The spectrum of pyridoxine dependent epilepsy across the age span: A nationwide retrospective observational study

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Abstract

Background: Pyridoxine-dependent epilepsy (PDE) is a rare seizure disorder usually presenting with neonatal seizures. Most cases are caused by biallelic pathogenic *ALDH7A1*variants. While anti-seizure medications are ineffective, pyridoxine provides seizure control, and dietary interventions may be of benefit. As the natural history beyond adolescence is insufficiently explored, our study aimed to assess the spectrum of PDE at various ages in Norway.

Methods: Patients were ascertained by contacting all Norwegian paediatric, neurological, and neurohabilitation departments and relevant professional societies. Medical records were collected and reviewed.

Results: We identified 15 patients treated for PDE; 13 had *ALDH7A1* variants (PDE-ALDH7A1), one had PNPO deficiency, and in one, aetiology remained obscure. Of those with PDE-ALDH7A1, 12 were alive at time of study; five were >18 years old and six were < 4 years. Median age was 10 years (range 2 months–53 years). Estimated minimum prevalence was 6.3/million among children and 1.2/million among adults. Ten had seizure onset on the first day of life. Perinatal complications and neuroradiological abnormalities suggested additional seizure aetiologies in several patients. Pyridoxine had immediate effect in six, while six had delayed (>1 hour) or uncertain effect. Median delay from first seizure to continuous treatment was 11 days (range 0–42). Nine experienced breakthrough seizures with intercurrent disease or due to pyridoxine discontinuation. Cognitive outcomes ranged from normal to severe intellectual disability. The condition appeared to remain stable in adult life.

Significance: We found a much higher prevalence of PDE-ALDH7A1 in children relative to adults, suggesting previous underdiagnosis and early mortality. Perinatal complications are common and can delay diagnosis and initiation of pyridoxine treatment. Lifelong and continuous treatment with pyridoxine is imperative. Due to better diagnostics and survival, the number of adult patients is expected to rise.

Keywords: *ALDH7A1*, PNPO, epidemiology, genetic variant, treatment, prognosis

1. Introduction

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive metabolic disorder, mainly caused by biallelic variants in the *ALDH7A1* gene (PDE-ALDH7A1, OMIM#266100). In most patients, seizures start within the first hours or days after birth, although the disorder may present later in childhood [1, 2], even up to adolescence [3, 4]. Conventional antiseizure medications (ASMs) are largely ineffective, but seizure control is achieved with pharmacological doses of pyridoxine (PN) [1, 2], reflecting PDE-ALDH7A1 as an archetypal example of precision epilepsy treatment [5]. Since seizures recur when PN is withdrawn, lifelong PN treatment is required [1, 2].

Several seizure types can be present at onset, including focal and bilateral clonic, myoclonic, tonic and atonic, as well as spasms [2, 6]. Early EEG abnormalities are variable, showing both focal and multifocal epileptiform activity with unilateral or bilateral distributions, including a burst suppression pattern [7, 8]. In some patients, magnetic resonance imaging (MRI) shows midline brain abnormalities, particularly corpus callosum hypoplasia and occasionally an enlarged cisterna magna and ventriculomegaly, as well as circulatory changes [9-13]. Despite early seizure control, the majority have cognitive deficits. In a recently reported Dutch cohort of 28 patients with PDE-ALDH7A1, cognition was normal in 25%, borderline in 29%, and in the remaining patients, there were variable degrees of developmental delay/intellectual disability (ID) [13].

The disorder was discovered in 1954 [1], and until the underlying genetic cause was identified in 2006, the response to PN alone defined the diagnosis [14]. Pathogenic variants in *ALDH7A1* cause a deficiency of α-aminoadipic semialdehyde dehydrogenase (antiquitin), a key enzyme in the lysine catabolism pathway [15]. The defect causes increased levels of α-aminoadipic semialdehyde (αAASA) and Δ^1-piperidine-6-carboxylate (P6C), which are biomarkers for the disease [14]. The accumulated P6C binds and inactivates the bioactive B6 vitamer pyridoxal 5'-phosphate (PLP), and the functional PLP deficiency causes disturbances in multiple brain enzyme systems. Increased levels of PN following supplementation allow rapid normalization of PLP-dependent enzymatic activity. Recent observational studies suggest that a lysine-restricted diet with enrichment of arginine (which inhibits lysine transport) is of additional benefit for cognitive outcomes, particularly when started in early life [10, 16-18].

The clinical course of PDE beyond childhood has not been studied in detail. Therefore, we set out to study all available Norwegian subjects with PDE to characterize the phenotypic spectrum and treatment response across the age span to address factors that might determine the overall prognosis of the disorder.

2. Methods

Invitations to take part in the study were sent to all paediatric, neurological, and neurohabilitation departments in Norway. Information was posted on the websites of the Norwegian Branch of the International League Against Epilepsy, the Norwegian Paediatric Neurology Society, the Medical Association for Neurohabilitation, and the National Unit of Newborn Screening and Advanced Laboratory Diagnostics in Inborn Errors of Metabolism, Oslo University Hospital. The study was also announced at the annual meeting and in the periodical of the Norwegian Neurological Association. Eligible professionals, such as physicians likely to be involved in the management of patients with PDE, were directly invited by e-mail.

Treatment providers were asked to contact available patients considered to have PDE or their parents/guardians for written informed consent to participate. Medical records of all responders were collected.

Definite cases of PDE-ALDH7A1 were defined as seizures responding to PN and biallelic pathogenic *ALDH7A1* variants. Siblings with the same phenotype as an affected case were considered probable PDE-ALDH7A1. Patients responding to PN without a confirmed genetic cause were considered possible cases requiring biochemical screening and DNA analysis.

