

# The genotypic spectrum of *ALDH7A1* mutations resulting in pyridoxine dependent epilepsy: A common epileptic encephalopathy

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## Abstract

Pyridoxine dependent epilepsy (PDE) is a treatable epileptic encephalopathy characterized by a positive response to pharmacologic doses of pyridoxine. Despite seizure control, at least 75% of individuals have intellectual disability and developmental delay. Current treatment paradigms have resulted in improved cognitive outcomes emphasizing the importance of an early diagnosis. As genetic testing is increasingly accepted as first tier testing for epileptic encephalopathies, we aimed to provide a comprehensive overview of *ALDH7A1* mutations that cause PDE. The genotypes, ethnic origin and reported gender was collected from 185 subjects with a diagnosis of PDE. The population frequency for the variants in this report and the existing literature were reviewed in the Genome Aggregation Database (gnomAD). Novel variants identified in population databases were also evaluated through in silico prediction software and select variants were over-expressed in an *E.coli*-based expression system to measure  $\alpha$ -amino adipic semialdehyde dehydrogenase activity and production of  $\alpha$ -amino adipic acid. This study adds 47 novel variants to the literature resulting in a total of 165 reported pathogenic variants. Based on this report, in silico predictions, and general population data, we estimate an incidence of approximately 1:64,352 live births. This report provides a comprehensive overview of known *ALDH7A1* mutations that cause PDE, and suggests that PDE may be more common than initially estimated. Due to the relative high frequency of the disease, the likelihood of under-diagnosis given the wide clinical spectrum and limited awareness among clinicians as well as the cognitive improvement noted with early treatment, newborn screening for PDE may be warranted.

## KEYWORDS

*ALDH7A1*,  $\alpha$  amino adipic semialdehyde, PDE, pyridoxine dependent epilepsy

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## 1 | INTRODUCTION

Pyridoxine dependent epilepsy (PDE; OMIM 266100) is a treatable epileptic encephalopathy characterized by a positive response to pharmacologic doses of pyridoxine.<sup>1,2</sup> The classic presentation of PDE is neonatal onset of treatment refractory seizures, with dramatic clinical and electroencephalogram (EEG) improvement following pyridoxine supplementation. The majority of patients are able to achieve adequate seizure control with pyridoxine alone, yet 75% of individuals with PDE have significant intellectual disability (ID) and developmental delay (DD).<sup>3,4</sup> Current treatment paradigms aim to reduce putative neurotoxic metabolites through a lysine restricted diet and competitive inhibition of lysine transport.<sup>5,6</sup> These therapies have been associated with improved neurologic outcomes, although optimal development is dependent on an early diagnosis and initiation of treatment in the first year of life.<sup>5,7</sup>

PDE results from a deficiency of  $\alpha$ -amino adipic semialdehyde ( $\alpha$ -AASA) dehydrogenase (E.C. 1.2.1.3), a key enzyme in lysine metabolism.<sup>8</sup>  $\alpha$ -AASA dehydrogenase deficiency results in the pathognomonic accumulation of  $\alpha$ -AASA and the cyclic equivalent  $\Delta^1$ -piperidine-6 carboxylate ( $\Delta^1$ -P6C). The accumulated  $\Delta^1$ -P6C is reported to sequester the active form of vitamin B<sub>6</sub> (pyridoxal 5'-phosphate, PLP) by forming a Knoevenagel condensation product.<sup>8</sup> The therapeutic strategy of pyridoxine supplementation is to overcome the secondary depletion of PLP. The degree of ID/DD in PDE does not correlate with seizure control or age at which pyridoxine treatment is initiated. Pyridoxine supplementation alone does not treat the underlying defect of lysine oxidation, and treatment with a lysine restricted diet<sup>6</sup> and with a lysine transport inhibitor (arginine)<sup>5,9</sup> has resulted in an improved cognitive outcome. Therefore, it is important to differentiate PDE from the other causes of pyridoxine responsive seizures, such as pyridoxamine 5'-phosphate oxidase deficiency and mutations in *PROSC*, where the recommended treatment is limited to be appropriate vitamin B<sub>6</sub> vitamin.

