COLD SPRING HARBOR
Molecular Case Studies

COMMENTARY

A case for newborn screening for pyridoxine-dependent epilepsy

Curtis R. Coughlin II,[1] Laura A. Tseng,[2,3] and Clara D.M. van Karnebeek[2,3,4]

[1]Section of Clinical Genetics and Metabolism, Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045, USA; [2]Department of Pediatrics, Emma Children's Hospital and Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam UMC, University of Amsterdam, 1105 AZ Amsterdam, the Netherlands; [3]On behalf of United for Metabolic Diseases, 1105 AZ Amsterdam, the Netherlands; [4]Department of Human Genetics, Amsterdam Reproduction and Development, Amsterdam UMC, University of Amsterdam, 1105 AZ Amsterdam, the Netherlands

Abstract Pyridoxine-dependent epilepsy due to mutations in *ALDH7A1* (PDH-ALDH7A1) is a highly treatable developmental and epileptic encephalopathy. Pharmacologic doses of pyridoxine are associated with dramatic clinical seizure improvement, and most patients achieve adequate seizure control with pyridoxine alone. Unfortunately, some patients with PDE-ALDH7A1 have died prior to when the diagnosis was made and subsequent treatment with pyridoxine could be implemented, highlighting the importance of a timely diagnosis. Although critical for seizure control, pyridoxine treatment alone is not sufficient for normal outcomes as most patients suffer intellectual and developmental delay. Adjunct lysine reduction therapies are associated with significant developmental improvements, although these treatments have limited efficacy if delayed after the first few months of life. Recently two biomarkers were identified that overcome previous technical hurdles for newborn screening. Herein we provide commentary that PDE-ALDH7A1 meets both current and historic criteria for newborn screening, and that a neonatal diagnosis and treatment can both reduce mortality from uncontrolled seizures and significantly improve the cognitive delay that is pervasive in this treatable disorder.

INTRODUCTION

Corresponding author:
Curtis.Coughlin@
cuanschutz.edu

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a006197

Pyridoxine-dependent epilepsy, historically referred to as antiquitin deficiency, due to biallelic mutations in *ALDH7A1* (PDE-ALDH7A1) is a developmental and epileptic encephalopathy often characterized by refractory seizures that are responsive to pyridoxine (vitamin B_6). Sixty-five years after the initial clinical description pyridoxine remains central to the treatment of seizures, although adjunct therapies are critical in the treatment and prevention of the intellectual disability or developmental delay (IDD) (Hunt et al. 1954; Coughlin et al. 2021). The delay in diagnosis and subsequent treatment results in significant mortality and morbidity for patients affected by this treatable disorder.

Although occasionally responsive to antiseizure medications, the majority of patients require pharmacologic doses of pyridoxine for adequate seizure control (Basura et al. 2009). A delay in diagnosis can result in poorly controlled seizures and numerous breakthrough seizure events (van Karnebeek et al. 2016). Tragically, patients have died prior to treatment with pyridoxine, suggesting that an early diagnosis would have been lifesaving (Marguet et al. 2016). Unfortunately, pyridoxine treatment alone is not sufficient for normal outcomes (Tseng et al. 2022), and most patients suffer IDD despite adequate seizure control (Bok et al.

Table 1. Vitamin B_6–responsive seizure disorders

Disorder	Gene	Vitamin B_6	Adjunct (to B_6) treatment
Pyridoxine-dependent epilepsy (PDE-ALDH7A1)	*ALDH7A1*	Pyridoxine	Lysine reduction therapies
Pyridoxamine 5′-phospate oxidase (PNPO) deficiency	*PNPO*	Pyridoxal phosphate[a]	NA
Pyridoxal phosphate binding protein (PLPBP)	*PLPBP*	Pyridoxine *or* pyridoxal phosphate	NA
Molybdenum cofactor deficiency	*MOCS1, MOCS2*	Pyridoxine[b]	Cysteine-restricted diet; cyclic pyranopterin monophosphate[c]
Hyperprolinemia type II	*ALDH4A1*	Pyridoxine	NA
Hypophosphatasia	*ALPL*	Pyridoxine	Enzyme replacement therapy

[a]Some patients are responsive to pyridoxine.
[b]Not all patients have a clinical response to vitamin B_6.
[c]Patients with *MOCS1*-related disease.

2012). It is critical to distinguish PDE-ALDH7A1 from other pyridoxine responsive seizure disorders, as adjunct treatment to pyridoxine is recommended for patients with PDE-ALDH7A1 (Table 1). Adjunct lysine reduction therapies (LRTs) are associated with significant developmental improvements (van Karnebeek et al. 2012; Coughlin et al. 2015), although these treatments have limited efficacy if delayed after the first few months of life (Al Teneiji et al. 2017).

The importance of early diagnosis and treatment has led many to suggest PDE-ALDH7A1 would be an ideal condition for newborn screening (NBS). Indeed, it was selected as a top priority for NBS of genetic epilepsies by members of the Child Neurology Society (Hess-Homeier et al. 2019). Until recently, the diagnosis of PDE-ALDH7A1 relied on biomarkers that were unstable at ambient temperature or genetic testing (Struys and Jakobs 2007; Jung et al. 2013). Two novel biomarkers were recently identified that are stable at room temperature and measurable using current laboratory techniques used in newborn screening laboratories worldwide (Wempe et al. 2019; Engelke et al. 2021). We believe PDE-ALDH7A1 meets historical and modern criteria for conditions that undergo newborn screening.