All available medical records were reviewed and relevant clinical, EEG, neuroimaging, genetic and biochemical data were extracted. DNA analysis, variant interpretation, and analysis of the biomarkers αAASA, P6C, 2S,6S-/2S,6R-oxopropylpiperidine-2-carboxylate (2-OPP) and 6-oxo-piperidine-2-carboxylic acid (6-oxo-pip) were performed in patients without known variants. In adults, cognitive function was assessed by standardized neuropsychological evaluation, whereas in children developmental delay was categorized by gross clinical neuropaediatric judgement and/or motor developmental scales. Adult patients or their parents were specifically asked about any perceived cognitive change above the age of 18 years.

The population of Norway < 18 and $\underline{\geq}$ 18 years of age for 2022 and the live birth rates for the last four years were collected from Statistics Norway [19].

The study was approved by the Regional Ethical Committee of Mid-Norway (No. 200284).

3. Results

3.1 Diagnosis and genetic findings

We identified 14 living patients on PN treatment and a diagnosis of PDE. At inclusion, nine were known to harbour biallelic pathogenic *ALDH7A1* variants (Patients 1-2, 4-9 and 13), whereas pathogenic variants were identified as part of the study in three adults (Patients 10-12). Of the 12 living patients with confirmed PDE-ALDH7A1, eight carried homozygous and four compound heterozygous variants. The founder variant NM_001182.4(ALDH7A1):c.1279G>C, p.(Glu427Gln) was the most prominent allele (67%), present in all but two subjects (Patients 4 and 10) (Table 1). Based on a similar phenotype, one deceased subject (Patient 3) was inferred to have the same genotype as his younger sibling (Patient 10). No other siblings were present in this cohort, but Patients 1 and 2 were double cousins. We classified all detected variants as pathogenic, class 5, according to the American College of Medical Genetics and Genomics [20].

In two possible PDE cases, both having infantile spasms responding to PN, no biomarkers consistent with PDE-ALDH7A1 were detected. A homozygous pathogenic variant in the gene encoding pyridoxamine 5'-phosphate oxidase, NM_018129.3(*PNPO*): c.674G>A, p.(Arg225His), was identified in one (Patient 15), whereas no likely pathogenic variants were identified in the other (Patient 14).

3.2 Findings in PDE-ALDH7A1

3.2.1 Demographics,prevalence and incidence. Of the 13 patients with PDE-ALDH7A1, six were born female (one subsequently identified as male), and seven were male. The median age of the 12 living patients was 10 years (IQR 24 years), range 2 months-53 years. Seven were below 18 years (Table 1), giving an estimated prevalence of 6.3/million (7/1,108,500) in Norwegian children and 1.2/million (5/4,316,700) in adults. Six children with PDE-ALDH7A1 were born during 2019-2022 in Norway (Patients 1, 2 and 4-7) (Table 1). According to the number of live births in this period, the estimated incidence of PDE-ALDH7A1 during the last four years was 6/215,943, i.e. 1/35,990.

3.2.2 Seizures prior to PN treatment. In retrospect, the mothers of six patients (44%) reported rhythmic foetal movements suggestive of intrauterine seizures during the last trimester. The median age at postnatal seizure onset was 9 h (IQR 41 h), range 1h-6 days after birth. Nine patients (69 %) presented with seizures within the first 24 h of life, while four had seizure onset between one and seven days postpartum. Prevailing seizure types included clonic, tonic, and myoclonic that could be focal, asymmetrical, and bilateral. In seven patients (53%), one or more episodes of status epilepticus (SE) (continuous or nearly continuous seizures $\geq$ 30 min) were reported in the neonatal period prior to PN treatment.

3.2.3 Perinatal complications. Three patients were delivered by Caesarean section, and three births were induced. Asphyxia was diagnosed in six (46%), whereas acidosis alone was found in the remaining. Twelve of the 13 patients (92%) needed postnatal respiratory support prior to PN administration (Table 1).

3.2.4 PN treatment. Median delay from first seizure to the first pyridoxine vitamer trial was 2 days (range 0-16). Six of the 11 living patients who received initial PN injections had a prompt effect on seizure activity, while the effect was uncertain or delayed (>1h) in five. No adverse clinical reactions were reported. Median delay from first seizure to continuous PN treatment was 11 days (range 0-42) for children, and for adults 12 days (range 1-17). Eight patients continued PN after two or more trials. Patient 13 tried to discontinue PN several times during childhood. The median time to seizure recurrence after all treatment interruptions was 6 d (IQR 4 d), range 1-61 days.

3.2.5 Dietary treatment. All children, but no adults, received treatment with lysine-restricted diet and arginine supplementation (Table 1).

3.2.6 Cognitive function. Of the adult patients, one had a normal developmental outcome, three had mild and one had moderate ID. Any apparent cognitive decline in adult life was denied. The neurodevelopment of the living children was variable; one remained with severe delay and dyskinetic quadriplegia, whereas two with early mild delay improved and were within normal limits later in childhood (Table 1).

3.2.7 EEG and neuroimaging. During the first 10 days, a burst suppression EEG pattern was described in eight patients (Table 2). Vascular lesions in brain parenchyma or substance loss were identified by neuroimaging in seven. Four patients had ventriculomegaly and two had mega cisterna magna. Normal MRI was found in four, apart from a somewhat thin corpus callosum in one. Another four patients had signs of corpus callosum hypoplasia (Table 2).

3.3 Clinical course in patients with PDE-ALDH7A1

Clinical, genetic and treatment details for each patient are summarized in Table 1. EEGs within the first year and MRI findings are reported in Table 2.

Patient 1 (female, 2 months): Delivery was induced by amniotomy 9 days after due date. She was well until 1 h post-partum when she had recurrent bilateral jerks with cyanosis. PN 100 mg i.v. was administered 2 h post-partum as first treatment due to the recent diagnosis of PDE-ALDH7A1 in her double cousin (Patient 2). Clinical effect occurred within 1 h and further seizures did not occur. Peroral

PN was continued. Transient respiratory distress was treated with CPAP for 2 days, and she is currently doing well.