The enzyme  $\alpha$ -AASA dehydrogenase is encoded by *ALDH7A1* (NM\_001182), and the majority of patients with PDE have biallelic mutations consistent with autosomal recessive inheritance.<sup>8</sup> *ALDH7A1* is located at 5q23.2 and contains a transcript of 4,964 base pairs encoding a protein of 539 amino acids. A number of publications have described *ALDH7A1* mutations in cohorts of patients with PDE,<sup>10–13</sup>

although a majority of published mutations are reported in small case series or single case reports. As a result, it is difficult to determine whether each genotype is unique to an individual patient limiting the ability to estimate mutation frequency. The c.1279G>C, p.Glu427Gln (historical name: p.Glu399Gln) mutation is the most commonly reported pathogenic variant in the literature, and is suggested to be present in 30% of patients with European ancestry.<sup>11,12</sup> A small number of patients were reported to have a single pathogenic variant identified,<sup>10,12</sup> and the majority of these patients were later identified to have an intragenic copy number variations (CNVs) within *ALDH7A1*.<sup>14</sup>

Genetic testing, either through specific gene panels or genomic sequencing, is increasingly accepted as first tier testing for epileptic encephalopathies, even when pathognomonic biomarkers exist. Genetic testing is a powerful diagnostic tool and limits the need for clinical suspicion of PDE and targeted testing. At the same time, the presence of variants of uncertain significance often complicates the interpretation of genetic tests. In order to provide a comprehensive overview of *ALDH7A1* mutations that are known to result in PDE, we compiled results from four clinical laboratories, an international patient registry and a review of the literature. Furthermore, we utilized this comprehensive review to estimate the carrier frequency of *ALDH7A1* variants in a general population database, which allowed us to estimate the incidence of this treatable disease.

Here we report 369 *ALDH7A1* variants in 185 individuals with the clinical diagnosis of PDE. Including the 47 novel variants in this report, there are now 165 pathogenic variants reported in the literature.

## 2 | METHODS

### 2.1 | Literature review

A systematic literature search was performed with the search criteria of pyridoxine dependent epilepsy, pyridoxine responsive epilepsy, pyridoxine dependent seizures, pyridoxine responsive seizures, antiquitin deficiency,  $\alpha$ -amino adipic semialdehyde dehydrogenase deficiency and *ALDH7A1*. All publications were reviewed for articles that contained clinical data on patients with PDE. PDE due to antiquitin deficiency was defined by either the elevation of  $\alpha$ -AASA or  $\Delta^1$ -P6C or biallelic mutations in *ALDH7A1*.

Publications that contained molecular data on patients with PDE were retained and systematically classified.

## 2.2 | Subject and variant identification

Subjects were identified through clinical genetics laboratories that perform clinical testing of *ALDH7A1*, the international registry for pyridoxine dependent epilepsy ([www.pdeonline.org](http://www.pdeonline.org)) and the pyridoxine dependent seizures patient registry.<sup>3</sup> Retrospective data was collected with ethics approval from each main site: University of Colorado, COMIRB 16–1325, COMIRB 16–0434 and Seattle Children's Research Institute IRB10863 and 15450, and deidentified laboratory data was collected in agreement with the rules of each local ethics committee. Inclusion criteria included any subject with a clinical suspicion of PDE and at least one pathogenic variant identified in *ALDH7A1*. Genetic testing results and reported gender were collected when available. Sequence variants in both the reported subjects and the literature were compared to the sequence of *ALDH7A1* (GenBank NM\_001182.4, Ensembl ENST00000409134). Coding nomenclature was corrected using the "A" of the ATG translation initiation codon as position number 1, as opposed to historic nomenclature which had failed to include the mitochondrial leader peptide.

## 2.3 | Population analysis

The minor allele frequency (MAF) was recorded as reported in the Genome Aggregation Database (gnomAD) (last accessed 10 November 2017). The gnomAD browser ([gnomad.broadinstitute.org](http://gnomad.broadinstitute.org)) includes 123,126 exome sequences and 15,496 whole-genome sequences from unrelated individuals. The MAF of all variants identified through the systematic literature review, and reported in this study, were recorded when available. All heterozygote frameshift, canonical splice-acceptor or splice-donor, and nonsense variants were presumed to be pathogenic. All single nucleotide variants (SNVs) were further evaluated to assess pathogenicity. Variants were defined as rare based upon a  $MAF \leq 3.34 \times 10^{-4}$ , which is the MAF of the common p.Glu427Gln mutation. Rare SNVs were further evaluated with in silico analysis tools: Protein Variation Effect Analyzer (PROVEAN; <http://provean.jcvi.org>), Sorting Intolerant From Tolerant (SIFT; <http://sift.jcvi.org>), and Polymorphism Phenotyping version 2.1.0 (Polyphen-2; <http://genetics.bwh.harvard.edu/pph2>).

