


REVIEW

Clinical and biochemical distinctions for a metabolite repair disorder caused by NAXD or NAXE deficiency

Nicole J. Van Bergen^{1,2}  | Adhish S. Walvekar³ | Myrto Patraskaki³ |
Tim Sikora¹ | Carole L. Linster³ | John Christodoulou^{1,2,4}

¹Brain and Mitochondrial Research Group, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Victoria, Australia

²Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

³Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Belvaux, Luxembourg

⁴Victorian Clinical Genetics Services, Royal Children's Hospital, Melbourne, Victoria, Australia

Correspondence

Nicole J. Van Bergen and John Christodoulou, Brain and Mitochondrial Research Group, Murdoch Children's Research Institute, Royal Children's Hospital, Melbourne, Victoria 3002, Australia.

Email: nicole.vanbergen@mcri.edu.au and john.christodoulou@mcri.edu.au

Carole L. Linster, Luxembourg Centre for Systems Biomedicine, University of Luxembourg, L-4367 Belvaux, Luxembourg.

Email: carole.linster@uni.lu

Funding information

Luxembourg National Research Fund, Grant/Award Number: C18/BM/12661133; The Royal Children's Hospital Foundation; Murdoch Children's Research Institute; Mito Foundation; State Government of Victoria's Operational Infrastructure Support Program; Juniclair Foundation

Abstract

The central cofactors NAD(P)H are prone to damage by hydration, resulting in formation of redox-inactive derivatives designated NAD(P)HX. The highly conserved enzymes NAD(P)HX dehydratase (NAXD) and NAD(P)HX epimerase (NAXE) function to repair intracellular NAD(P)HX. Recently, pathogenic variants in both the *NAXD* and *NAXE* genes were associated with rapid deterioration and death after an otherwise trivial fever, infection, or illness in young patients. As more patients are identified, distinct clinical features are emerging depending on the location of the pathogenic variant. In this review, we carefully catalogued the clinical features of all published NAXD deficiency patients and found distinct patterns in clinical presentations depending on which sub-cellular compartment is affected by the enzymatic deficiency. Exon 1 of *NAXD* contains a mitochondrial propeptide, and a unique cytosolic isoform is initiated from an alternative start codon in exon 2. NAXD deficiency patients with variants that affect both the cytosolic and mitochondrial isoforms present with neurological defects, seizures and skin lesions. Interestingly, patients with *NAXD* variants exclusively affecting the mitochondrial isoform present with myopathy, moderate neuropathy and a cardiac presentation, without the characteristic skin lesions, seizures or neurological degeneration. This suggests that cytosolic NAD(P)HX repair may protect from neurological damage, whereas muscle fibres may be more sensitive to mitochondrial NAD(P)HX damage. A deeper understanding of the clinical phenotype may facilitate rapid identification of new cases and allow earlier therapeutic intervention. Niacin-based therapies are promising, but advances in disease modelling for both NAXD and NAXE deficiency may identify more specific compounds as targeted treatments. In this review, we found distinct patterns in the clinical presentations of NAXD deficiency patients based on the location of the pathogenic variant,

Nicole J. Van Bergen and Adhish S. Walvekar contributed equally to this work and should be considered joint first authors. Carole L. Linster and John Christodoulou contributed equally to this work and should be considered joint last authors.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Journal of Inherited Metabolic Disease* published by John Wiley & Sons Ltd on behalf of SSIEM.

which determines the subcellular compartment that is affected by the enzymatic deficiency.

KEYWORDS

febrile illness, metabolism, metabolite repair, NAXD deficiency, NAXE deficiency, neurodegeneration, skin lesions