Patient 2 (male, 3 months): Caesarean section was performed due to protracted labour at term, and he needed immediate positive pressure ventilation. Approximately 1 h after birth he developed motor seizures treated with phenobarbital (PB), which was repeated after 12 h. On day 2 he received 100 mg PN i.v. when seizure-free. EEG improved, PDE-ALDH7A1 metabolites were detected, and PN was continued without seizure recurrence.

Patient 3 (male, 10 months at death): Delivery was normal but complicated by asphyxia requiring resuscitation. Seizures evolving to SE started 3 h after birth. Despite treatment with diazepam (DZP) and other ASMs, seizures returned with multiple episodes of SE. During a severe SE at age 2.5 months, PN 300 mg i.v. was given twice without immediate effect and was not continued. Next, he received xylocaine with an apparent response. Fatal SE occurred at 10 months.

Patient 4 (female, 18 months): This patient is of Afghan ancestry. Five hours after an uneventful birth, she developed multifocal myoclonic seizures, which recurred despite ASMs until PN 100 mg i.v. was administered on day 6. The seizures abated within one day. PDE-ALDH7A1 biomarkers were identified, and she continued PN. Following levetiracetam (LEV) discontinuation at 2 months, she had transient episodes with gaze deviation/head turning. LEV was reintroduced at age 18 months due to recurrent febrile seizures. Early motor development was mildly delayed, but cognitive, language and social development was considered normal at age 13 months.

Patient 5 (female, 2 years 3 months): Caesarean section was performed 11 days after due date. Immediately after birth, she developed respiratory distress/asphyxia requiring respiratory support. Prolonged clonic seizures occurred at age 22 h. On day 15, she developed SE for 3 days. Elevated urine vanillactic acid suggested PNPO deficiency, and on day 18, peroral PLP was administered with effect. PDE-ALDH7A1 was confirmed 6 days later, and PLP was substituted by PN. At age 2 months, she had a febrile SE and later a non-febrile seizure. Severe feeding problems required gastrostomy. A moderate developmental delay was evident.

Patient 6 (*male, 3 years 3 months)*: Delivery was induced due to preeclampsia and completed by emergency Caesarean section because of foetal bradycardia. Subtle orofacial twitching occurred 1 h after birth. At 3 h he was apnoeic with combined respiratory and lactic acidosis. From 12 h, he developed motor seizures resistant to ASMs. PN 100 mg i.v. was administered on day 2 with uncertain effect within 24 h. Seizures culminating into SE recurred on day 4. High spinal fluid levels of threonine and urinary vanillactic acid suggested PNPO deficiency and he responded to PLP from day 4 with

fewer seizures and improved respiration. PLP was replaced by PN when PDE-ALDH7A1 was confirmed by genetic analysis on day 9. He had four breakthrough seizures during intercurrent illness up to age 18 months despite double PN doses on all occasions, with one episode classified as SE. Motor development was mildly delayed during the first two years of life. Apart from moderately slowed expressive language development, he is currently achieving adequate milestones.

Patient 7 (male, 3 years 4 months): Birth was uneventful. On day 4, he presented with SE with a transient response to midazolam (MDZ) and ASMs. He developed a combined respiratory and lactic acidosis and required respiratory support. On day 5, 100 mg PN was injected while seizure free. An initial EEG burst suppression normalized and a diagnosis of benign familial neonatal seizures (BFNS) was assumed. Seizures reappeared 6 days after the first PN bolus. He received another PN dose and MDZ and remained seizure-free for 4 days. Following MDZ discontinuation, the seizures returned, and he subsequently received both ASMs and PN. Since BFNS was the working diagnosis, all medication was discontinued at age 27 days. One week later, seizures returned and ASM treatment was resumed. The genetic diagnosis was established at one month. Early language acquisition appeared delayed but was considered normal from age 3 years.

Patient 8 (male, 16 years): Delivery was normal. At age 6 days, he developed seizures refractory to PB. Two days later, he received 50 mg PN i.v., but more frequent focal seizures led to the addition of phenytoin (PHT), and he became seizure-free. One week later, he developed severe intractable seizures and subtle episodes of SE associated with desaturations as low as 24%. On day 20, he received a second i.v. bolus of PN plus valproate and was seizure-free for 7 days. Another PN trial during thiopental infusion for SE had some clinical effect, and PN was continued. Progressive microcephaly was noted from age 10 months. MRI showed widespread abnormalities (Table 2). PDE-ALDH7A1 was confirmed at 2 years of age, when increased urine αAASA and one heterozygous pathogenic variant in *ALDH7A1* were detected. At age 16, a second pathogenic variant (deletion of exon 7) was identified. Sporadic seizures occurred during PN treatment, also when combined with LEV from age 4 years. PN was substituted by PLP but changed back to PN at age 13. Lysine reduction therapy was introduced at age 10 years, and subsequent mild improvement of alertness and communication was reported. He remains seizure-free with severe ID and dyskinetic quadriplegic cerebral palsy (CP) and is fed via gastrostomy.

Patient 9 (female, 25 years): Birth was induced 12 days post-term. Neonatal respiratory distress with pneumomediastinum, hypoxia and acidosis were reported. Bilateral myoclonic jerks appeared at 6 h. DZP and PB had a temporary effect. At 9 days, she had recurring serial myoclonic/clonic seizures

lasting several hours unresponsive to PHT and xylocaine. Following 100 mg PN i.v., seizures ceased within two minutes. Focal seizures occurred 5 days later but responded promptly to PN. Seizures recurred after one week, and PN was continued. A single febrile seizure occurred at age 1 year, but she has since remained seizure-free. Motor development was mildly delayed during the first year, but she caught up, and further milestones were reached within normal limits. The genetic diagnosis was confirmed at age 15 years. Neuropsychological testing at age 21 showed an overall normal cognitive level, apart from slight problems with maintained attention and processing speed. Visuospatial abilities were above average. She exhibited normal executive functions and age-adequate learning abilities.

