in whom there is neurophysiological evidence of progressive neuropathy, a reduction in pyridoxine dose may need to be considered and weighed against a possible deterioration of seizure control.

Statement #24: All patients treated with a lysine-restricted diet should have plasma amino acids measured at least every 3 (<3 years of age) to 6 months (>3 years of age)

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (72.4%), mostly agree (20.7%), partially agree (6.9%), mostly disagree (0%), completely disagree (0%)*

Amino acid concentrations are used to individually tailor the lysine-restricted diet, and the previous guidelines recommended plasma lysine should remain in the lower quartile of the reference range.[35] Lysine is an essential amino acid and over restriction of lysine has the potential risk of malnutrition.[82] In patients with glutaric aciduria type I, an appropriately administered low lysine diet prevented malnutrition and was able to promote normal weight gain,[83] although patients should be closely monitored especially during periods of rapid growth. As a result, dietary treatment should be supervised by a metabolic dietitian.

Statement #25: All patients on lysine-reduction therapies should have plasma and urine biomarkers Δ^1-P6C and/or α-AASA measured every 6-12 months to assess treatment efficacy

- *Level of evidence: D*

- *Strength of recommendation: 2*
- *Expert opinion: completely agree (41.4%), mostly agree (41.4%), partially agree (17.2%), mostly disagree (0%), completely disagree (0%)*

Statement #26: If a lumbar puncture is performed, CSF Δ^1-P6C, α-AASA, pyridoxal 5'-phosphate, pipecolic acid, neurotransmitters, and amino acids should be measured to assess treatment efficacy

- *Level of evidence: D*
- *Strength of recommendation: 2*
- *Expert opinion: completely agree (58.6%), mostly agree (20.7%), partially agree (13.8%), mostly disagree (6.9%), completely disagree (0%)*

A small number of observational studies have reported the impact of treatment on CSF metabolites. Compared to pre-treatment levels, patients treated with lysine reduction therapy have shown an increase in CSF arginine and a decrease in CSF lysine and α-AASA.[29–31] Further studies are required to determine if there is an association between these metabolites and clinical outcomes. Minor elevations of other CSF amino acids and decrease in 5-methyltetrahydrofolate have been noted in patients with PDE-ALDH7A1, although these findings may be due to a deficiency of pyridoxal 5'-phosphate.[25,84] Historically abnormal gamma-aminobutyric acid (GABA) metabolism was suggested to be the underlying cause of PDE, although CSF glutamate and GABA are typically in the normal range.[85–87] As noted previously, most lysine-free formulas are low in tryptophan. One PDE patient treated with a lysine-free and low tryptophan formula was noted to have a mild serotonin deficiency (low 5-hydroxyindoleacetic acid).[30,67]

Statement #27 All patients with PDE-ALDH7A1 should have developmental evaluations to assess treatment efficacy. Developmental assessments should be age-appropriate, started at the time of diagnosis, and repeated at the start of school or when there is clinical concern of developmental delay

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (82.8%), mostly agree (13.8%), partially agree (3.4%), mostly disagree (0%), completely disagree (0%)*

Emergency treatment

Statement #28: In times of seizure relapse during febrile illness, the dose of pyridoxine may be doubled up to a maximum of 60 mg/kg/day (in children) or 500 mg/day (adolescents and adults) for up to 3 days

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (62.1%), mostly agree (31%), partially agree (3.4%), mostly disagree (3.4%), completely disagree (0%)*

Statement #29: In times of illness, ensure adequate caloric intake to prevent catabolism of endogenous protein and reduce protein intake

- *Level of evidence: D*
- *Strength of recommendation: 1*
- *Expert opinion: completely agree (67.9%), mostly agree (25%), partially agree (3.6%), mostly disagree (3.6%), completely disagree (0%)*

In the previous versions of the guidelines, higher dosages of pyridoxine during the first three days of febrile illness[25] and maintenance of caloric intake to prevent catabolism[35] were recommended. These recommendations are based on expert opinion as there is no data on efficacy or safety of the increased dose of pyridoxine in this setting. Further studies are needed to elucidate whether and which emergency treatments are warranted during times of illness or other catabolic stress.

