A case for newborn screening for pyridoxine-dependent epilepsy

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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, patients with PDE-ALDH7A1 have died prior to diagnosis and subsequent treatment with pyridoxine 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

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 B₆). 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 achieve 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 (L Tseng, J Abdenur, A Andrews et al., pers. comm.), and most patients suffer IDD despite adequate seizure control (Bok et al. 2012). It is critical to distinguish PDE-*ALDH7A1* from other vitamin B₆ responsive seizure disorders as adjunct treatment is recommended for most patients with PDE-*ALDH7A1* (Table 1). Adjunct lysine reduction therapies (LRT) 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 and (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 stable at room temperature and measurable using current laboratory techniques employed 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 (Andermann et al. 2008; Petros 2012; Dobrow et al. 2018) (Table 2). 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 HSS has provided a list of disorders recommended for states to screen referred to as the recommended uniform screening panel (RUSP). In The Netherlands, the 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 (UKNSC) 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 significantly higher after newborn screening. 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 birth following screening (Boneh et al. 2006; Spiekerkoetter et al. 2003).

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. Using only this strict criterion, the majority of patients would go undiagnosed and remain poorly treated.

Although most patients present in the newborn period, approximately 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 one 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 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 pyridoxine 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 (Gül-Mert et al. 2015; Nam et al. 2012; Yang et al. 2014). 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 (Marguet et al. 2016; Mills et al. 2010; Toldo et al. 2018; Baumgart et al. 2014). In these cases, a correct diagnosis and subsequent 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 (L Tseng, J Abdenur, A Andrews et al., pers. comm.).

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 due to 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 LRT 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 include 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). LRT often include 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 the 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 LRT 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 LRT 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 six 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 readably treatable seizure disorder based 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 B₆ 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 -piperidine-6-carboxylate (Δ^1 -P6C) and α -AASA (Mills et al. 2006; Struys and Jakobs 2007; Struys et al. 2012a) (Figure 1). 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 diagnosis patients with PDE-ALDH7A1 (Plecko et al. 2000, 2005). Pipecolic acid may not be an ideal candidate for a 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 evaluated in patients 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-pipecolate (Wempe et al. 2019) and 2S,6S – 2S,6R – oxopropylpiperidene-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 employed in newborn screening laboratories such as liquid chromatography tandem mass spectrometry. Although only limited studies have been

performed to date (Hong et al. 2022; Kuhara et al. 2020), 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 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 improve 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. Life-long 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 (Leandro and Houten 2020; Crowther et al. 2019). 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 life-long cognitive disability requiring dependence on a care giver and a typical adult.

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Figure Legends

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 oxopropylpiperidine-2-carboxylic acid.

Figure

Table 1. Vitamin B₆ responsive seizure disorders

Disorder	Gene	Vitamin B ₆	Adjunct (to B ₆) treatment
Pyridoxine dependent epilepsy (PDE-ALDH7A1)	<i>ALDH7A1</i>	Pyridoxine	Lysine reduction therapies
Pyridoxamine 5'-phosphate oxidase (PNPO) deficiency	<i>PNPO</i>	Pyridoxal phosphate ¹	NA
Pyridoxal phosphate binding protein (PLPBP)	<i>PLPBP</i>	Pyridoxine <i>or</i> Pyridoxal phosphate	NA
Molybdenum cofactor deficiency	<i>MOCS1</i> , <i>MOCS2</i>	Pyridoxine ²	Cysteine-restricted diet; Cyclic pyranopterin monophosphate ³
Hyperprolinemia type II	<i>ALDH4A1</i>	Pyridoxine	NA
Hypophosphatasia	<i>ALPL</i>	Pyridoxine	Enzyme replacement therapy

¹Some patients are responsive to pyridoxine²Not all patients have clinical response to vitamin B₆³Patients with *MOCS1* – related disease**Table 2. Suggested principles for population-based screening**

Wilson & Jungner, World Health Organ, 1968	Andermann et al, Bull. World Health Organ, 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

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