Patient 10 (*born female, identifies as male, 25 years):* Labour was induced at 39 weeks due to birth complications of the elder brother (Patient 3). The patient had respiratory distress and asphyxia complicated by pneumomediastinum. Motor seizures occurred on day 1. MDZ/ASMs had only a temporary effect. On day 3, the seizures stopped 1 minute after 50 mg PN i.v. Tonic seizures recurred after 10 days and again responded to PN within 5 minutes. Peroral PN was maintained, and ASMs were discontinued. Febrile seizures occurred at ages 1 and 8 years. Motor and intellectual functions were delayed, and autistic features with self-harming behaviour developed. A left-sided esotropia was noticed. At age 13, altered gender identification was acknowledged. Moderate ID was diagnosed by cognitive testing.

Patient 11 (*male, 26 years):* Less than 2 h after a normal birth, he developed severe hypoxia/acidosis with pneumothorax requiring respiratory support. An hour later, facial twitching evolved to bilateral seizures. DZP had only temporary effect. The following day 100 mg PN i.v. was administered with prompt effect. Seizures recurred after one week despite PB treatment and were immediately terminated by another i.v. PN bolus. PN treatment was maintained and PB was discontinued. At age 1 year, he had serial febrile seizures for 2 h that resolved with i.v. PN, DZP and PB. Throughout childhood, he was hyperactive with slowed development, particularly for motor and verbal functions. Left esotropia was present. Neuropsychological assessment at age 20 revealed IQ 68.

Patient 12 (female, 32 years): Following normal birth, she developed hypoxia and acidosis requiring respiratory support and treatment of a pneumothorax. On day 2, she had a series of motor seizures subsiding with DZP/PB and PHT. On day 11, she developed treatment resistant SE that terminated 1 minute after PN i.v. Seizures recurred 6 days later and were promptly terminated by another PN bolus followed by continued peroral PN. Psychomotor development was delayed with motor symptoms suggesting CP. Sporadic clonic jerks in the upper extremities persisted. Another SE occurred at the age of 10 months, and she had further seizures at the age of 1.5 and 4 years, all associated with

intercurrent disease. During gastroenteritis, she had a tonic-clonic seizure at the age of 22 years. IQ was 53. She has mild left hemiplegia. At age 32 years, she developed auditory hallucinations successfully treated with aripiprazole.

Patient 13 (male, age 53 years): He developed severe hypoxia and pneumonia with acidosis and series of motor seizures 4 h after normal birth. DZP/PB had no effect. Next day, PN 50 mg i.m. stopped the seizures within minutes. Seizures recurred 4 days later and again responded to PN i.m. Despite continued PN and PB, he had several seizures during intercurrent illness. Tonic-clonic SE (1.5 h) occurred at age 8 years. Initial hyperactive behaviour subsided with age. While seizure-free, PN discontinuation was attempted several times, but seizures recurred within 2-3 days, except once, when he remained seizure-free for 2 months. He received carbamazepine, which was continued due to occasional episodes of reduced consciousness. Age 28 years, he developed gastroenteritis and suffered a severe SE while holidaying abroad. Due to vomiting, PN was not ingested, but was reintroduced when family members arrived 3 days later. He made full recovery. At age 53 years, he refused formal neuropsychological testing but was considered to have mild ID by adaptive behaviour assessment.

3.4 Patients without *ALDH7A1* variants

*Patient 14 (male, 10 years)***:** Birth was induced due to post-mature pregnancy. The neonatal period was unremarkable. Infantile spasms with EEG hypsarrhythmia followed episodes of eye-rolling and disturbed consciousness from 5 months. He received vigabatrin and prednisolone without full seizure control or EEG improvement. Two months later, he received 100 mg PN i.v. with prompt EEG normalization and seizure termination. Peroral PN 200 mg/d was continued as single treatment, and he remained seizure free from age 7 months. EEG at age 1.5 years was normal. By genetic testing at age 1 year, a presumed pathogenetic homozygous *ALDH7A1* variant (c.2T>G, p.Met1) was reported. At age 10 years, PDE-ALDH7A1 biomarkers were absent in plasma, and the variant could not be reproduced by genome sequencing. A gene panel including genes associated with pyridoxine-responsive seizures and brain channelopathies did not identify any pathogenic variants. He exhibited mild developmental delay during the first years of life. Development has since been normal with adequate school performance. The aetiology of his seizure disorder remains unknown.

*Patient 15 (female, 29 years)**:*** This patient of Balkan ancestry was born at gestational week 34. She developed bilateral motor seizures after 15 minutes, which stopped with rectal DZP. EEG was normal. At age 3 months, treatment-resistant seizures occurred, developing into infantile spasms with EEG hypsarrhythmia. She received ACTH, steroids and vigabatrin as well as other ASMs, but had 7 episodes of SE until 100 mg PN i.v. stopped ongoing seizures within 20-30 minutes at age 10 months.

PN treatment was continued and ASMs were tapered off. MRI was normal apart from a thin corpus callosum, which had normalized at 8 years. After initial delayed development, she gradually caught up with adequate school performances. She still had sporadic tonic-clonic seizures, both spontaneously and triggered by infections and missed medication. The PN dose was gradually increased to 520 mg b.i.d. At age 13, nicotinamide was dispensed by error in place of PN, and she developed serial GTCs on the first day. Further seizures occurred at ages 19 and 21 years when missing single morning doses. Neurological examination at age 29 revealed areflexia in the legs, but no motor or sensory deficits. This patient has previously been published as a case of PDE [21], but PDE-ALDH7A1 biomarkers were recently absent in plasma. Subsequent genetic analyses revealed a homozygous pathogenic variant in *PNPO* c.674G>A, p.(Arg225His).