Guideline update

Statement 30: In order to assess the efficacy of these recommendations, patient outcomes should be systematically collected in the PDE patient registry. New evidence on treatment efficacy or impact of treatment on patients will be reviewed by the International PDE Consortium for immediate response or more detailed consideration of an update to the existing guideline.

- *Strength of recommendation: 1*
- *Expert opinion: completely agree (96.4%), mostly agree (3.6%), partially agree (0%), mostly disagree (0%), completely disagree (0%)*

Using the AGREE II instrument as a guide,[39] we propose a procedure for updating the guideline for diagnosis and management of patients with PDE-ALDH7A1. Specifically, we recommend using the existing PDE patient registry to collect patient outcomes which will be evaluated by the International PDE Consortium. Patient registries can provide a helpful structure for the collection of uniform data to evaluate specified outcomes for a specific population. Patient registries can be used for several purposes including elucidation of the natural history of the disease course or treatment outcomes. Patient registries can be used for clinical trial designs in many ways and if well designed and well-performed, studies from patient registries can provide a real-world view of these outcomes.[88,89] Due to low prevalence and often scarcity of data, (international) collaboration and optimal use of limited resources by data sharing is recommended.

CONCLUSIONS

These guidelines address the diagnosis and management of PDE-ALDH7A1 and are based on the best available evidence. With these guidelines, we aim to facilitate clinical decision making and improve the care for patients with PDE-ALDH7A1 in a standardized manner.

The ability to diagnose PDE-ALDH7A1 has dramatically improved with the identification of multiple biomarkers and the increased availability of genetic testing. One limitation may be that not every clinician is aware of the significant phenotypic heterogeneity in this disease.[4] This may explain why the diagnosis of PDE-ALDH7A1 is delayed even when patients present with classical symptoms such as neonatal epileptic encephalopathy.[46] The clinical semiology of this rare disease may be improved through the collaboration of the International PDE Consortium and the PDE patient registry. Although there is limited evidence, the present guidelines support that lysine-reduction therapies should be started early in life for optimal neurologic outcomes.[28] Next-generation sequencing epilepsy panels and genomic sequencing aid in the diagnosis of new patients all around the world, especially in countries with difficult access to biochemical analysis. In the future, newborn screening may provide an opportunity to identify all patients with PDE-ALDH7A1 regardless of presentation[57,90] and ensure treatment is initiated before further damage occurs.

Although the phenotype of PDE was described over sixty-five years ago,[9] the most frequent underlying genetic defect, namely a deficiency of α-AASA dehydrogenase, was described less than 15 years ago.[11] Evidence for the benefit of lysine reduction therapies has been limited given that only observational studies have been performed. Questions remain about whether lysine reduction therapies are beneficial in all patients. Even so, there is optimism that the combination of pyridoxine and lysine reduction therapies can improve the poor cognitive outcome pervasive in this disease. We did not reach consensus on the statement regarding the dosage of arginine for both children and adolescents.

The evidence for many of the recommendations are limited and, as a result, these guidelines are highly dependent on expert opinion. It is imperative to continually evaluate the evidence that supports these recommendations and establish meaningful clinical outcomes to evaluate current and future therapies for PDE-ALDH7A1. Since prospective randomized controlled trials in rare diseases are difficult, the International PDE registry (www.pdeonline.org) provides a great opportunity to systematically collect the impact of these recommendations for the diagnosis and management of these patients.