## 2.4 | Expression analysis

An *ALDH7A1* gene construct (containing the complete open reading frame of human *ALDH7A1*) was cloned into the

pET15b expression vector as previously described, and was the generous gift of Dr. Coulter-Mackie and colleagues.<sup>15</sup> In vitro mutagenesis was done with the Qiagen Quikchange II XL kit (Agilent) and the sequence verified by Sanger sequencing. This expression vector was transformed into BL21 Star™ (DE3) *E. coli* (ThermoFisher). Cultures were grown overnight in LB media at 37 °C and aniquitin expression was induced by the addition of 1.0 mM IPTG. Cells were harvested after 3 hours, snap frozen in liquid nitrogen and stored at -80°C. Enzymatic conversion of P6C to AAA was carried out using the previously published aniquitin activity assay using bacterial cell lysate combined with 2.2 mM NAD<sup>+</sup> and 0.6 mM P6CH in a total volume of 1.0 mL at 37°C following the production of NADH at 320 nm in a Cary 300 spectrophotometer.<sup>15,16</sup> After 5 minutes, 500 µL of the reaction mixture was combined with 100 µL of glacial acetic acid and analyzed by LC-MS/MS methods.

## 3 | RESULTS

### 3.1 | Literature review

We retrieved 118 unique variants in *ALDH7A1* reported in 62 publications (Table S1). The frequency of each pathogenic variant was not recorded as the authors could not ensure that a patient was unique to each report. The majority of pathogenic variants (49% of all reported variants) were SNVs that result in single amino acid substitutions (i.e. missense mutations). A high number of missense mutations were identified in exons 15 (25%), 10 (16%), 6 (12%) and 5 (11%) compared to exons 1,2,3,7,8 and 11 where no missense mutations were reported. Other pathogenic variants reported in the literature included variants at the intron/exon boundary (splice site mutations) (21%), insertion–deletion (InDel) mutations (16%), SNVs that result in premature stop codons (nonsense mutations) (7%), CNVs (6%) and SNVs not at the boundary that still result in splicing errors (2%).

### 3.2 | Subjects identified from clinical laboratories and the PDE registry

Molecular genetic results were available for 185 subjects and gender was reported in 139 subjects with 54% of subjects reported to be female. Biallelic mutations were identified in 182 of 185 (98%) subjects (Table S2). All mutation types were identified in this cohort and were similar to those reported in the literature. This study allowed for the evaluation of the frequency of pathogenic variants. Missense mutations were the most frequently identified variant and were present in 57% of all alleles (Figure S1). The p.Glu427Gln was the most common variant identified and was present in

25.6% of all alleles, and the p.Gly505Arg missense mutation was identified in 3.5% of all alleles. The synonymous variant p.Val278Val (historical nomenclature: c.750G>A, r.748\_787del) results in a cryptic splice site,<sup>17</sup> and was the second most common recurrent mutation noted in 5.4% of all disease alleles. Despite the presence of these recurrent variants, the majority of missense mutations were private or familial. In fact, 32 of the 49 missense mutations reported in this study were only identified in a single individual. Splice acceptor and splice donor variants were the second most common mutation type present in 18% of all alleles. The c.1566-1G>C variant was identified in 3.5% of all alleles.

### 3.3 | Population analysis

A total of 60 variants were identified in gnomAD which were either previously reported in the literature or reported in this study. These reported variants had an allele frequency of 2.11E-03. There were 23 novel, protein-truncating variants and 9 novel splice acceptor/donor variants, which were assumed to be pathogenic with an allele frequency of 3.18E-04 and 3.66E-05 respectively. A total of 240 rare (MAF  $\leq$  3.34E-04) novel SNVs were identified; 117 of these SNVs were predicted to be deleterious by at least two in silico programs with an allele frequency of 1.45E-03 (Table S3). Based on the gnomAD data alone, the MAF was calculated at 0.00394. Using the Hardy–Weinberg principle, the estimated carrier frequency of *ALDH7A1* mutations is 1:127 and the estimated incidence of  $\alpha$ -AASA dehydrogenase deficiency is 1:64,352 births.