4. Discussion

4.1 Epidemiology of PDE-ALDH7A1

The present study is the first population-based survey of PDE-ALDH7A1 prevalence and is based on a Norwegian nationwide inclusion of patients treated with PN for PDE spanning a wide age spectrum. Thirteen of the 15 included patients had PDE-ALDH7A1, one had PNPO deficiency, and in one the aetiology remained obscure.

We estimated the prevalence of PDE-ALDH7A1 among children in Norway to be 6.3/million, with a much lower estimate of 1.2/million in adults. This discrepancy might reflect: 1) *Reduced survival,* as illustrated by Patient 3. 2) *Lack of awareness of the condition*. PDE was first recognized in 1954 and most likely underdiagnosed for many years. Unrecognised adult patients with severe ID and uncontrolled epilepsy may have remained undiagnosed or presumed to have hypoxic-ischemic encephalopathy. This diagnosis was considered in several of our cases, particularly in Patient 3, but also in Patient 8, who was diagnosed after considerable delay due to blunted responses to PN. Noteworthy, six of the 12 living patients in this study were below four years of age, and to our knowledge no new cases were identified in Norway in the 13 preceding years. Recently, awareness of PDE in neonatal medicine has increased because of the development of specific diagnostic biomarkers available in Norway from January 2021 as well as rapid genetic testing. 3) *Ascertainment bias*. Some subjects with recognized PDE-ALDH7A1 may no longer be followed in the specialist health service and thus be unavailable for inclusion in the present study. Moreover, patients with milder phenotypes may present late and remain undiagnosed [4, 22].

Epidemiological studies on PDE are scarce. Previously reported birth incidences were 1:396,000 in the Netherlands [23] and 1:783,000 in the UK and Ireland [6]; however, these studies used only clinical

criteria for diagnosis. The more recent Dutch PDE cohort of 28 patients gave a calculated PDE-ALDH7A1 birth incidence of at least 1:181,726 [13]. Interestingly, analysis of population data from the Genome Aggregation Database (gnomAD) has estimated a carrier frequency of *ALDH7A1* variants predicted to be pathogenic by in silico analysis tools of 1:127, corresponding to an estimated conception rate with biallelic variants of 1:64,352 [3]. These data suggested a much higher incidence of PDE-ALDH7A than recognized, a discrepancy that partly was thought to be explained by intrauterine demise and early mortality of undiagnosed infants [3]. However, the present data indicates an even higher live birth incidence of 1/35,990 in Norway during the last four years. This high rate might be accidental during this short period of time, but there is reason to assume that it is related to the increasing access to biomarkers and genetic testing as well as an emerging awareness and clinical recognition of this treatable condition.

4.2 Recognition of PDE-ALDH7A1

All patients with PDE-ALDH7A1 in this study presented with seizures within the first days of life (Table 1), but delayed onset later in childhood or adolescence may occur with milder symptoms escaping the clinical suspicion of PDE [13, 22, 24, 25]. It is important that awareness of the condition is high in all departments treating seizure disorders, including emergency departments. PDE should be considered in all cases of epilepsy of unknown aetiology, including those in conjunction with asphyxia or other neonatal complications, and particularly in those with recurrent SE [4, 26].

All five adult patients experienced an immediate and striking effect of the first PN bolus (Table 1). As biomarkers or genetic tests for PDE-ALDH7A1 were not available at the time of their births, all were initially diagnosed by a PN treatment trial only [4]. Among the children, a delayed or uncertain effect of the first PN bolus was common, but swift metabolic or genetic analyses established the final diagnosis. Some patients may have been left undiagnosed even when exposed to PN, as seemed to be the case in Patient 3, who died from SE in infancy. In Patients 3 and 8, a delayed effect of PN might mistakenly have been interpreted as a response to ASMs. With the current availability of genetic testing and biomarkers, recurrence after withdrawal of PN treatment as a diagnostic criterion is considered obsolete [27].

An apparent response to PN does not by itself imply that the patient has a disease caused by variants involving a known gene related to PDE. Several cases of infantile spasms have shown improvement with PN [28], and the list of genes associated with PN responsiveness is expanding. Infantile spasms rarely occur in PDE, so when present, the diagnosis must rely on finding the appropriate genotype [25]. In the present study, genetic analysis failed to identify pathogenic *ALDH7A1*-variants in two

patients with infantile spasms receiving long-term PN treatment. In Patient 14, the aetiology remains unknown, while PNPO deficiency was identified in Patient 15. PLP, the active form of vitamin B6, is considered to be the treatment of choice in PNPO deficiency, but a considerable proportion of patients also respond to PN, particularly those with this patient's specific variant [29]. Thus, all patients in whom the PDE diagnosis was based solely on a response to PN prior to the availability of biomarkers should currently have their diagnosis confirmed.

4.3 Potential prognostic factors

4.3.1 Initial response to PN. All adult patients in this study responded promptly to the first PN administration, while several children had a delayed or uncertain response to the first PN trial, possibly influenced by perinatal complications (Table 1) expanding the phenotypic presentation. With the current availability of disease-specific biomarkers and genetic analysis, we suggest that patients with an ambiguous PN response should continue treatment until results from these tests are obtained. All children in this study receive dietary treatment, and their final outcomes are not yet established, whereas the adult patients appeared to have a stable cognitive function with mainly mild or no ID (Table 1). The association between a first convincing PN response and the final outcomes should be further explored in long-term studies taking the effect of perinatal complications and dietary interventions into consideration. Notably, the time to onset of both PN and dietary treatment was shorter in the youngest subjects (particularly Patients 1 and 2) (Table 1), reflecting a currently increased awareness of PDE-ALDH7A1 in neonatal medicine, which hopefully may improve the long-term prognosis.