1 | INTRODUCTION

1.1 | NAD(P)H damage and the repair system

Metabolism is not perfect. Metabolites are prone to spontaneous chemical damage and metabolic enzymes often show promiscuity, both leading to the generation of unwanted metabolites.¹ NADH and NADPH are central metabolic cofactors that can get damaged by hydration. The hydrated forms, R-NAD(P)HX and S-NAD(P)HX, are redox inactive and can further cyclize to form cyclic NAD(P)HX (Figure 1). To counter the accumulation of damaged metabolites, a pair of highly conserved metabolite repair enzymes revert both NADHX and NADPHX

back to the functional cofactors.^{2,3} The ATP-dependent metabolite repair enzyme NAD(P)HX dehydratase (NAXD) acts specifically on the S epimer of NAD(P)HX. The second enzyme, NAD(P)HX epimerase (NAXE), catalyses the interconversion between the S- and R-forms of NAD(P)HX, and essentially supplies the substrate for the stereospecific enzyme NAXD (Figure 1). The importance of these metabolite repair enzymes is highlighted by the central roles played by NAD(P)H in a multitude of biochemical processes including key redox reactions related to energy production (i.e., glycolysis, Krebs cycle, fatty acid β -oxidation, the mitochondrial electron transport chain, pentose phosphate pathway, fatty acid synthesis), signalling and cell survival. Both NAXD and NAXE proteins are highly expressed in mitochondria.⁴ Apart

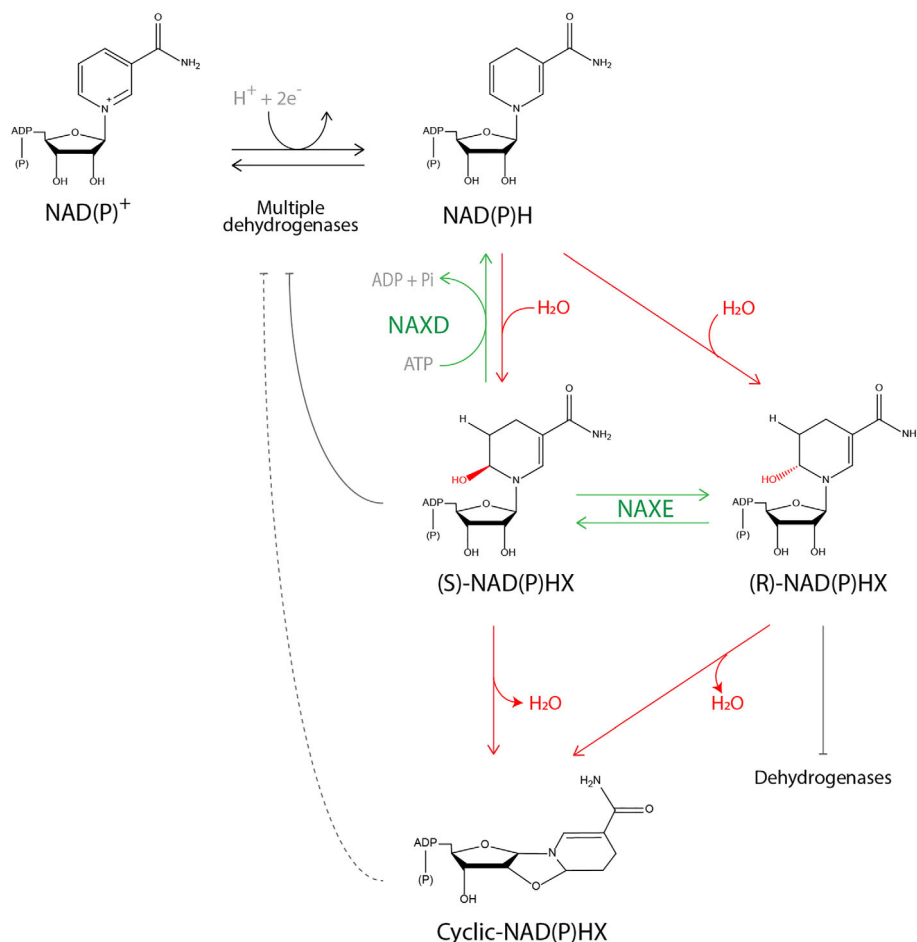


FIGURE 1 NAD(P)H damage and NAD(P)HX repair by NAXD and NAXE. NAD⁺/NADH and NADP⁺/NADPH are critical cofactors for numerous dehydrogenase enzymes. NAD(P)H can be spontaneously hydrated to form two stereoisomers S-NAD(P)HX or R-NAD(P)HX. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) can also facilitate the hydration of NAD(P)H. S-NAD(P)HX or R-NAD(P)HX can be further and irreversibly (dehydrated) to a cyclic derivative (C-NAD(P)HX). The epimerase NAXE facilitates interconversion between the S- and the R-forms of NAD(P)HX. The ATP-dependent NAXD stereospecifically converts S-NAD(P)HX back to NAD(P)H. The NAD(P)HX stereoisomers are expected to interfere with many key dehydrogenases and repair of these are critical to prevent wide-ranging metabolic perturbations.