SCREENING CRITERIA

With the establishment of NBS for phenylketonuria (PKU) came ethical questions about which conditions should undergo screening (Lesser 1963; Holm et al. 1970). In response, the World Health Organization commissioned a report on screening and established basic principles of screening centered on early disease detection and treatment (Wilson and Jungner 1968). Although this criterion is still influential today, modified NBS criteria have been proposed with the increase in available testing technology and the ever-expanding treatment options (Table 2; Andermann et al. 2008; Petros 2012; Dobrow et al. 2018). Expert advisory committees have been established to evaluate new conditions for NBS programs (Franková et al. 2021). For example, in the United States the Advisory Committee on Heritable Disorders in Newborns and Children provides advice about newborn and child screening to the Secretary of Health and Human Services (HHS; Howell and Lloyd-Puryear 2010). In turn, the HHS has provided a list of disorders recommended for states to screen referred to as the recommended uniform screening panel (RUSP). In the Netherlands, the

Table 2. Suggested principles for population-based screening

Wilson and Jungner 1968	Andermann et al. 2008
1. The condition sought should be an important health problem.	1. The screening program should respond to recognized need.
2. There should be an accepted treatment for patients with recognized disease.	2. The objectives of screening should be defined at the outset.
3. Facilities for diagnosis and treatment should be available.	3. There should be a defined target population.
4. There should be a recognizable latent or early symptomatic stage.	4. There should be scientific evidence of screening program effectiveness.
5. There should be a suitable test or examination.	5. The program should integrate education, testing, clinical services and program management. There should be quality assurance, with mechanisms to minimize potential risks of screening.
6. The test should be acceptable to the population.	
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.	6. The program should ensure informed choice, confidentiality, and respect for autonomy.
8. There should be an agreed policy on whom to treat as patients.	7. The program should promote equity and access to screening for the entire target population.
9. The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.	8. Program evaluation should be planned from the outset.
10. Case finding should be a continuing process and not a "once and for all" project.	9. The overall benefits of screening should outweigh the harm.

Dutch Health Council provides advice and regulation on inclusion of disorders in the national NBS program (Bolhuis and Page-Christiaens 2005). In Australia, policies on newborn screening are determined by Australian Health Minister's Advisory Council (AHMAC) through a Standing Committee on Screening (O'Leary and Maxwell 2015), and the UK National Screening Committee (UK NSC) advises ministers and the National Health Services on issues regarding general population screening (www.gov.uk).

PDE-ALDH7A1 Is a Relatively Common Disorder

Although there is no recognized "minimal incidence" for a condition to be considered for NBS, it has been suggested that conditions with an incidence of at least 1:100,000 live births should have a higher priority score. A recent study estimated the incidence of PDE-ALDH7A1 at 1:65,000 live births based on publicly available genomic data (Coughlin et al. 2019). This estimated prevalence may be conservative, and it is not unusual for rare disease prevalence to be found significantly higher after newborn screening is implemented. For example, very long-chain acyl-CoA dehydrogenase deficiency had an estimated disease incidence of 1:250,000 live births prior to newborn screening and an incidence of 1:31,500 live births following screening (Spiekerkoetter et al. 2003; Boneh et al. 2006).

Although there are limitations to this study, previous estimates of disease incidence were performed prior to the identification of biochemical or genetic marker. Not only is this estimated incidence more common than 1:100,000 live births, it is similar to other conditions, such as galactosemia and biotinidase deficiency, that currently undergo NBS in many developed countries (Suzuki et al. 2001; Porta et al. 2017).

A Recognizable Latent or Asymptomatic Stage

Historically, patients with PDE-ALDH7A1 were described as presenting in the first few days of life and dramatically responding to the initial dose of pyridoxine (Hunt et al. 1954). This would suggest that all patients would be easily recognized and treated in the newborn

period. However, using only this strict criterion, patients who presented after the neonatal period would go undiagnosed and remain poorly treated.

Although most patients present in the newborn period, ~25%–30% of patients have onset of seizures in infancy or early childhood (Basura et al. 2009; Falsaperla et al. 2018). Patients have also been reported with seizure onset after 1 year of life (Haidar et al. 2018; Jiao et al. 2019) and into late adolescence (Srinivasaraghavan et al. 2018). These patients are often described as having a "late presentation," as the epileptic phenotype is an often described prominent feature of this disorder. Yet patients may have a long-standing history of IDD prior to onset of seizures (Srinivasaraghavan et al. 2018). These cases emphasize the importance of starting treatment prior to the onset of epilepsy.

Severity of the Disorder

It is crucial that PDE-ALDH7A1 patients are treated with pyridoxin, as patients are rarely responsive to other antiseizure medications (Basura et al. 2009). Withdrawal of pyridoxine is associated with recurrence of seizures emphasizing the importance of pyridoxine supplementation (Nam et al. 2012; Yang et al. 2014; Gül-Mert et al. 2015). Several familial studies reported patients who died before pyridoxine was provided only to be diagnosed with PDE-ALDH7A1 after the birth of an affected sibling (Mills et al. 2010; Baumgart et al. 2014; Marguet et al. 2016; Toldo et al. 2018). In these cases, a correct diagnosis at birth and subsequent early treatment with pyridoxine may have been lifesaving.

Even in those patients who are diagnosed and treated with pyridoxine in the neonatal period, the clinical outcome is often poor. Approximately 75% of patients are reported to have IDD (Basura et al. 2009; Bok et al. 2012), although recent studies suggest that this could be an underestimate of the IDD among this patient population (Tseng et al. 2022).