### 3.4 | Expression analysis

A total of 10 variants were selected for  $\alpha$ -AASADH activity and AAA production. The p.Glu427Gln and p.Cys478Ser variants were selected as positive controls as they have been previously reported to have 0 and 19% of wild type enzyme activity,<sup>16</sup> and in our laboratory had 0.3% (SD  $\pm$ 0.2) and 14%; (SD  $\pm$ 1.5) of wild type activity respectively (Figure 1). Four groups of variants were also selected to represent various aspects of this study including variants previously reported in the literature (p.Gly274Glu, p.Ser317Leu) and novel variants reported in this study (p.Arg122Trp in subject PDE-51 and p.Tyr516Cys in subjects PDE-19, 76 and 87). Rare SNVs identified from gnomAD were also selected including variants predicted to be deleterious by in silico tools (p.His339Arg, p.Ala533Thr), and variants not predicted to be deleterious by in silico tools (p.Thr68Asn, p.Phe337Cys). In these 10 variants, the residual  $\alpha$ -AASADH activity as measured by NAD reduction rate correlated closely with the amount of AAA production ( $R^2 = 0.9673$ ), and both provided an equivalent measure of relative enzyme

activity. Enzyme activity was quantified for all 10 selected variants and enzyme activity ranged from 0.34% to 89.8% of wild type activity (Figure 1).

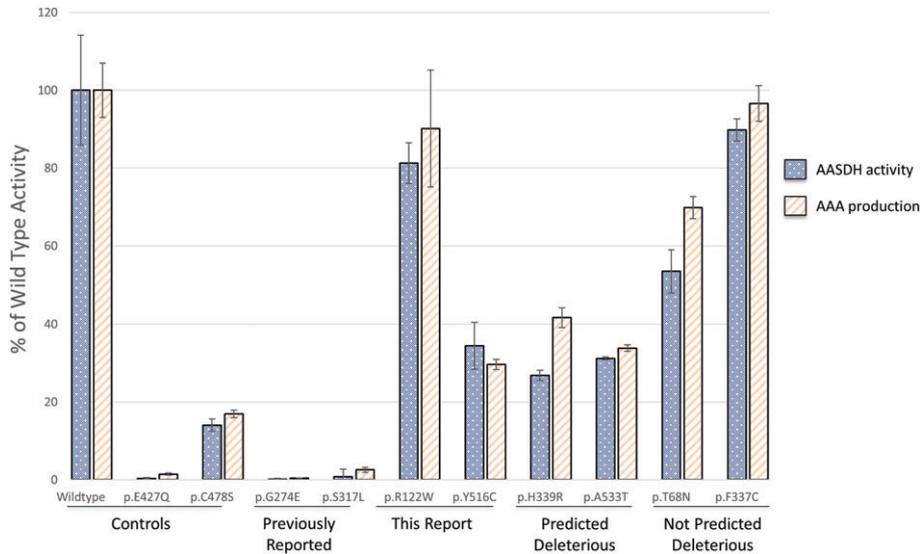
## 4 | DISCUSSION

Herein we report a comprehensive review of *ALDH7A1* variants identified in patients with clinical or biochemical suspicion or diagnosis of  $\alpha$ -AASA dehydrogenase deficiency. Genetic testing identified biallelic pathogenic variants in 98% of subjects, although biochemical testing using pathognomonic biochemical markers of  $\alpha$ -AASA and  $\Delta^1$ -P6C often complements these studies. This result suggests that genetic testing is a reliable method for the diagnosis of  $\alpha$ -AASA dehydrogenase deficiency, but eight subjects (4.3%) had at least one CNV present, including one subject that was compound heterozygous for two CNVs. Thus, CNV analysis is important when only one pathogenic variant is identified, or in the case of a high clinical suspicion of PDE, even if no variants are identified through traditional sequencing methods. And, in cases with high clinical suspicion, mRNA studies<sup>17</sup> and testing for pyridoxine dependent seizure disorders should also be considered.<sup>18,19</sup> This study did not differentiate subjects in whom molecular testing was performed first as opposed to molecular testing performed after a diagnostic biochemical result.