4.3.2 Genetic findings. As in previous studies [13, 30], we could not identify any clear genotype-phenotype relationship in this small number of patients (Table 1). The c.1279G>C variant was the most prevalent in the cohort, appearing biallelic in six patients; two of them were adults, one with normal development and one with mild ID. Two individuals (Patients 6 and 9) were compound heterozygous for c.1279G>C and a previously reported pathogenic in-frame deletion encompassing exon 7 [31]. One had a borderline IQ of 68 (Patient 9), the other a severe ID and quadriplegic CP (Patient 6), mainly attributed to neonatal SE with severe postnatal asphyxia and a delayed diagnosis of nearly two months, corroborating the role of other factors than the genotype for the outcome.

4.3.3 Neonatal complications. In line with previous studies, asphyxia, lactic acidosis and the need for respiratory support were common [12]. We speculate whether these complications partly may be related to undetected, early subtle seizure activity or possibly result from other pathophysiological mechanisms of PN deficiency. New-borns with PDE-ALDH7A1 are susceptible to asphyxia,

sometimes complicated by focal vascular brain affection. Interestingly, it has been proposed that ALDH7A1 deficiency increases the vulnerability to oxidative stress [32, 33]. Two patients with asphyxia were left with CP (Patients 9 and 13, Tables 1 and 2). Neonatal complications should never lead to negligence of considering PDE in infants with seizures.

4.3.4 Seizures, status epilepticus and EEG findings. Patients with mothers reporting abnormal intrauterine motor activity suggesting prenatal seizures (Table 1) had varying developmental outcomes ranging from normal (Patient 9) to moderate delay with uncontrolled seizures (Patient 5), suggesting that noticeable seizure onset in foetal life is not an invariable poor prognostic sign. Among the six patients who had one or more SE in the neonatal period, one died and two had moderate to severe neurodevelopmental delay/ID. In the eight patients exhibiting burst suppression patterns during the first days of life, the outcomes were also variable (Tables 1 and 2).

4.3.5 MRI findings. Brain imaging findings varied and sometimes raised the suspicion of alternative causes of epilepsy. In one child with moderate developmental delay, brain haemorrhages and venous thromboses were demonstrated in the neonatal period (Patient 5). The two patients diagnosed with CP (Patients 8 and 12) had extensive MRI abnormalities with substance loss (Table 2) [34, 35]. Several patients had corpus callosum abnormalities, as previously reported [11-13]. Expectedly, ventriculomegaly was accompanied by more severe developmental delay/ID, whereas a normal MRI signified a favourable cognitive outcome.

4.3.6 The sum of numerous factors. All these potential negative prognostic factors are dependent variables which in concert suggest a more severe brain dysfunction and cannot be considered alone. The outcome is apparently determined by additive effects of biochemical disturbances and perinatal complications, including structural brain abnormalities and prolonged seizures. Noteworthy, Patient 9 stands out with a favourable course and a normal developmental outcome in adult life. She had only minor neonatal problems, no SE and a prompt effect of the first PN administration.

4.4 PDE in adult life

A recent Dutch study described the clinical characteristics of a young adult cohort with PDE-ALDH7A1 comprising 10 patients aged 18-30 years, seven of whom carried homozygous c.1279G>C variants. The overall seizure control was good, but cognitive function varied considerably with one patient attending university. Noteworthy, four patients had hand tremor [36], which was not reported in our patients. However, in the present study, one patient developed psychotic symptoms with auditory hallucinations at the age of 32 years (Patient 12).

Importantly, PN treatment only compensates for the inactivation of the bioactive B6 vitamer and does not influence the underlying metabolic disruption, such as the accumulation of lysine degradation metabolites that are suggested to have a neurotoxic effect. A lysine-restricted diet is therefore recommended in PDE-ALDH7A1, and recent observational studies in children are promising [11, 18], but long-term studies are needed to evaluate any effect on adult cognitive outcome. According to available information in the present study, cognition appeared to be stable in adult life up to the age of 53 in one patient. Whether adult patients might benefit from dietary interventions, or if this effect is restricted to the developing brain should be further explored.

Seizure breakthroughs were mostly precipitated by intercurrent disease with fever. Their occurrence did not seem to be influenced by dietary treatment but appeared to decrease with age (Table 1). However, withdrawal of PN leads to the recurrence of seizures at all ages, and treatment should be lifelong. Life-threatening SE may occur when treatment is missed or cannot be ingested, such as in Patient 13 during gastroenteritis when the need for continuous PN was not acknowledged by treatment providers. First, this episode illustrates the danger of missed PN treatment; second, that awareness about PDE is needed in hospital departments treating SE and protracted seizures. Likewise, prolonged SE during pregnancy due to probable non-adherence has been reported [37]. Adults with PDE should carry emergency cards or amulets providing information about the necessity of continuous treatment. Noteworthy, the adult patient who turned out to have PNPO deficiency exhibited a striking vulnerability to PN non-adherence with seizures recurring soon after missed intake, in line with previous reports demonstrating a pronounced sensitivity to vitamer B6 dose and timing in this condition [38]. In contrast, the patients with pathogenic *ALDH7A1* variants experienced seizure recurrence several days after PN termination. This discrepancy might represent a phenotypic characteristic that could raise suspicion of PNPO deficiency in patients without genetic confirmation.

Patient 9 is a well-functioning woman of fertile age and needs pregnancy advice, an issue that has received little attention in the literature [22, 24, 39]. Guidelines concerning follow-up of women with PDE during gestation are called for.