from its role in NAD(P)HX repair, NAXE has been reported to moonlight as an apolipoprotein A-1 binding protein (hence the prior name AIBP) with roles in cholesterol efflux, angiogenesis, haematopoiesis, atherogenesis,⁵ neuroinflammation,⁷ and other processes.^{7,8} If and how these additional reported functions are involved in the progressive encephalopathy developing in young NAXE deficiency patients remains unclear.

1.2 | Pathogenic variants in NAXD and NAXE cause human disease

It is now well established that pathogenic variants in *NAXD* lead to human disease (OMIM # 618321, Early-onset progressive encephalopathy with brain edema and/or leukoencephalopathy-2, PEBEL2).^{9,10–14} In the majority of cases, this severe disease evolves rapidly following an episode of otherwise benign mild fever or infection in young, previously healthy children. The majority of children with NAXD deficiency died within days or months following a severe neurodegenerative course.

Common features of the NAXD neurodegenerative phenotype include cytotoxic oedema of the basal ganglia suggestive of a mitochondrial disorder, generalised asymmetrical cortical atrophy in the temporal and frontal lobes,⁹ oedema or atrophy of the cerebellum,^{11,14} paraspinous muscle oedema and grey matter abnormalities in the cervical cord at C2. During the acute disease phase several patients experienced movement abnormalities including unsteady gait, dystonia and ataxia.

The rapid regression is not surprising given the critical and constant demand of NAD(P)H for mitochondrial energy production, antioxidant protection and other crucial processes in the brain. Children with NAXE deficiency have an almost identical, nearly always fatal neurodegenerative course (OMIM # 617186, PEBEL1), also generally triggered after mild fever, respiratory or urinary tract infection or other illnesses including gastroenteritis or tonsillitis.^{15–21} NAXD and NAXE are critical metabolite repair enzymes, and physical or heat stress can damage the central metabolite NAD(P)H to form a toxic compound NAD(P)HX, and it is likely that the fever or illness-induced stress in children triggers a rapid accumulation of NAD(P)HX which cannot be effectively cleared due to a lack of NAXD (or NAXE) enzymes. Additionally, because the brain is one of the most energy-demanding organs in the body, failure of key metabolite repair enzymes involved in energy production will have devastating consequences on brain function. How a febrile illness triggers the deterioration of brain and heart function and causes severe skin lesions

remains, however, unknown for both NAXD and NAXE deficiency.

2 | LOSS OF DIFFERENT ISOFORMS LEADS TO DIFFERENT CLINICAL PHENOTYPES IN NAXD DEFICIENCY PATIENTS

The *NAXD* gene (formerly known as *CARKD*) encodes three proteins generated from different transcription initiation sites and alternative splicing, one with a mitochondrial propeptide (referred to as mitoNAXD or mCARKD), one with a putative signal peptide (spCARKD), and a shorter one lacking a targeting sequence (cytoNAXD or cCARKD), which accumulates in the cytosol.⁴ Exon 1 of *NAXD* contains the mitochondrial propeptide, and a cytosolic isoform is initiated from an alternative start codon in exon 2.⁴ The mitochondrial transcript is the most highly expressed⁹ and the presence of NAXD activity has been confirmed in mitochondria and the cytoplasm in liver tissue.⁴ An alternative exon 1 contains a signal peptide, and there is evidence of endoplasmic reticulum localisation of NAXD.⁴

The first six case reports of NAXD deficiency featured pathogenic variants across the *NAXD* gene, and of these, two patients had pathogenic variants in the mitochondrial propeptide.⁹ At the time, there was insufficient evidence to differentiate clinically between any patient sub-groups based on the location of the pathogenic variant. Since then, there have been new cases reported^{10–14} and pathogenic variants are now featured across the gene.