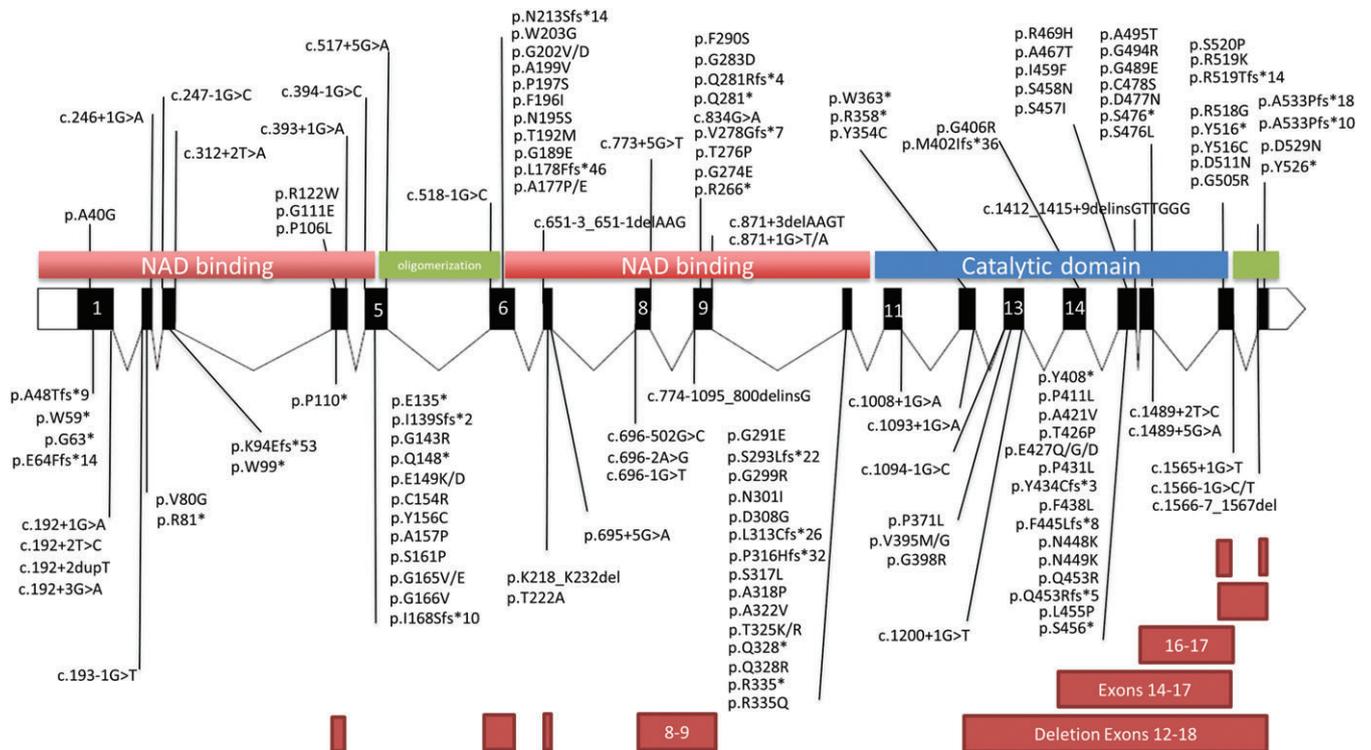
SNVs resulting in single amino acid substitutions were the most common variant type reported in the literature (49% of unique variants reported) and in this cohort (57% of all disease-causing alleles). The p.Glu427Gln variant was the single most common pathogenic variant identified and 32% of all subjects were at least heterozygous for this variant. Ethnicity was not available for the majority of our subjects, but we assume that a significant proportion of our cohort is of European ancestry, where this variant is reported to be present in 30% of patients.<sup>11,12</sup> Although a number of recurrent variants were identified, twenty-percent of our cohort had at least one private missense mutation. Determining the pathogenic nature of a novel SNV can be difficult, and this report highlights a few strategies for evaluating novel variants in *ALDH7A1*.

First, the location of the SNV in the gene structure appears relevant. Although pathogenic variants were identified throughout the gene, a significant number of unique variants were identified within exon 15, similar to previous reports that the majority of pathogenic variants occur in exon 15 within the catalytic domain of *ALDH7A1* (Figure 2). Missense mutations are equally prominent within exons 5 and 6 within the linker domain and exon 10 within the NAD binding domain of *ALDH7A1*. The paucity of missense mutations within exons 3,7,8 and 11 is noteworthy.