Acceptable Treatment

As described above, pyridoxine supplementation is central to the treatment of epilepsy. Pyridoxine is generally well-tolerated and widely available, although there is a small risk of neuropathy at high doses, which is reversible (Rankin et al. 2007; Ghavanini and Kimpinski 2014). Several descriptive studies have reported antenatal (Bok et al. 2010a; Srinivasaraghavan et al. 2018) and immediate newborn treatment with pyridoxine because of a known family history of the disorder (Bennett et al. 2009; Mills et al. 2010; Alfadhel et al. 2012). Children who received perinatal treatment with pyridoxine are often reported to have no clinical or electroencephalogram evidence of seizures, although some of these patients are still reported to have significant IDD (Rankin et al. 2007; Yeghiazaryan et al. 2011). This suggests that pyridoxine treatment alone is not sufficient to achieve a normal cognitive outcome.

Adjunct LRTs were developed to address the IDD pervasive in this disorder and are recommended for most patients (Coughlin et al. 2021). Of note, this recommendation was highly dependent on expert opinion. Unfortunately, this is relatively common for clinical guidelines focused on inborn errors of metabolism (Vockley et al. 2013). LRT includes a lysine-restricted diet (van Karnebeek et al. 2012), supplementation of arginine as competitive inhibitor of lysine transport over the blood–brain barrier (Mercimek-Mahmutoglu et al. 2014), or a combination of both LRT strategies and pyridoxine referred to as "triple therapy" (Coughlin et al. 2015). A lysine-restricted diet often requires the use of low protein foods and medical foods and should be monitored by a multidisciplinary team that includes a metabolic dietitian. Although LRT is more difficult than pyridoxine treatment alone, it is accessible, affordable, and similar to those used for other disorders—such as glutaric aciduria type 1—currently identified through newborn screening.

Significant Benefit to Early Treatment

The association between adjunct LRTs and improved developmental outcomes has been described in numerous observational studies, but not all patients benefit from treatment. In one retrospective study, only patients treated with LRTs in the first few months of life had a normal developmental outcome (Al Teneiji et al. 2017). Preliminary data from the International Registry for Patients with Pyridoxine-Dependent Epilepsy (hereafter referred to as the PDE Registry) suggests that LRT started in the first 6 months of life is associated with a significant improvement in developmental test scores (C Coughlin, L Tseng, L Bok, et al., pers. comm.). The delay of diagnosis and subsequent delay in LRT may be a main contributor to IDD in this treatable disorder.

Natural History of the Disorder Is Well-Understood

PDE-ALDH7A1 was characterized as readily treatable seizure disorder based on a number of small case reports. Regional natural history studies suggested a heterogenous phenotype including late-onset seizures, abnormal brain imaging findings, and IDD (Baxter et al. 1996; Basura et al. 2009). The International PDE consortium was established in 2011 with the goal of improving knowledge dissemination and formalizing international collaborations (Stockler et al. 2011). The PDE Consortium has since published clinical recommendations and consensus guidelines for the treatment of patients with PDE-ALDH7A1 (van Karnebeek et al. 2014; Coughlin et al. 2021). The PDE Registry (www.pdeonline.org) was established shortly thereafter with the goal to understand the effect of current therapies and evaluate novel treatment strategies.

A Suitable Screening Test

There is no pathognomonic electrographic finding for PDE-ALDH7A1, and a trial of pyridoxine has long been recommended for all patients with refractory epilepsy (Scriver 1960). Unfortunately, a single trial of pyridoxine is neither sensitive nor specific. Up to 80% of patients with PDE-ALDH7A1 will have an incomplete response to the initial dose of pyridoxine (Bok et al. 2010b) or require repeated pyridoxine trials (Bass et al. 1996). Furthermore, a number of other genetic epilepsies are responsive to pyridoxine (Wilson et al. 2019). It is critical to establish the specific genetic diagnosis as other B_6 vitamers may be necessary for complete seizure remission and other therapies may be recommended. Furthermore, patients may present with concomitant findings such as hypoglycemia and lactic acidosis (van Karnebeek et al. 2016). These atypical presentations have resulted in delayed clinical suspicion, diagnosis, and treatment (Russell et al. 2012).

PDE-ALDH7A1 is due to the deficiency of α-aminoadipic semialdehyde (α-AASA) dehydrogenase, a key enzyme in lysine metabolism, with subsequent accumulation of Δ^1-piperideine-6-carboxylate (Δ^1-P6C) and α-AASA (Fig. 1; Mills et al. 2006; Struys and Jakobs 2007; Struys et al. 2012a). The biomarkers α-AASA and Δ^1-P6C are sensitive and specific for PDE-ALDH7A1, although patients with molybdenum cofactor deficiency or isolated sulfite oxidase deficiency have been reported with mild elevations of α-AASA (Mills et al. 2012; Struys et al. 2012b). Unfortunately, these biomarkers are unstable at ambient temperature making them impractical for NBS (Jung et al. 2013).

Lysine catabolism occurs through two separate pathways although the majority of L-lysine catabolism is through the saccharopine pathway (Pena et al. 2017; Crowther et al. 2019). Despite the unclear role in lysine degradation, pipecolic acid was the first biomarker used to diagnose patients with PDE-ALDH7A1 (Plecko et al. 2000, 2005). Pipecolic acid may not be an ideal candidate for an NBS marker. Patients with PDE-ALDH7A1 have had normal pipecolic acid levels, although this has only been reported in patients following pyridoxine treatment (Mercimek-Mahmutoglu et al. 2013). Pipecolic acid is also elevated in patients