Interestingly, recent reports have shed light on potential phenotype–genotype correlations for NAXD deficiency. A recently reported patient was confirmed to have an 8 kilobase microdeletion spanning exons 1–2 of *NAXD* and a single nucleotide variant (SNV) within exon 1, predicted to affect splicing.²² Although not yet confirmed experimentally, the SNV likely abrogates the mitochondrial isoform, but allows expression of the cytosolic isoform, which could explain the “milder” clinical presentation of this individual. Other reports of patients with mitoNAXD deficiency highlight an emerging clinical sub-phenotype. A previously reported patient had an early frameshift pathogenic variant, p.(Ala20Phefs*9), which was predicted to prevent expression of the mitochondrial NAXD isoform, but allow expression of the cytosolic isoform.⁹ The clinical phenotype of the case reported in Van Bergen et al¹ with the p.(Ala20Phefs*9) pathogenic variant also differed from the majority of other reported cases in the original report, and this individual did not present with neurodegeneration or skin

lesions, although death occurred after an episode of gastroenteritis. This variant was also one of the two variants, the other being p.(Arg15Glnfs*3), reported by Borna et al.¹⁰ Interestingly, this case had the most prolonged survival of NAXD deficiency patients reported to date. Overexpression and cell fractionation studies indicated that the p.(Ala20Phefs*9) variant prevents mitochondrial, but not cytosolic NAXD expression.¹⁰

Although the mitochondrial isoform is the most abundant NAXD transcript, rescue studies overexpressing the cytosolic isoform in fibroblasts from NAXD deficiency patients showed NADHX levels restored back to that of control cells.¹ Therefore, it is possible that some level of protection is provided by expression of the cytosolic isoform in the patients reported by Manor et al.¹³ and Borna et al.^{10,23} Interestingly, fibroblasts of a previously reported NAXD deficiency case with a loss-of-function variant (p.(Ala20Phefs*9)) in the mitochondrial isoform only showed NADHX levels which were just above the detection threshold, and were much lower compared to NADHX levels in patients with pathogenic variants in NAXD affecting both the mitochondrial and cytosolic forms.¹

The recent report by Manor et al.²² brings the number of patients predicted to present with mitoNAXD deficiency to a total of four, as opposed to 11 for a combined cytoNAXD and mitoNAXD deficiency.^{9,10–14} Comparison of the clinical features of mitoNAXD deficiency patients to patients having the combined cytoNAXD and mitoNAXD deficiency (see Table 1, Figure 2, Supplemental Table 1), yields interesting insights that shed some light on the disease aetiology. Perhaps the most striking observation is that none of the four patients with mitoNAXD deficiency alone developed the skin lesions that occurred in all of the patients with combined cytoNAXD and mitoNAXD deficiency.^{9,10–14} Skin lesions generally occurred in regions of high-skin folding or movement including in the neck, chin, groin, buttocks, fingers, and axillae (Supplemental Figure 1). Skin lesions appeared inflamed, red, and blistered, with a burn-like or rash-like appearance. Histological examination revealed epidermal detachment, separation at the dermo-epidermal junction, vacuolar damage of the basal membrane, subepidermal blistering and extensive epidermal necrosis.⁹ No microorganism infections were identified in the affected skin lesion regions. Seizure activity was reported for almost all of the patients with combined cytoNAXD and mitoNAXD deficiency but was not reported for any of the mitoNAXD deficiency patients.

Overall, it appears from this comparison that isolated mitoNAXD deficiency may lead to a more slowly progressive and less aggressive disease, characterised

predominantly by myopathy (muscle weakness, increased creatine kinase), elevated lactate, and is accompanied by a more moderate neuropathy, which can be exacerbated by febrile illness (Figure 2). Combined cytoNAXD and mitoNAXD deficiency depends on the same types of triggers for onset of disease, but is, however, dominated by episodes of neurological regression accompanied by severe skin lesions.