4.5 Methodological issues

This study was conducted retrospectively and thus has limitations. We reviewed the medical records of available patients and did not have the opportunity to examine all patients in person. Developmental assessments were not standardized in all patients; in children, grading was based mostly on clinical judgement and the outcomes could not yet be finally determined for the youngest subjects. However,

thorough clinical notes from the early histories were available for all patients providing detailed insights on circumstances with potential impact on the outcome.

A strength of the study is its implementation in the context of the Norwegian National Health Service, which incorporates the entire population of the country. Close and transparent cooperation between the hospital-based specialist services in all geographical areas facilitates this type of national survey. However, by the applied screening methods for inclusion of patients directed towards the specialist services, some subjects with PDE may have been missed due to failure of reaching out to treating physicians, particularly adult patients with follow-up in primary care only.

5. Concluding remarks

According to this nationwide survey, the prevalence of identified subjects with PDE-ALDH7A1 was considerably higher among children compared to adults. This discrepancy may be due to increased mortality in childhood, a previous lower detection rate of the disease or shortcomings of the ascertainment procedure, particularly leaving older patients unavailable for the study. It should be acknowledged that an apparent response to PN also may occur in other seizure disorders, such as in other forms of vitamin B6-responsive epilepsies, including some cases of infantile spasms. Therefore, genetic testing is mandatory in all patients suspected to have PDE.

As in previous studies, the cognitive outcomes of patients with PDE-ALDH7A1 were variable, ranging from normal to severe deficits. Perinatal complications and early and recurrent SE appeared to influence neurodevelopment. Two patients were left with CP. The presence of alternative causes of epilepsy should never lead to the disregard of PDE in early-onset refractory epilepsy. No other clear predictors for emergent developmental delay/ID were identified, neither genetic nor clinical or therapeutic. The overall pathophysiological mechanisms contributing to the evolving phenotype remain unresolved and should be further explored.

Due to the recent emergence of more accurate diagnostic procedures, an increasing number of children with PDE will be identified, rendering a higher proportion of adult patients in the future. Hence, there is a need for increased awareness of this rare seizure disorder among adult neurologists. Knowledge about the course of PDE outside childhood has hitherto been scarce. In the present study, available information did not suggest cognitive deterioration with time, but this issue should be further studied by standardized procedures. Seizure breakthroughs in adults were uncommon and usually related to non-adherence to PN treatment. Life-threatening status epilepticus associated with discontinuation of PN occurred in one adult patient unable to convey the diagnosis when acutely admitted to hospital. It is extremely important that life-long treatment is maintained despite seizure freedom for decades.

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Table 1. Clinical, genetic and treatment characteristics of 13 patients with PDE-ALDH7A1 according to age at last follow-up.

Patient/ sex/ age at last follow-up	*ALDH7A1 (NM_001182.4)*	*Foetal seizure activity*	*Neonatal complications*	*Seizure onset age*	*Age at, and effect from first PN injection*	*Onset of continuous PN treatment*	*Dietary intervention[a]*	*Breakthrough seizures*	*Status epilepticus*	*Current PN dose (mg)*	*Neurodevelopment*
1 F/2 mo	c.1279G>C, p.(Glu427Gln) homozygous	+	Lactic acidosis/respiratory distress/ respiratory support	1 h	2 h, effect within 1 h	1 d	From 2 mo	None	No	140	Early development unremarkable
2 M/ 3 mo	c.1279 G>C, p.(Glu427Gln) homozygous	+	Mild asphyxia/ lactic acidosis/ respiratory support	1 h	2 d when seizure free	2 d	From 2 mo	None	No	156	Early hypotonia
3 M/10 mo at death	Likely same as the younger sibling, Patient 10	+	Asphyxia/ respiratory support/ Meconium-stained AF	3 h	Not evident at age 2.5 mo	NA	NA	NA	Several - leading to death	NA	Severe delay
4 F/18 mo	c.1008+1 G>A homozygous	NR	Acidosis/ intracranial haemorrhage	1 h	6 d, effect within 1 d	6 d	From 3 w	Febrile 11 mo, 18 mo, afebrile 16 mo	No	300	Early mild delay, later normal
5 F/ 2 y, 3 mo	c.1279 G>C, p.(Glu427Gln) homozygous	+	Asphyxia/ brain haemorrhage/ respiratory support	22 h	NA (Peroral PLP from 18 d; PN from 24 d)	24 d	From 3 mo	Febrile (2 mo)/ afebrile (1 y 9 mo)	15 d/2 mo	300	Moderate delay
6 M/ 3 y, 3 mo	c.1279 G>C, p.(Glu427Gln) homozygous	NR	Meconium-stained AF/ lactic acidosis/ respiratory support	12 h	2 d, effect within 1 day	4 d	From 2 mo	Febrile/gastroenteritis/non-adherence (3, 7, 9, 18 mo)	4 d/18 mo	264	Moderate language delay
7 M/ 3 y, 4 mo	c.1279 G>C, p.(Glu427Gln); c.834 G>A, p.(Val 278 =)	NR	Lactic acidosis/ respiratory support	4 d	5 d, uncertain effect	1 mo	From 5 w	None	4 d	324	Early mild delay, later normal