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References

1. Scriver, C. R. Vitamin B6-dependency and infantile convulsions. *Pediatrics* **26**, 62–74 (1960).
2. Basura, G. J., Hagland, S. P., Wiltse, A. M. & Gospe, S. M. Clinical features and the management of pyridoxine-dependent and pyridoxine-responsive seizures: review of 63 North American cases submitted to a patient registry. *Eur. J. Pediatr.* **168**, 697–704 (2009).
3. Bok, L. A. *et al.* The EEG response to pyridoxine-IV neither identifies nor excludes pyridoxine-dependent epilepsy. *Epilepsia* **51**, 2406–2411 (2010).
4. van Karnebeek, C. D. M. *et al.* Pyridoxine-Dependent Epilepsy: An Expanding Clinical Spectrum. *Pediatr. Neurol.* **59**, 6–12 (2016).
5. Wang, S., Sun, J., Tu, Y., Zhu, L. & Feng, Z. Clinical and genetic characteristics of pyridoxine-dependent epilepsy: Case series report of three Chinese patients with phenotypic variability. *Exp Ther Med* **14**, 1989–1992 (2017).
6. Mills, P. B. *et al.* Genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy (ALDH7A1 deficiency). *Brain* **133**, 2148–2159 (2010).
7. Haidar, Z. *et al.* Atypical pyridoxine dependent epilepsy resulting from a new homozygous missense mutation, in ALDH7A1. *Seizure* **57**, 32–33 (2018).
8. Srinivasaraghavan, R., Parameswaran, N., Mathis, D., Bürer, C. & Plecko, B. Antiquitin Deficiency with Adolescent Onset Epilepsy: Molecular Diagnosis in a Mother of Affected Offsprings. *Neuropediatrics* **49**, 154–157 (2018).
9. Hunt, A. D., Stokes, J., McCRORY, W. W. & Stroud, H. H. Pyridoxine dependency: report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics* **13**, 140–145 (1954).
10. Wilson, M. P., Plecko, B., Mills, P. B. & Clayton, P. T. Disorders affecting vitamin B6 metabolism. *J. Inherit. Metab. Dis.* **42**, 629–646 (2019).

11. Mills, P. B. *et al.* Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat. Med.* **12**, 307–309 (2006).

12. Plecko, B. *et al.* Pipecolic acid as a diagnostic marker of pyridoxine-dependent epilepsy. *Neuropediatrics* **36**, 200–205 (2005).

13. Plecko, B. *et al.* Pipecolic acid elevation in plasma and cerebrospinal fluid of two patients with pyridoxine-dependent epilepsy. *Ann. Neurol.* **48**, 121–125 (2000).

14. Willemsen, M. A. A. P., Mavinkurve-Groothuis, A. M. C., Wevers, R. A., Rotteveel, J. J. & Jakobs, C. Pipecolic acid: a diagnostic marker in pyridoxine-dependent epilepsy. *Ann. Neurol.* **58**, 653 (2005).

15. Plecko, B. *et al.* Biochemical and molecular characterization of 18 patients with pyridoxine-dependent epilepsy and mutations of the antiquitin (ALDH7A1) gene. *Hum. Mutat.* **28**, 19–26 (2007).

16. Struys, E. A. & Jakobs, C. α-Aminoadipic semialdehyde is the biomarker for pyridoxine dependent epilepsy caused by α-aminoadipic semialdehyde dehydrogenase deficiency. *Molecular Genetics and Metabolism* **91**, 405 (2007).

17. Bok, L. A., Struys, E., Willemsen, M. A. A. P., Been, J. V. & Jakobs, C. Pyridoxine-dependent seizures in Dutch patients: diagnosis by elevated urinary alpha-aminoadipic semialdehyde levels. *Arch. Dis. Child.* **92**, 687–689 (2007).

18. Struys, E. A. *et al.* The measurement of urinary Δ^1-piperideine-6-carboxylate, the alter ego of α-aminoadipic semialdehyde, in Antiquitin deficiency. *J. Inherit. Metab. Dis.* **35**, 909–916 (2012).

19. Sadilkova, K., Gospe, S. M. & Hahn, S. H. Simultaneous determination of alpha-aminoadipic semialdehyde, piperideine-6-carboxylate and pipecolic acid by LC-MS/MS for pyridoxine-dependent seizures and folinic acid-responsive seizures. *J. Neurosci. Methods* **184**, 136–141 (2009).

20. Coughlin, C. R. *et al.* The genotypic spectrum of ALDH7A1 mutations resulting in pyridoxine dependent epilepsy: A common epileptic encephalopathy. *J. Inherit. Metab. Dis.* **42**, 353–361 (2019).

21. Scharer, G. *et al.* The genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy due to mutations in ALDH7A1. *J. Inherit. Metab. Dis.* **33**, 571–581 (2010).

22. Salomons, G. S. *et al.* An intriguing 'silent' mutation and a founder effect in antiquitin (ALDH7A1). *Ann. Neurol.* **62**, 414–418 (2007).

23. Mefford, H. C. *et al.* Intragenic deletions of ALDH7A1 in pyridoxine-dependent epilepsy caused by Alu-Alu recombination. *Neurology* **85**, 756–762 (2015).