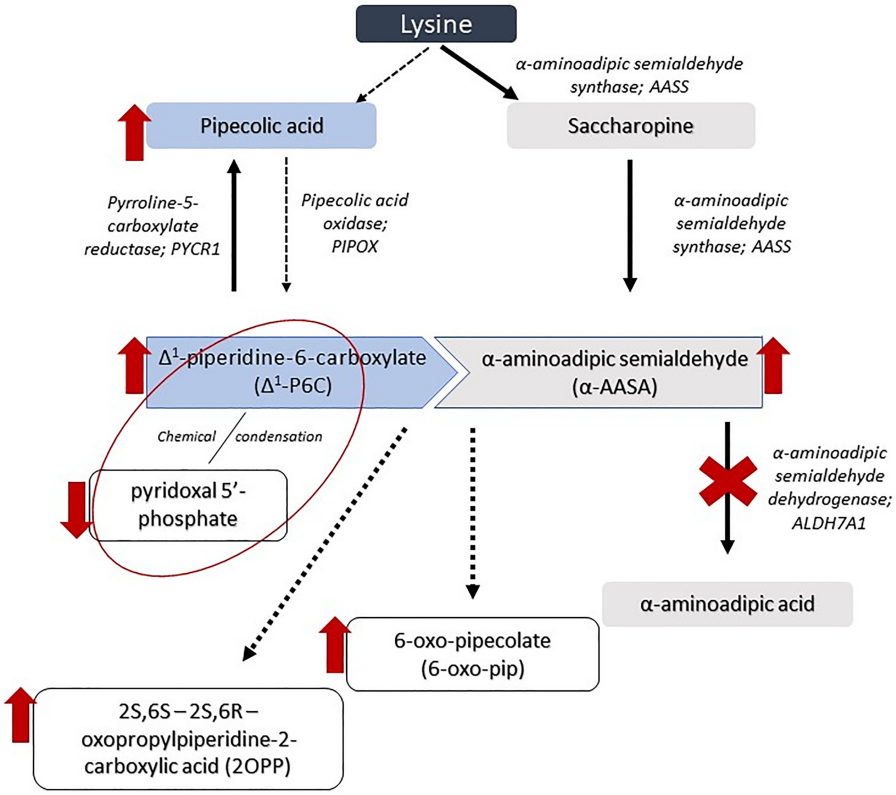

Figure 1. PDE-ALD7A1 and lysine metabolism. PDE-ALDH7A1 is caused by the deficiency of α-aminoadipic semialdehyde (α-AASA) dehydrogenase, a key enzyme in lysine metabolism. This results in the accumulation of α-AASA and its cyclic equivalent Δ^1-piperidine-6-carboxylate (Δ^1-P6C). Recently two biomarkers have been identified that can be applied to current newborn screening programs: 6-oxo-pipecolate and 2S,6S—2S,6R oxopropylpiperidince-2-carboxylic acid.

with peroxisomal disorders (Peduto et al. 2004), which makes using this single metabolite less than ideal for newborn screening of PDE-ALDH7A1.

Recently two novel biomarkers have been identified: 6-oxo-pipecoalte (Wempe et al. 2019) and 2S,6S—2S,6R—oxopropylpiperidine-2-carboxylic acid (Engelke et al. 2021; van Outersterp et al. 2021). These biomarkers are stable at room temperature and can be quantified in dried blood spots using current laboratory techniques used in newborn screening laboratories such as liquid chromatography tandem mass spectrometry. Although only limited studies have been performed to date (Kuhara et al. 2020; Hong et al. 2022), these biomarkers appear to have overcome the previous technical hurdle associated with NBS for PDE-ALDH7A1. If future studies confirm these preliminary findings, adding this condition to existing public health programs would require little laboratory investment. Molecular genetic testing is increasingly incorporated into newborn screening approaches (Godler et al. 2022). Many patients with PDE-ALDH7A1 appear to have a private mutation (Coughlin et al. 2019), although a combination of genetic testing and biochemical testing may be ideal.

CONCLUSION AND FUTURE DIRECTIONS

One must be cautious about adding any condition to an existing NBS panel, especially as "mild" or adult-onset patients will most likely be identified. The International PDE

Consortium is well-positioned to aid investigators, clinicians, patients, and public health agencies who pursue NBS for PDE-ALDH7A1. This group of international experts has established treatment guidelines, an international prospective natural study, and an international collaboration to share quality control specimens.

Although LRT significantly improves the IDD in this disorder, even patients treated in the first few months are reported to have mild developmental delay or behavioral difficulties. Furthermore, these dietary treatments are not benign. Lifelong consumption of similar medical formulas has been associated with poor compliance (Boy et al. 2018) and imposes personal and financial burdens on caregivers (MacDonald et al. 2010, 2016). Other disease mechanisms have also been suggested (Minenkova et al. 2021; Yazdani and Elgstøen 2021). As a result, new therapeutic strategies for patients with PDE-ALDH7A1 are needed. New treatment options, such as upstream enzyme inhibition therapy, may eliminate the exposure to neurotoxic metabolites (Crowther et al. 2019; Leandro and Houten 2020). For these novel therapies to be successful, patients would need to be diagnosed and treated in the newborn period.

One may argue that there are significant benefits of NBS for PDE-ALDH7A1. Patients have died prior to the initiation of pyridoxine only to be diagnosed later, after the birth of an affected sibling. In these rare circumstances, NBS may have been lifesaving. As described above, LRT in the first few months of life have also been associated with improved developmental and cognitive outcomes. A newborn diagnosis would allow for the initiation of treatment at a time of critical development. This may be the difference between a lifelong cognitive disability requiring dependence on a caregiver and a typical adult.

ADDITIONAL INFORMATION

Acknowledgments

We are grateful to the patients and families as well as our laboratory and clinical colleagues for their collaborations, which have generated new insights and underpinnings for NBS inclusion.

Competing Interest Statement

The authors have declared no competing interest.

Referees

Mari Mori
Walla Al-Hertani

Author Contributions

C.R.C., L.A.T., and C.D.M.v.K. contributed to the conception and drafting of the text and reviewed the figure. The manuscript is approved for submission by all authors listed.