8 M/ 16 y, 9 mo	c.1279 G>C, p.(Glu427Gln); c.(650 +244_650 + 254)_(695 +951_695 +962)del	+	Severe asphyxia at seizure onset/ respiratory support	6 d	8 d, uncertain effect	42 d	From 10 y	Sporadic during childhood (febrile/ afebrile)	Several subtle episodes 8–30 d	420	Severe delay/ severe dyskinetic quadriplegia
9 F/ 25 y	c.1279 G>C, p.(Glu427Gln) homozygous	+	Meconium-stained AF/ acidosis/ pneumomediastinum/ respiratory support	6 h	9 d, prompt effect	12 d	No	Febrile (1 y)	No	320	Normal
10 F/ 25 y	c.1513 G>C, p.(Gly505Arg) homozygous	NR	Respiratory distress/ Asphyxia/ pneumomediastinum/ respiratory support	1 d	3 d, prompt effect	12 d	No	Febrile (1 and 8 y)	No	500	Moderate ID
11 M/26 y	c.1279 G>C, p.(Glu427Gln); c.(650 +244_650 + 254)_(695 +951_695 +962)del	Denied	Acidosis/ pneumothorax/ respiratory support	2 h	2 d, prompt effect	10 d	No	Febrile (1 y)	No	170	Mild ID
12 F/ 32 y	c.1279 G>C, p.(Glu427Gln); c.1513 G>C, p.(Gly505Arg)	NR	Asphyxia/ respiratory support	2 d	11 d, prompt effect	17 d	No	Intercurrent illness (1.5 and 4 y); gastroenteritis (24 y)	11 d	40	Mild ID/ mild hemiplegic CP
13 M/53 y	c.1279 G>C, p.(Glu427Gln) homozygous	NR	Lactic acidosis/ Sepsis/ respiratory support	4 h	2 d, prompt effect	5 d	No	Sporadic during childhood (fever, vomiting, diarrhoea)	1.5 y/28 y (gastroenteritis, PN withdrawal)	240	Mild ID

PN, pyridoxine; M, male; mo, month; h, hour; NR, not reported; NA, not applicable; F, female: d, day; y, year; w, week; PLP, pyridoxal-5′-phosphate; AF, amniotic fluid; ID, intellectual disability; CP, cerebral palsy

[a)] Lysine restriction, arginine supplementation

Table 2. EEG within the first year of life and brain MRI findings in 13 patients with PDE-ALDH7A1.

Patient	*EEG within the first year*	*Main MRI findings*
1	• Side-shifting sharp potentials (2 d)	• Scattered surface blood and small haemorrhages posteriorly. Possibly thin corpus callosum. Enlarged cisterna magna. Periventricular and caudothalamic groove cysts. Deep medullary vein thrombosis/stasis (3d)
2	• Bilateral/side-shifting sharp potentials and seizure activity, episodic background attenuation (1 d) • Bilateral rhythmic activity, episodic background attenuation (2 d) • Normal (10 d)	• Intracerebral haemorrhage L frontal lobe. Intraventricular haemorrhage L anterior horn. Bilateral occipital micro-haemorrhages and deep medullary vein thrombosis (1 d) • Additional diffusion restriction R occipital lobe, periventricular and L frontal lobe (2d)
3	• Burst suppression (3, 16 d) • Bursts of slow activity (27 d) • Burst suppression (1, 2.5; 10 mo)	• MRI not performed (CT: ischaemic changes both hemispheres, 1 mo; brain substance loss and ventriculomegaly, 3.5 mo)
4	• Burst suppression (2, 6 d) • EA, L temporo-occipital (13 d) • Normal (2.5 mo) • EA temporo-central-parietal (3 mo)	• Surface cerebellar micro-haemorrhages (5 d) • Normal (3 mo)
5	• Burst suppression (SE) (1, 2 d) • Multifocal/ bilateral EA (9, 13, 21 27; 1 mo) • Normal (7 mo)	• Intraventricular and cerebellar haemorrhages, perimedullary venous thrombosis, corpus callosum hypoplasia (2 d). • Small haemorrhages in basal temporal lobes, thrombus in sinus rectus, wide ventricles and prepontine and infratemporal arachnoid cysts (15d) • Substance loss corresponding to haemorrhagic lesions; large cisterna magna (3, 5 mo)
6	• Burst suppression (1, 3 d) • Slow background (7, 14 d, 4; 9 mo) • Normal (11 mo)	• Normal (4 d)
7	• Burst suppression (4, 5, 6 d) • Normal (8 d) • Bilateral async EA (18, 19 d) • Normal (23, 29, 32 d) • EA R frontal /bilateral (1 mo)	• Normal (5, 23 d)
8	• EA L hemisphere (8, 14 d) • EA L occipital (16, 20 d) • EA (27 d) • EA (SE, 1 mo) • Normal (2 mo) • Bilateral EA, slowing (11 mo)	• Corpus callosum hypoplasia, wide cisterna magna (1.5 mo) • White matter substance loss, poor myelinization, (10.5 mo) • Ventriculomegaly, sparse white matter, thin cortex and corpus callosum, absent supra tentorial myelinization (1 y)
9	• Burst suppression (2, 6, 9 d) • EA L frontotemporal/slow background (12, 17 d) • Bilateral asynchronous EA (21 d) • Slow background (1, 1.5 mo) • Normal (6 mo) • Bilateral asynchronous EA, temporal (9 mo) • Normal (10 mo)	• Normal (13 d) • Thin isthmus corpus callosum (22 y)
10	• Burst suppression (2 d) • EA (3 d) • Normal (5 d)	• Corpus callosum hypoplasia and low brain volume (5 y)

11	• Multifocal EA (1 d) • Multifocal EA (7, 16 d) • High amplitude slow background (10 mo)	• Large cisterna magna (4.5 y)
12	• Normal (7 d) • Burst suppression (10 d) • Normal (1, 1,5, 6)	• Periventricular white matter lesions and ventriculomegaly (18, 24 y)
13	• Alternating bilateral high amplitude delta slow wave (15 d) • Normal (3 mo) • Slow activity L temporo-occipital area (7 mo) • Slow background (9 mo)	• Normal (47 y)

d, day; mo, month; y, year; CT, computerised tomography; EA, epileptiform activity; SE, status epilepticus; L, left; R, right.