24. Bok, L. A. *et al.* Long-term outcome in pyridoxine-dependent epilepsy. *Dev Med Child Neurol* **54**, 849–854 (2012).

25. Stockler, S. *et al.* Pyridoxine dependent epilepsy and antiquitin deficiency: clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. *Mol. Genet. Metab.* **104**, 48–60 (2011).

26. van Karnebeek, C. D. M. *et al.* Lysine restricted diet for pyridoxine-dependent epilepsy: first evidence and future trials. *Mol. Genet. Metab.* **107**, 335–344 (2012).

27. de Rooy, R. L. P. *et al.* Pyridoxine dependent epilepsy: Is late onset a predictor for favorable outcome? *Eur. J. Paediatr. Neurol.* **22**, 662–666 (2018).

28. Al Teneiji, A. *et al.* Phenotype, biochemical features, genotype and treatment outcome of pyridoxine-dependent epilepsy. *Metab Brain Dis* **32**, 443–451 (2017).

29. Mercimek-Mahmutoglu, S. *et al.* Novel therapy for pyridoxine dependent epilepsy due to ALDH7A1 genetic defect: L-arginine supplementation alternative to lysine-restricted diet. *Eur. J. Paediatr. Neurol.* **18**, 741–746 (2014).

30. Mahajnah, M. *et al.* A Prospective Case Study of the Safety and Efficacy of Lysine-Restricted Diet and Arginine Supplementation Therapy in a Patient With Pyridoxine-Dependent Epilepsy Caused by Mutations in ALDH7A1. *Pediatr. Neurol.* **60**, 60–65 (2016).

31. Coughlin, C. R. *et al.* Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: Neurodevelopmental outcome. *Mol. Genet. Metab.* **116**, 35–43 (2015).

32. Yuzyuk, T. *et al.* Effect of dietary lysine restriction and arginine supplementation in two patients with pyridoxine-dependent epilepsy. *Mol. Genet. Metab.* **118**, 167–172 (2016).

33. Navarro-Abia, V. *et al.* Hydrocephalus in pyridoxine-dependent epilepsy: New case and literature review. *Brain Dev.* **40**, 348–352 (2018).

34. Toldo, I. *et al.* Brain malformations associated to Aldh7a1 gene mutations: Report of a novel homozygous mutation and literature review. *Eur. J. Paediatr. Neurol.* **22**, 1042–1053 (2018).

35. van Karnebeek, C. D. M. *et al.* Lysine-Restricted Diet as Adjunct Therapy for Pyridoxine-Dependent Epilepsy: The PDE Consortium Consensus Recommendations. *JIMD Rep* **15**, 1–11 (2014).

36. Westgate, M. J. revtools: An R package to support article screening for evidence synthesis. *Res Synth Methods* **10**, 606–614 (2019).

37. Guyatt, G. H. *et al.* GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* **336**, 924–926 (2008).

38. Vockley, J., Chapman, K. A. & Arnold, G. L. Development of clinical guidelines for inborn errors of metabolism: commentary. *Mol. Genet. Metab.* **108**, 203–205 (2013).

39. Brouwers, M. C. *et al.* AGREE II: advancing guideline development, reporting and evaluation in health care. *J Clin Epidemiol* **63**, 1308–1311 (2010).

40. Bennett, C. L., Chen, Y., Hahn, S., Glass, I. A. & Gospe, S. M. Prevalence of ALDH7A1 mutations in 18 North American pyridoxine-dependent seizure (PDS) patients. *Epilepsia* **50**, 1167–1175 (2009).

41. Mercimek-Mahmutoglu, S., Donner, E. J. & Siriwardena, K. Normal plasma pipecolic acid level in pyridoxine dependent epilepsy due to ALDH7A1 mutations. *Mol. Genet. Metab.* **110**, 197 (2013).

42. Ebinger, M., Schultze, C. & König, S. Demographics and diagnosis of pyridoxine-dependent seizures. *J. Pediatr.* **134**, 795–796 (1999).

43. Baxter, P. Epidemiology of pyridoxine dependent and pyridoxine responsive seizures in the UK. *Arch. Dis. Child.* **81**, 431–433 (1999).