Functional studies are often utilized to evaluate the effect of novel variants, and a number of *ALDH7A1* variants have previously been evaluated in an *E.coli* based expression



**FIGURE 1** AASADH activity of *ALDH7A1* variants. Legend: The human *ALDH7A1* open reading frame was cloned into an *E.coli*-based expression vector. The enzyme activity of 10 expressed variants is shown for  $\alpha$ -AASD dehydrogenase activity as reduction of NAD (dotted bar) and as AAA production (lined bar), each represented as % of the wild type, with standard deviation provided. The variants expressed included negative and positive controls (Controls), variants previously reported in the literature (Previously Reported), variants identified in subjects within this report (This report), rare variants identified in gnomAD and predicted to be pathogenic (Predicted Deleterious) and rare variants identified in gnomAD and not predicted to be pathogenic (Not Predicted Deleterious)



**FIGURE 2** *ALDH7A1* pathogenic variants. Legend: The *ALDH7A1* gene is represented as a schematic with exons to scale and introns at 1/10th scale. Placement of predicted protein changes are denoted by solid black line. CNVs are denoted by rectangular boxes

system (Table 1). In this *E.coli* based system, decreased AAA production correlated closely with decreased enzyme activity by NAD reduction. In our laboratory, previously reported pathogenic variants had <14% of wild type activity consistent

with the previous experiments noted in the literature. Rare variants that were not predicted to be disease causing also had significant residual enzyme activity (>53% of wild type activity) supporting the use of the in silico prediction programs,

**TABLE 1** Variants in *ALDH7A1* and in vitro expression analysis

Codon	Predicted Protein Change	Activity of $\alpha$ -AASADH <sup>1</sup>	Expression at 30o <sup>2</sup> (nmol NADH/min/mg ATQ)	Expression at 37o <sup>2</sup> (nmol NADH/min/mg ATQ)	References
	p.Thr68Asn			53.5%	This report
c.328C>T	p.Arg110*	0%			8
c.332G>A	p.Gly111Glu	0%			15
	p.Arg122Trp			81.2%	This report
c.469G>C	p.Ala157Pro	0%			32
c.494G>T	p.Gln165Val	0%			32
c.497G>T	p.Gln166Val	0%			32
c.530C>A	p.Ala177Glu	0%			32
c.596C>T	p.Ala199Val	0%			( <sup>8,15</sup> )
c.607T>G	p.Trp203Gly	0%			15
c.821G>A	p.Gly274Glu			0.22%	This report
c.848G>A	p.Gly283Asp	0%			32
c.872G>A	p.Gly291Glu	0%			32
c.902A>T	p.Asn301Ile	0%			15
c.950C>T	p.Ser317Leu			0.80%	This report
c.974C>A	p.Thr325Lys	0%			15
c.974C>G	p.Thr325Arg	0%			15
c.1004G>A	p.Arg335Gln	0%			15
	p.Phe337Cys			89.8%	This report
	p.His339Arg			26.8%	This report
c.1184T>G	p.Val395Gly		64%	12%	16
c.1224T>G	p.Tyr408*	1.8%			8
c.1314C>A	p.Phe438Leu		80%	13%	16
c.1279G>C	p.Glu427Gln	0%			( <sup>8,15</sup> )
c.1280A>G	p.Glu427Gly	0%			15
c.1373G>A	p.Ser458Asn	0%			15
c.1375A>T	p.Ile459Phe	0%			15
c.1432T>A	p.Cys478Ser		27%	19%	16
c.1513G>C	p.Gly505Arg	0%			15
	p.Tyr516Cys			34.4%	This report
	p.Ala533Thr			31.2%	This report
c.1596delG	p.Ala533Profs*	0%			8

<sup>1</sup>Represented as a % of wild type activity; <sup>2</sup>Represented as a % of wild type activity calculated from the mean specific activity.

however in other diseases discrepancies have been reported.<sup>20</sup> Some findings were surprising. For instance, the p.Arg122Trp variant had the second highest enzyme activity of any SNV studied at 81% (SD  $\pm$  5.2) of wild type. This variant was identified in a subject (PDE-51) who was compound heterozygous with the p.Gly63\* allele (Table S2). The p.Arg122Trp variant is relatively rare with a MAF of 9.02E-05, which is similar to well-reported variants such as p.Gly505Arg. Based on this enzyme activity, more studies are needed to determine whether this SNV is indeed a pathogenic variant.