REFERENCES

Alfadhel M, Sirrs S, Waters PJ, Szeitz A, Struys E, Coulter-Mackie M, Stockler-Ipsiroglu S. 2012. Variability of phenotype in two sisters with pyridoxine dependent epilepsy. *Can J Neurol Sci* **39:** 516–519. doi:10.1017/S0317167100014050

Al Teneiji A, Bruun TUJ, Cordeiro D, Patel J, Inbar-Feigenberg M, Weiss S, Struys E, Mercimek-Mahmutoglu S. 2017. Phenotype, biochemical features, genotype and treatment outcome of pyridoxine-dependent epilepsy. *Metab Brain Dis* **32:** 443–451. doi:10.1007/s11011-016-9933-8

Andermann A, Blancquaert I, Beauchamp S, Déry V. 2008. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. *Bull World Health Organ* **86:** 317–319. doi:10.2471/BLT.07.050112

Bass NE, Wyllie E, Cohen B, Joseph SA. 1996. 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. doi:10.1177/088307389601100519

Basura GJ, Hagland SP, Wiltse AM, Gospe SM. 2009. 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. doi:10.1007/s00431-008-0823-x

Baumgart A, von Spiczak S, Verhoeven-Duif NM, Møller RS, Boor R, Muhle H, Jähn JA, Klitten LL, Hjalgrim H, Lindhout D, et al. 2014. Atypical vitamin B6 deficiency: a rare cause of unexplained neonatal and infantile epilepsies. *J Child Neurol* **29:** 704–707. doi:10.1177/0883073813505354

Baxter P, Griffiths P, Kelly T, Gardner-Medwin D. 1996. Pyridoxine-dependent seizures: demographic, clinical, MRI and psychometric features, and effect of dose on intelligence quotient. *Dev Med Child Neurol* **38:** 998–1006. doi:10.1111/j.1469-8749.1996.tb15060.x

Bennett CL, Chen Y, Hahn S, Glass IA, Gospe SM. 2009. Prevalence of ALDH7A1 mutations in 18 North American pyridoxine-dependent seizure (PDS) patients. *Epilepsia* **50:** 1167–1175. doi:10.1111/j.1528-1167.2008.01816.x

Bok LA, Been JV, Struys EA, Jakobs C, Rijper EAM, Willemsen MA. 2010a. Antenatal treatment in two Dutch families with pyridoxine-dependent seizures. *Eur J Pediatr* **169:** 297–303. doi:10.1007/s00431-009-1020-2

Bok LA, Maurits NM, Willemsen MA, Jakobs C, Teune LK, Poll-The BT, de Coo IF, Toet MC, Hagebeuk EE, Brouwer OF, et al. 2010b. The EEG response to pyridoxine-IV neither identifies nor excludes pyridoxine-dependent epilepsy. *Epilepsia* **51:** 2406–2411. doi:10.1111/j.1528-1167.2010.02747.x

Bok LA, Halbertsma FJ, Houterman S, Wevers RA, Vreeswijk C, Jakobs C, Struys E, Van Der Hoeven JH, Sival DA, Willemsen MA. 2012. Long-term outcome in pyridoxine-dependent epilepsy. *Dev Med Child Neurol* **54:** 849–854. doi:10.1111/j.1469-8749.2012.04347.x

Bolhuis PA, Page-Christiaens GCML. 2005. [The advisory report "Neonatal screening" from the Health Council of The Netherlands]. *Ned Tijdschr Geneeskd* **149:** 2857–2860.

Boneh A, Andresen BS, Gregersen N, Ibrahim M, Tzanakos N, Peters H, Yaplito-Lee J, Pitt JJ. 2006. VLCAD deficiency: pitfalls in newborn screening and confirmation of diagnosis by mutation analysis. *Mol Genet Metab* **88:** 166–170. doi:10.1016/j.ymgme.2005.12.012

Boy N, Mengler K, Thimm E, Schiergens KA, Marquardt T, Weinhold N, Marquardt I, Das AM, Freisinger P, Grünert SC, et al. 2018. Newborn screening: a disease-changing intervention for glutaric aciduria type 1. *Ann Neurol* **83:** 970–979. doi:10.1002/ana.25233

Coughlin CR, van Karnebeek CDM, Al-Hertani W, Shuen AY, Jaggumantri S, Jack RM, Gaughan S, Burns C, Mirsky DM, Gallagher RC, et al. 2015. Triple therapy with pyridoxine, arginine supplementation and dietary lysine restriction in pyridoxine-dependent epilepsy: neurodevelopmental outcome. *Mol Genet Metab* **116:** 35–43. doi:10.1016/j.ymgme.2015.05.011

Coughlin CR, Swanson MA, Spector E, Meeks NJL, Kronquist KE, Aslamy M, Wempe MF, van Karnebeek CDM, Gospe SM, Aziz VG, et al. 2019. The genotypic spectrum of *ALDH7A1* mutations resulting in pyridoxine dependent epilepsy: a common epileptic encephalopathy. *J Inherit Metab Dis* **42:** 353–361. doi:10.1002/jimd.12045

Coughlin CR, Tseng LA, Abdenur JE, Ashmore C, Boemer F, Bok LA, Boyer M, Buhas D, Clayton PT, Das A, et al. 2021. Consensus guidelines for the diagnosis and management of pyridoxine-dependent epilepsy due to α-aminoadipic semialdehyde dehydrogenase deficiency. *J Inherit Metab Dis* **44:** 178–192. doi:10.1002/jimd.12332

Crowther LM, Mathis D, Poms M, Plecko B. 2019. New insights into human lysine degradation pathways with relevance to pyridoxine-dependent epilepsy due to antiquitin deficiency. *J Inherit Metab Dis* **42:** 620–628. doi:10.1002/jimd.12076