44. Been, J. V., Bok, L. A., Andriessen, P. & Renier, W. O. Epidemiology of pyridoxine dependent seizures in the Netherlands. *Arch. Dis. Child.* **90**, 1293–1296 (2005).

45. Rankin, P. M., Harrison, S., Chong, W. K., Boyd, S. & Aylett, S. E. Pyridoxine-dependent seizures: a family phenotype that leads to severe cognitive deficits, regardless of treatment regime. *Dev Med Child Neurol* **49**, 300–305 (2007).

46. Falsaperla, R. *et al.* Pyridoxine-dependent epilepsies: an observational study on clinical, diagnostic, therapeutic and prognostic features in a pediatric cohort. *Metab Brain Dis* **33**, 261–269 (2018).

47. Xue, J. *et al.* Simultaneous quantification of alpha-aminoadipic semialdehyde, piperideine-6-carboxylate, pipecolic acid and alpha-aminoadipic acid in pyridoxine-dependent epilepsy. *Sci Rep* **9**, 11371 (2019).

48. Jiao, X. *et al.* Clinical and genetic features in pyridoxine-dependent epilepsy: a Chinese cohort study. *Dev Med Child Neurol* **62**, 315–321 (2020).

49. Gospe, S. M. Natural history of pyridoxine-dependent epilepsy: tools for prognostication. *Dev Med Child Neurol* **54**, 781–782 (2012).

50. Mercimek-Mahmutoglu, S. *et al.* Prevalence of inherited neurotransmitter disorders in patients with movement disorders and epilepsy: a retrospective cohort study. *Orphanet J Rare Dis* **10**, 12 (2015).

51. van Karnebeek, C. D. M. *et al.* Metabolic Evaluation of Epilepsy: A Diagnostic Algorithm With Focus on Treatable Conditions. *Front Neurol* **9**, 1016 (2018).

52. Mills, P. B. *et al.* Urinary AASA excretion is elevated in patients with molybdenum cofactor deficiency and isolated sulphite oxidase deficiency. *J. Inherit. Metab. Dis.* **35**, 1031–1036 (2012).

53. Struys, E. A. *et al.* Pyridoxine-dependent epilepsy with elevated urinary α-amino adipic semialdehyde in molybdenum cofactor deficiency. *Pediatrics* **130**, e1716-1719 (2012).

54. Veldman, A. *et al.* Successful treatment of molybdenum cofactor deficiency type A with cPMP. *Pediatrics* **125**, e1249-1254 (2010).

55. Hyland, K. *et al.* Folinic acid responsive seizures: a new syndrome? *J. Inherit. Metab. Dis.* **18**, 177–181 (1995).

56. Gallagher, R. C. *et al.* Folinic acid-responsive seizures are identical to pyridoxine-dependent epilepsy. *Ann. Neurol.* **65**, 550–556 (2009).

57. Wempe, M. F. *et al.* Identification of a novel biomarker for pyridoxine-dependent epilepsy: Implications for newborn screening. *J. Inherit. Metab. Dis.* **42**, 565–574 (2019).

58. Kuhara, T. *et al.* Identification of new biomarkers of pyridoxine-dependent epilepsy by GC/MS-based urine metabolomics. *Anal. Biochem.* 113739 (2020) doi:10.1016/j.ab.2020.113739.

59. Baumgart, A. *et al.* Atypical vitamin B6 deficiency: a rare cause of unexplained neonatal and infantile epilepsies. *J. Child Neurol.* **29**, 704–707 (2014).

60. Marguet, F. *et al.* Pyridoxine-dependent epilepsy: report on three families with neuropathology. *Metab Brain Dis* **31**, 1435–1443 (2016).

61. Gül-Mert, G., İncecik, F., Hergüner, M. Ö., Ceylaner, S. & Altunbaşak, Ş. Pyridoxine-dependent epilepsy in two Turkish patients in Turkey and review of the literature. *Turk. J. Pediatr.* **57**, 394–397 (2015).

62. Nam, S. H. *et al.* Clinical and genetic analysis of three Korean children with pyridoxine-dependent epilepsy. *Ann. Clin. Lab. Sci.* **42**, 65–72 (2012).

63. Russell, K. E., Mulligan, S. R. & Mallory, L. A. Diagnosis of pyridoxine-dependent seizures in a nineteen-year-old patient. *Pediatr. Neurol.* **47**, 141–143 (2012).