For the  $\alpha$ -AASA enzyme to be functional, the protein must have enzyme activity (which was analyzed in this study) but the protein must also be stable in its natural environment (mitochondria in eukaryotic cells) and must reach its targeted environment (the mitochondrion). Overexpression in *E. coli* only interrogates a single function, and thus may miss other functions that are equally relevant, and which may well explain the discrepancy between the observed high residual activity of 81% on this study and the proposed pathogenic nature of the mutations. Many variants affect more than one

functionality with the ultimate impact being a sum of all required functionalities. This also highlights the difficulty in a genetic-first approach to diagnosis. Fortunately, for PDE caused by  $\alpha$ -AASA dehydrogenase deficiency, diagnostic biochemical testing is available in body fluids with the determination of biomarkers such as  $\alpha$ -AASA or  $\Delta^1$ -P6C. Although these biomarkers appear excellent, the sensitivity and specificity of biochemical testing still needs to be determined in a sufficiently large study. The combination of clinical findings, molecular genetic testing, and biochemical testing allows clinicians and researchers to be certain of the diagnosis with a high degree of confidence.

Functional studies have been proposed as a primary mechanism to assign pathogenicity of novel variants in general, and high throughput screening of very large numbers of novel genetics variants has been proposed.<sup>21</sup> However, this study highlights some of the difficulty associated when only one type of functional study is performed. Moreover, a challenge in functional studies is to determine what level of impairment of the functionality will be sufficient for a variant to be pathogenic, which, thus far, has not been sufficiently determined in PDE. Evidence from other inborn errors of metabolism suggests that if a sufficient number of mutations are interrogated, a complex gradation was identified and defining a sharp cut-off was often difficult or even impossible.

**Population incidence of PDE:** Previous studies have attempted to utilize population databases, such as the ExAC database, in order to estimate a disease incidence for autosomal recessive and X-linked recessive disorders. This has been most successful when a large number of pathogenic variants are known a priori.<sup>22</sup> We expanded the limited information on pathogenic variants in *ALDH7A1* from the literature of case reports and small case series in this large cohort, but the number of variants identified remains limited in comparison to those identified in the gnomAD database. In order to provide a more comprehensive estimate of disease incidence, we further evaluated rare SNVs noted within gnomAD using computational (in silico) predictive programs as one piece of evidence to evaluate novel variants.<sup>23</sup> We attempted to apply the same rigor to variants identified in the general population as is used in the context of clinical testing. We first eliminated any SNVs that were either present in homozygous state or had a significantly high prevalence in the general population. We further filtered novel SNVs though in silico prediction programs, resulting in a

blend of known, pathogenic variants and predicted pathogenic variants described above. Using this approach, we identified 212 variants within the gnomAD database. Based upon these results and the Hardy–Weinberg principle, we were able to estimate the incidence of  $\alpha$ -AASA dehydrogenase deficiency at 1:64352 births, with incidences in specific subpopulations (Table 2).

This estimate of disease incidence relies on in silico prediction programs. It does not include the carrier frequency of CNVs, which is a known, but infrequent, disease mechanism, or of non-coding mutations that would impact *ALDH7A1* expression. Previous estimates of the incidence of PDE based on clinical diagnosis have varied widely between 1:20,000 in a single center in Germany,<sup>24</sup> 1:396,000 in the Netherlands,<sup>25</sup> and 1:783,000 in the United Kingdom.<sup>26</sup> These previous studies only included patients who responded to a pyridoxine trial, often provided as a single dose, as the underlying genetic and biochemical cause had yet to be identified. Since then, multiple limitations of such approaches have been identified including the recognition that EEG improvement following a pyridoxine trial is neither sensitive nor specific<sup>27</sup>, seizures may not occur until childhood, patients may have delayed or incomplete response to pyridoxine, and patients may present with atypical symptoms.<sup>11,28,29</sup> As a result, if a therapeutic pyridoxine trial is relied upon as the sole diagnostic test, it is likely that a significant number of patients will remain undiagnosed. Indeed, our estimates imply that the incidence is 6 to 12 times higher than that recognized on clinical grounds by a pyridoxine trial. Given this substantial under ascertainment by clinical diagnosis, the treatable nature of PDE, and the improved outcome with early treatment, newborn screening for this disorder should be considered.<sup>30</sup> Future studies will be required to see if a genomic or a biochemical approach to newborn screening is preferred.