Dobrow MJ, Hagens V, Chafe R, Sullivan T, Rabeneck L. 2018. Consolidated principles for screening based on a systematic review and consensus process. *CMAJ* **190:** E422–E429. doi:10.1503/cmaj.171154

Engelke UF, van Outersterp RE, Merx J, van Geenen FA, van Rooij A, Berden G, Huigen MC, Kluijtmans LA, Peters TM, Al-Shekaili HH, et al. 2021. Untargeted metabolomics and infrared ion spectroscopy identify biomarkers for pyridoxine-dependent epilepsy. *J Clin Invest* **131:** e148272. doi:10.1172/JCI148272

Falsaperla R, Vari MS, Toldo I, Murgia A, Sartori S, Vecchi M, Suppiej A, Burlina A, Mastrangelo M, Leuzzi V, et al. 2018. Pyridoxine-dependent epilepsies: an observational study on clinical, diagnostic, therapeutic and prognostic features in a pediatric cohort. *Metab Brain Dis* **33:** 261–269. doi:10.1007/s11011-017-0150-x

Franková V, Driscoll RO, Jansen ME, Loeber JG, Kožich V, Bonham J, Borde P, Brincat I, Cheillan D, Dekkers E, et al. 2021. Regulatory landscape of providing information on newborn screening to parents across Europe. *Eur J Hum Genet* **29:** 67–78. doi:10.1038/s41431-020-00716-6

Ghavanini AA, Kimpinski K. 2014. Revisiting the evidence for neuropathy caused by pyridoxine deficiency and excess. *J Clin Neuromuscul Dis* **16:** 25–31. doi:10.1097/CND.0000000000000049

Godler DE, Ling L, Gamage D, Baker EK, Bui M, Field MJ, Rogers C, Butler MG, Murgia A, Leonardi E, et al. 2022. Feasibility of Screening for Chromosome 15 Imprinting Disorders in 16 579 Newborns by Using a Novel Genomic Workflow. *JAMA Netw Open* **5:** e2141911. doi:10.1001/jamanetworkopen.2021.41911

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

Haidar Z, Jalkh N, Corbani S, Fawaz A, Chouery E, Mégarbané A. 2018. Atypical pyridoxine dependent epilepsy resulting from a new homozygous missense mutation, in ALDH7A1. *Seizure* **57:** 32–33. doi:10.1016/j.seizure.2018.03.010

Hess-Homeier DL, Cunniff C, Grinspan ZM. 2019. Priorities for newborn screening of genetic epilepsy. *Pediatr Neurol* **101:** 83–85. doi:10.1016/j.pediatrneurol.2019.07.009

Holm VA, Deering WM, Penn RL. 1970. Some factors influencing the development of a voluntary PKU screening program: possible implication for other screening procedures for newborns. *J Am Med Assoc* **212:** 1835–1842. doi:10.1001/jama.1970.03170240039005

Hong KM, Heath O, Halligan R, Francescon A, Greaves R, Pitt J. 2022. Pyridoxine dependent epilepsy: diagnostic proficiency of new biomarkers. *Pathology* **54:** S17–S18. doi:10.1016/j.pathol.2021.12.064

Howell RR, Lloyd-Puryear MA. 2010. From developing guidelines to implementing legislation: actions of the US Advisory Committee on Heritable Disorders in Newborns and Children toward advancing and improving newborn screening. *Semin Perinatol* **34:** 121–124. doi:10.1053/j.semperi.2009.12.004

Hunt AD, Stokes J, McCRORY WW, Stroud HH. 1954. Pyridoxine dependency: report of a case of intractable convulsions in an infant controlled by pyridoxine. *Pediatrics* **13:** 140–145. doi:10.1542/peds.13.2.140

Jiao X, Xue J, Gong P, Wu Y, Zhang Y, Jiang Y, Yang Z. 2019. Clinical and genetic features in pyridoxine-dependent epilepsy: a Chinese cohort study. *Dev Med Child Neurol* **62:** 315–321. doi:10.1111/dmcn.14385

Jung S, Tran N-TB, Gospe SM, Hahn SH. 2013. 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. doi:10.1016/j.ymgme.2013.07.017

Kuhara T, Akiyama T, Ohse M, Koike T, Shibasaki J, Imai K, Cooper AJL. 2020. Identification of new biomarkers of pyridoxine-dependent epilepsy by GC/MS-based urine metabolomics. *Anal Biochem* **604:** 113739. doi:10.1016/j.ab.2020.113739

Leandro J, Houten SM. 2020. The lysine degradation pathway: subcellular compartmentalization and enzyme deficiencies. *Mol Genet Metab* **131:** 14–22. doi:10.1016/j.ymgme.2020.07.010

Lesser AJ. 1963. Phenylketonuria and the Guthrie test. *Pediatrics* **32:** 940. doi:10.1542/peds.32.5.940

MacDonald A, Gokmen-Ozel H, van Rijn M, Burgard P. 2010. The reality of dietary compliance in the management of phenylketonuria. *J Inherit Metab Dis* **33:** 665–670. doi:10.1007/s10545-010-9073-y

MacDonald A, Smith TA, de Silva S, Alam V, van Loon JMT. 2016. The personal burden for caregivers of children with phenylketonuria: a cross-sectional study investigating time burden and costs in the UK. *Mol Genet Metab Rep* **9:** 1–5. doi:10.1016/j.ymgmr.2016.08.008

Marguet F, Barakizou H, Tebani A, Abily-Donval L, Torre S, Bayoudh F, Jebnoun S, Brasseur-Daudruy M, Marret S, Laquerriere A, et al. 2016. Pyridoxine-dependent epilepsy: report on three families with neuropathology. *Metab Brain Dis* **31:** 1435–1443. doi:10.1007/s11011-016-9869-z