64. Yang, Z. *et al.* Clinical diagnosis, treatment, and ALDH7A1 mutations in pyridoxine-dependent epilepsy in three Chinese infants. *PLoS ONE* **9**, e92803 (2014).

65. Ben Younes, T. *et al.* Pyridoxine-dependent epilepsy: A novel mutation in a Tunisian child. *Arch Pediatr* **24**, 241–243 (2017).

66. Coci, E. G. *et al.* Novel homozygous missense mutation in ALDH7A1 causes neonatal pyridoxine dependent epilepsy. *Mol. Cell. Probes* **32**, 18–23 (2017).

67. Mercimek-Mahmutoglu, S. *et al.* Lysine-restricted diet and mild cerebral serotonin deficiency in a patient with pyridoxine-dependent epilepsy caused by ALDH7A1 genetic defect. *Mol Genet Metab Rep* **1**, 124–128 (2014).

68. Bass, N. E., Wyllie, E., Cohen, B. & Joseph, S. A. Pyridoxine-dependent epilepsy: the need for repeated pyridoxine trials and the risk of severe electrocerebral suppression with intravenous pyridoxine infusion. *J. Child Neurol.* **11**, 422–424 (1996).

69. Grillo, E., da Silva, R. J. & Barbato, J. H. Pyridoxine-dependent seizures responding to extremely low-dose pyridoxine. *Dev Med Child Neurol* **43**, 413–415 (2001).

70. Kölker, S. *et al.* Diagnosis and management of glutaric aciduria type I--revised recommendations. *J. Inherit. Metab. Dis.* **34**, 677–694 (2011).

71. van Karnebeek, C. D. M. & Jaggumantri, S. Current treatment and management of pyridoxine-dependent epilepsy. *Curr Treat Options Neurol* **17**, 335 (2015).

72. Schmidt, Z., Murthy, G., Ennis, M., Stockler-Ipsiroglu, S. & Elango, R. Impact of enteral arginine supplementation on lysine metabolism in humans: A proof-of-concept for lysine-related inborn errors of metabolism. *J. Inherit. Metab. Dis.* (2020) doi:10.1002/jimd.12233.

73. Shah, P. S., Shah, V. S. & Kelly, L. E. Arginine supplementation for prevention of necrotising enterocolitis in preterm infants. *Cochrane Database Syst Rev* **4**, CD004339 (2017).

74. El-Shimi, M. S. *et al.* Enteral L-Arginine and Glutamine Supplementation for Prevention of NEC in Preterm Neonates. *Int J Pediatr* **2015**, 856091 (2015).

75. Polycarpou, E. *et al.* Oral L-arginine supplementation and faecal calprotectin levels in very low birth weight neonates. *J Perinatol* **33**, 141–146 (2013).

76. Häberle, J. *et al.* Suggested guidelines for the diagnosis and management of urea cycle disorders: First revision. *J. Inherit. Metab. Dis.* **42**, 1192–1230 (2019).

77. Koenig, M. K. *et al.* Recommendations for the Management of Strokelike Episodes in Patients With Mitochondrial Encephalomyopathy, Lactic Acidosis, and Strokelike Episodes. *JAMA Neurol* **73**, 591–594 (2016).

78. El-Hattab, A. W., Almannai, M. & Scaglia, F. Arginine and citrulline for the treatment of MELAS syndrome. *Journal of inborn errors of metabolism and screening* **5**, (2017).

79. McLachlan, R. S. & Brown, W. F. Pyridoxine dependent epilepsy with iatrogenic sensory neuronopathy. *Can J Neurol Sci* **22**, 50–51 (1995).

80. Echaniz-Laguna, A., Mourot-Cottet, R., Noel, E. & Chanson, J.-B. Regressive pyridoxine-induced sensory neuronopathy in a patient with homocystinuria. *BMJ Case Rep* **2018**, (2018).

81. Ghavanini, A. A. & Kimpinski, K. Revisiting the evidence for neuropathy caused by pyridoxine deficiency and excess. *J Clin Neuromuscul Dis* **16**, 25–31 (2014).