Finally, previous population based studies of PDE have focused on European populations. The ethnicity data present in gnomAD allowed a further preliminary analysis of other populations, even though European subjects remained the largest population present in gnomAD at 44% of all subjects. When we further stratified our findings based on the reported ethnicity in gnomAD, we noted a higher carrier frequency (1:66) and therefore estimated incidence (1:16,556) of PDE in individuals reported to be of East Asian ancestry (Table 2). Unfortunately, the number of individuals of East Asian descent in gnomAD is still limited to only 16,556

**TABLE 2** Estimated incidence of PDE based upon purported pathogenic variants in gnomAD

Ancestry	European							
	East Asian	European (Finnish)	African	Non-(Finnish)	Latino	South Asian	Other	Ashkenazi Jewish
<b>Carrier frequency</b>	1:66	1:123	1:128	1:134	1:143	1:147	1:160	1:1,231
<b>Incidence</b>	1:16,556	1:60,303	1:65,474	1:71,659	1:81,427	1:86,299	1:101,807	1:6,058,365

alleles. Despite this limitation, it may highlight the need for population-targeted testing in populations outside those of European ancestry.

Although it is likely that a significant number of affected patients remain undiagnosed, there may be other reasons for the discrepancy between the estimated incidence of PDE and previous clinical observations. Most notably, the rate of intrauterine demise and neonatal mortality for PDE has not been established. The estimated disease incidence is also limited by current *in silico* tools used to evaluate many of the variants identified in the general population.<sup>31</sup> In order to overcome this limitation, each variant was assessed based on the MAF and with multiple *in silico* tools before determining the pathogenic nature of the variant. Population-based studies are required to confirm the incidence of PDE.

## 5 | CONCLUSION

Even in a disorder with well-defined biochemical markers, genetic diagnosis (i.e. sequencing analysis) is common in PDE. A genetic-first approach to inherited disorders is often challenged by determining the pathogenic nature of novel variants. This report provides a comprehensive review of *ALDH7A1* variants, which should aid academic and commercial molecular diagnostic laboratories. Using population based genomic sequencing databases we estimated the frequency of  $\alpha$ -AASA dehydrogenase deficiency at approximately 1:64,000 live births.

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## DETAILS OF THE CONTRIBUTION OF INDIVIDUAL AUTHORS

C Coughlin and JLK Van Hove conceived of study design, reviewed data and drafted the manuscript. MA Swanson, E Spector, NJL Meeks, KE Kronquist, M Aslamy, MF Wempe, CDM van Karnebeek, SM Gospe Jr, VG Aziz, PL Nagy, K Hyland, SJM van Dooren, GS Salomons produced, reviewed

and interpreted laboratory data. All authors critically reviewed and contributed to the final manuscript and agreed to be accountable for the final version of the manuscript.

## COMPLIANCE WITH ETHICAL STANDARD

### CONFLICT OF INTEREST

Coughlin II, M.A. Swanson, E. Spector, NJL Meeks, KE Kronquist, M Aslamy, MF Wempe, CDM van Karnebeek, SM Gospe Jr, VG Aziz, PL Nagy, K Hyland, SJM van Dooren, GS Salomons and JLK Van Hove declare that they have no conflict of interest. H Gao and B Tsai are both employees and stockholders of Fulgent Genetics, a DNA sequencing laboratory. There is no apparent financial gain or loss expected as a result of this study.

### DETAILS OF ETHICS APPROVAL

Retrospective data was collected with ethics approval from each main site: University of Colorado, COMIRB 16-1325, COMIRB 16-0434 and Seattle Children's Research Institute IRB10863 and 15450, and deidentified laboratory data was collected in agreement with the rules of each local ethics committee.

### A PATIENT CONSENT STATEMENT

Individual written informed consent was obtained from patients (or parents/guardians) for registry studies.

### DOCUMENTATION OF APPROVAL FROM THE INSTITUTIONAL COMMITTEE FOR CARE AND USE OF LABORATORY ANIMALS

Not applicable.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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