Mercimek-Mahmutoglu S, Donner EJ, Siriwardena K. 2013. Normal plasma pipecolic acid level in pyridoxine dependent epilepsy due to *ALDH7A1* mutations. *Mol Genet Metab* **110:** 197. doi:10.1016/j.ymgme.2013.04.018

Mercimek-Mahmutoglu S, Cordeiro D, Cruz V, Hyland K, Struys EA, Kyriakopoulou L, Mamak E. 2014. 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. doi:10.1016/j.ejpn.2014.07.001

Mills PB, Struys E, Jakobs C, Plecko B, Baxter P, Baumgartner M, Willemsen MAAP, Omran H, Tacke U, Uhlenberg B, et al. 2006. Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med* **12:** 307–309. doi:10.1038/nm1366

Mills PB, Footitt EJ, Mills KA, Tuschl K, Aylett S, Varadkar S, Hemingway C, Marlow N, Rennie J, Baxter P, et al. 2010. Genotypic and phenotypic spectrum of pyridoxine-dependent epilepsy (*ALDH7A1* deficiency). *Brain* **133:** 2148–2159. doi:10.1093/brain/awq143

Mills PB, Footitt EJ, Ceyhan S, Waters PJ, Jakobs C, Clayton PT, Struys EA. 2012. Urinary AASA excretion is elevated in patients with molybdenum cofactor deficiency and isolated sulphite oxidase deficiency. *J Inherit Metab Dis* **35:** 1031–1036. doi:10.1007/s10545-012-9466-1

Minenkova A, Jansen EEW, Cameron J, Barto R, Hurd T, MacNeil L, Salomons GS, Mercimek-Andrews S. 2021. Is impaired energy production a novel insight into the pathogenesis of pyridoxine-dependent epilepsy due to biallelic variants in *ALDH7A1*? *PLoS ONE* **16:** e0257073. doi:10.1371/journal.pone.0257073

Nam SH, Kwon M-J, Lee J, Lee CG, Yu HJ, Ki C-S, Lee M. 2012. Clinical and genetic analysis of three Korean children with pyridoxine-dependent epilepsy. *Ann Clin Lab Sci* **42:** 65–72.

O'Leary P, Maxwell S. 2015. Newborn bloodspot screening policy framework for Australia. *Australas Med J* **8:** 292–298. doi:10.4066/AMJ.2015.2482

Peduto A, Baumgartner MR, Verhoeven NM, Rabier D, Spada M, Nassogne M-C, Poll-The B-TT, Bonetti G, Jakobs C, Saudubray J-M. 2004. Hyperpipecolic acidaemia: a diagnostic tool for peroxisomal disorders. *Mol Genet Metab* **82:** 224–230. doi:10.1016/j.ymgme.2004.04.010

Pena IA, Marques LA, Laranjeira ÂBA, Yunes JA, Eberlin MN, MacKenzie A, Arruda P. 2017. Mouse lysine catabolism to aminoadipate occurs primarily through the saccharopine pathway; implications for

pyridoxine dependent epilepsy (PDE). *Biochim Biophys Acta* **1863:** 121–128. doi:10.1016/j.bbadis.2016.09.006

Petros M. 2012. Revisiting the Wilson–Jungner criteria: how can supplemental criteria guide public health in the era of genetic screening? *Genet Med* **14:** 129–134. doi:10.1038/gim.0b013e31823331d0

Plecko B, Stöckler-Ipsiroglu S, Paschke E, Erwa W, Struys EA, Jakobs C. 2000. Pipecolic acid elevation in plasma and cerebrospinal fluid of two patients with pyridoxine-dependent epilepsy. *Ann Neurol* **48:** 121–125. doi:10.1002/1531-8249(200007)48:1<121::AID-ANA20>3.0.CO;2-V

Plecko B, Hikel C, Korenke G-C, Schmitt B, Baumgartner M, Baumeister F, Jakobs C, Struys E, Erwa W, Stöckler-Ipsiroglu S. 2005. Pipecolic acid as a diagnostic marker of pyridoxine-dependent epilepsy. *Neuropediatrics* **36:** 200–205. doi:10.1055/s-2005-865727

Porta F, Pagliardini V, Celestino I, Pavanello E, Pagliardini S, Guardamagna O, Ponzone A, Spada M. 2017. Neonatal screening for biotinidase deficiency: a 30-year single center experience. *Mol Genet Metab Rep* **13:** 80–82. doi:10.1016/j.ymgmr.2017.08.005

Rankin PM, Harrison S, Chong WK, Boyd S, Aylett SE. 2007. Pyridoxine-dependent seizures: a family phenotype that leads to severe cognitive deficits, regardless of treatment regime. *Dev Med Child Neurol* **49:** 300–305. doi:10.1111/j.1469-8749.2007.00300.x

Russell KE, Mulligan SR, Mallory LA. 2012. Diagnosis of pyridoxine-dependent seizures in a nineteen-year-old patient. *Pediatr Neurol* **47:** 141–143. doi:10.1016/j.pediatrneurol.2012.04.018

Scriver CR. 1960. Vitamin B6-dependency and infantile convulsions. *Pediatrics* **26:** 62–74. doi:10.1542/peds.26.1.62

Spiekerkoetter U, Sun B, Zytkovicz T, Wanders R, Strauss AW, Wendel U. 2003. MS/MS-based newborn and family screening detects asymptomatic patients with very-long-chain acyl-CoA dehydrogenase deficiency. *J Pediatr* **143:** 335–342. doi:10.1067/S0022-3476(03)00292-0

Srinivasaraghavan R, Parameswaran N, Mathis D, Bürer C, Plecko B. 2018. Antiquitin deficiency with adolescent onset epilepsy: molecular diagnosis in a mother of affected offsprings. *Neuropediatrics* **49:** 154–157. doi:10.1055/s-0037-1621721