82. Müller, E. & Kölker, S. Reduction of lysine intake while avoiding malnutrition--major goals and major problems in dietary treatment of glutaryl-CoA dehydrogenase deficiency. *J. Inherit. Metab. Dis.* **27**, 903–910 (2004).

83. Boy, N. *et al.* Low lysine diet in glutaric aciduria type I--effect on anthropometric and biochemical follow-up parameters. *J. Inherit. Metab. Dis.* **36**, 525–533 (2013).

84. Ramos, R. J. *et al.* Vitamin B6 is essential for serine de novo biosynthesis. *J. Inherit. Metab. Dis.* **40**, 883–891 (2017).

85. Kurlemann, G. *et al.* Disturbance of GABA metabolism in pyridoxine-dependent seizures. *Neuropediatrics* **23**, 257–259 (1992).

86. Gospe, S. M., Olin, K. L. & Keen, C. L. Reduced GABA synthesis in pyridoxine-dependent seizures. *Lancet* **343**, 1133–1134 (1994).

87. Goto, T., Matsuo, N. & Takahashi, T. CSF glutamate/GABA concentrations in pyridoxine-dependent seizures: etiology of pyridoxine-dependent seizures and the mechanisms of pyridoxine action in seizure control. *Brain Dev.* **23**, 24–29 (2001).

88. *Registries for Evaluating Patient Outcomes: A User's Guide*. (Agency for Healthcare Research and Quality (US), 2014).

89. Jansen-van der Weide, M. C. *et al.* Rare disease registries: potential applications towards impact on development of new drug treatments. *Orphanet J Rare Dis* **13**, 154 (2018).

90. Jung, S., Tran, N.-T. B., Gospe, S. M. & Hahn, S. H. Preliminary investigation of the use of newborn dried blood spots for screening pyridoxine-dependent epilepsy by LC-MS/MS. *Mol. Genet. Metab.* **110**, 237–240 (2013).

Figure Legends:

Figure 1: Lysine metabolism and pyridoxine-dependent epilepsy

Legend: Pyridoxine-dependent epilepsy is caused by the deficiency of α-aminoadipic semialdehyde (α-ASSA) dehydrogenase, which results in the accumulation of multiple metabolites including α-AASA, Δ^1-piperideine-6-carboxylate (Δ^1-P6C), and pipecolic acid.

Supplemental Tables:

Supplemental Table 1: Final survey results from PDE Consortium

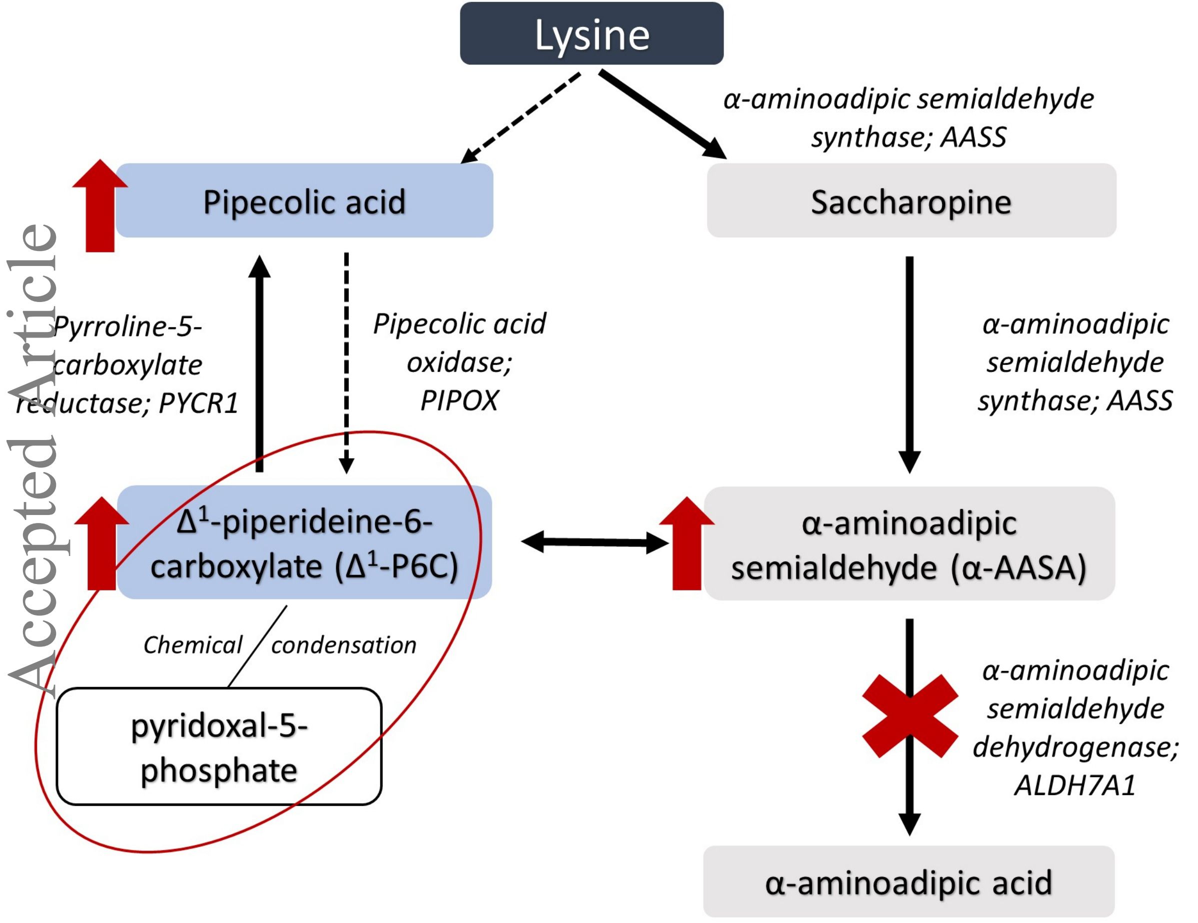
Supplemental Figure Legends:

Supplemental Figure 1: PRISMA flow diagram for systematic review on PDE-ALDH7A1

Legend: A systematic review was performed using the search terms included pyridoxine dependent epilepsy, pyridoxine dependent seizures, pyridoxine responsive epilepsy, pyridoxine responsive seizures, antiquitin deficiency, α-AASA dehydrogenase, and *ALDH7A1*. The review was limited to articles published in English language and included clinical findings of patients with PDE due to α-aminoadipic semialdehyde dehydrogenase. A total of 109 full text articles were included in the final review.

Supplemental Figure 2: Overview of consensus procedure

Legend: The consensus procedure was initiated with a review of the literature and summary of evidence. Based on this, statements were formed and designed into a survey. The guidelines development group (GDG) was consisted of members of the PDE Consortium, which consists of clinicians and scientists with an expertise in PDE-ALDH7A1 The GDG included representation from 29 institutions across Africa, Asia, Australia, Europe, North America and South America. The consensus procedure included four rounds, with in the first round the invitation for participation and online first survey, followed by an in-person meeting where the group response was discussed and statements where refined. In the third round, a second online survey was sent where answers could be received. In the fourth round, final approval of the recommendations formed out of the statements which reached consensus, was obtained.



Lysine
α-aminoadipic semialdehyde synthase; AASS
Pipecolic acid
Saccharopine
Pyrroline-5-carboxylate reductase; PYCR1
Pipecolic acid oxidase; PIPOX
α-aminoadipic semialdehyde synthase; AASS
Δ1-piperideine-6-carboxylate (Δ1-P6C)
α-aminoadipic semialdehyde (α-AASA)
Chemical condensation
pyridoxal-5-phosphate
α-aminoadipic semialdehyde dehydrogenase; ALDH7A1
α-aminoadipic acid

Table 1: Evidence levels and strength of recommendations

Level of evidence		Definitions
A	High-quality evidence	Randomized control studies, High confidence in effect estimates
B	Moderate-quality evidence	Cohort studies, Moderately confident in effect estimate
C	Low-quality evidence	Observational studies, Confidence in effect estimate is limited
D	Very-low quality evidence	Expert opinions, Limited confidence in the effect estimate
Strength of recommendations		**Definitions**
1	Strong recommendation	Recommendation applies to most patients in most circumstances and with high expert agreement[1] (≥ 90% of respondents)
2	Conditional recommendation	Alternative approaches are reasonable depending on circumstances and with limited expert agreement (< 90% of respondents)

[1]Agreement was defined by the percentage of respondents who either answered "completely agree" or "mostly agree"