Stockler S, Plecko B, Gospe SM, Coulter-Mackie M, Connolly M, van Karnebeek C, Mercimek-Mahmutoglu S, Hartmann H, Scharer G, Struijs E, et al. 2011. Pyridoxine dependent epilepsy and antiquitin deficiency: clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. *Mol Genet Metab* **104:** 48–60. doi:10.1016/j.ymgme.2011.05.014

Struys EA, Jakobs C. 2007. α-Aminoadipic semialdehyde is the biomarker for pyridoxine dependent epilepsy caused by α-aminoadipic semialdehyde dehydrogenase deficiency. *Mol Genet Metab* **91:** 405. doi:10.1016/j.ymgme.2007.04.016

Struys EA, Bok LA, Emal D, Houterman S, Willemsen MA, Jakobs C. 2012a. 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. doi:10.1007/s10545-011-9443-0

Struys EA, Nota B, Bakkali A, Al Shahwan S, Salomons GS, Tabarki B. 2012b. Pyridoxine-dependent epilepsy with elevated urinary α-amino adipic semialdehyde in molybdenum cofactor deficiency. *Pediatrics* **130:** e1716–e1719. doi:10.1542/peds.2012-1094

Suzuki M, West C, Beutler E. 2001. Large-scale molecular screening for galactosemia alleles in a pan-ethnic population. *Hum Genet* **109:** 210–215. doi:10.1007/s004390100552

Toldo I, Bonardi CM, Bettella E, Polli R, Talenti G, Burlina A, Sartori S, Murgia A. 2018. Brain malformations associated to *Aldh7a1* gene mutations: report of a novel homozygous mutation and literature review. *Eur J Paediatr Neurol* **22:** 1042–1053. doi:10.1016/j.ejpn.2018.06.010

Tseng LA, Abdenur JE, Andrews A, Aziz VG, Bok LA, Boyer M, Buhas D, Hartmann H, Footitt EJ, Grønborg S, et al. 2022. Timing of therapy and neurodevelopmental outcomes in 18 families with pyridoxine-dependent epilepsy. *Mol Genet Metab* doi:10.1016/j.ymgme.2022.02.005

van Karnebeek CDM, Hartmann H, Jaggumantri S, Bok LA, Cheng B, Connolly M, Coughlin CR, Das AM, Gospe SM, Jakobs C, et al. 2012. Lysine restricted diet for pyridoxine-dependent epilepsy: first evidence and future trials. *Mol Genet Metab* **107:** 335–344. doi:10.1016/j.ymgme.2012.09.006

van Karnebeek CDM, Stockler-Ipsiroglu S, Jaggumantri S, Assmann B, Baxter P, Buhas D, Bok LA, Cheng B, Coughlin CR, Das AM, et al. 2014. Lysine-restricted diet as adjunct therapy for pyridoxine-dependent epilepsy: the PDE consortium consensus recommendations. *JIMD Rep* **15:** 1–11. doi:10.1007/8904_2014_296

van Karnebeek CDM, Tiebout SA, Niermeijer J, Poll-The BT, Ghani A, Coughlin CR, Van Hove JLK, Richter JW, Christen HJ, Gallagher R, et al. 2016. Pyridoxine-dependent epilepsy: an expanding clinical spectrum. *Pediatr Neurol* **59:** 6–12. doi:10.1016/j.pediatrneurol.2015.12.013

van Outersterp RE, Engelke UFH, Merx J, Berden G, Paul M, Thomulka T, Berkessel A, Huigen MCDG, Kluijtmans LAJ, Mecinović J, et al. 2021. Metabolite identification using infrared ion spectroscopy—novel biomarkers for pyridoxine-dependent epilepsy. *Anal Chem* **93:** 15340–15348. doi:10.1021/acs.analchem.1c02896

Vockley J, Chapman KA, Arnold GL. 2013. Development of clinical guidelines for inborn errors of metabolism: commentary. *Mol Genet Metab* **108:** 203–205. doi:10.1016/j.ymgme.2013.01.013

Wempe MF, Kumar A, Kumar V, Choi YJ, Swanson MA, Friederich MW, Hyland K, Yue WW, Van Hove JLK, Coughlin CR. 2019. Identification of a novel biomarker for pyridoxine-dependent epilepsy: implications for newborn screening. *J Inherit Metab Dis* **42:** 565–574. doi:10.1002/jimd.12059

Wilson JMG, Jungner G. 1968. *Principles and practice of screening for disease*. World Health Organization, Geneva.

Wilson MP, Plecko B, Mills PB, Clayton PT. 2019. Disorders affecting vitamin B6 metabolism. *J Inherit Metab Dis* **42:** 629–646. doi:10.1002/jimd.12060

Yang Z, Yang X, Wu Y, Wang J, Zhang Y, Xiong H, Jiang Y, Qin J. 2014. Clinical diagnosis, treatment, and *ALDH7A1* mutations in pyridoxine-dependent epilepsy in three Chinese infants. *PLoS ONE* **9:** e92803. doi:10.1371/journal.pone.0092803

Yazdani M, Elgstøen KBP. 2021. Is oxidative stress an overlooked player in pyridoxine-dependent epilepsy? A focused review. *Seizure* **91:** 369–373. doi:10.1016/j.seizure.2021.07.014

Yeghiazaryan NS, Striano P, Spaccini L, Pezzella M, Cassandrini D, Zara F, Mastrangelo M. 2011. Long-term follow-up in two siblings with pyridoxine-dependent seizures associated with a novel *ALDH7A1* mutation. *Eur J Paediatr Neurol* **15:** 547–555. doi:10.1016/j.ejpn.2011.05.011