The absence of a skin phenotype in isolated mitoNAXD deficiency suggests that replenishing the NAD(P)H pool in the cytosol (from the presence of cytoNAXD) is sufficient to prevent skin lesions. Furthermore, niacin supplementation efficiently and entirely resolved the characteristic skin lesions (described above), without scarring, in the combined NAXD deficiency patient,¹⁴ indicating that increasing the pool of “healthy” NAD (as opposed to decreasing NAD(P)HX forms) achieves the same results. This is a critical positive factor for potential therapeutic interventions, since replenishing the NAD pool by supplementing with NAD precursor compounds has potential benefit for a range of skin, neuro-metabolic and age-related conditions.^{24–26} Milder neurological symptoms and absence of seizures in mitoNAXD deficiency cases also suggest that cytosolic NAD(P)HX repair can partially protect from neurological damage, whereas muscle fibres seem to be more sensitive to mitochondrial NAD(P)HX damage. Considering the above observations, one must be cautious when using skin fibroblasts to study the effect of pathogenic variants in NAXD predicted to only affect the mitochondrial expression of the enzyme. Given the emergence of clinical subtypes depending on the location of the genetic variants, careful selection of the cell or tissue type could be critical to reveal the phenotype(s) caused by different NAXD alleles.

3 | CELLULAR AND METABOLIC CONSEQUENCES OF NAXD DEFICIENCY

NAXD deficiency has been studied in several simple cellular disease models (Figure 3), namely yeast, HAP1 cells (human near-haploid cell line), and NAXD deficiency patient-derived fibroblasts. These models show characteristic accumulation of NAD(P)HX forms, predominantly the NAXD substrate, S-NADHX.^{9,27} This is in contrast to the predominant accumulation of cyclic or R-NADHX forms observed in NAXE deficient cell models.^{17,27}

In the yeast (*Saccharomyces cerevisiae*) deletion mutants lacking the NAD(P)HX dehydratase gene (*YKL151C*), as a model of NAXD deficiency,²⁷ both growth phase and temperature influenced the intracellular levels

TABLE 1 Comparison of clinical features of patients with defective NAXD expression/function at mitochondrial versus whole cell level and with NAXE deficiency

Features	Patients with defects (predicted or experimentally verified) in mitochondrial expression of NAXD				Patients with deficiency of NAXD at the whole cell level		Patients with NAXE deficiency	
	Case 11	Case 5	Case 7	Case 2	11 cases	23 cases		
Patient number in Figure 2A	22	(Case 3 in the original publication) ⁹	(Case 5 in the original publication) ⁹	10	9,13,10,14,12,11	15–21		
Gender	Male	Female	Female	Male	Six males, five females	14 males, 9 females		
Pathogenic variant in the NAXD locus	c.46G > A: p.(Val16Ile) and microdeletion encompassing exons 1 and 2 of NAXD	c.51_54delAGAA: p.(Ala20Phefs*9)	c.54_57delAAGA: p.(Ala20Phefs*9)	c.44delG: p.(Arg15GlnfsTer3) and (c.51_54delAGAA): p.(Ala20Phefs*9)	—	—		
Evidence for the absence of NAXD expression in mitochondria	Computational prediction	Computational prediction	Computational prediction	Experimentally verified	—	—		
Age at severe presentation	16 years	1 year 7 months	2 years 10 months	7 years	3 months–16 years (median of all NAXD cases is 1 year 7 months)	2 days–22 years (median of all NAXE cases is 1 year 2 months)		
Current status/Outcome	Alive with the ongoing niacin treatment	Deceased (1 year 7 months)	Deceased (2 year 10 months)	Deceased (7 years 3 months)	Two alive, nine deceased (median of all NAXD cases is 2 year 10 months)	3 alive, 19 deceased, one not reported (median of all NAXE cases is 1 year 9 months)		
Febrile illness (or other trigger) prior to deterioration	Yes	Yes	Yes	Yes	Yes	Yes, for 11/11 reported cases		
Skin lesions	No	No	No	No	Yes 7/11 reported cases	Yes 3/5 reported cases		
Seizures	No	No	No	No	Yes 4/8 reported cases	Yes 10/10 reported cases		
Myopathy	Yes	Not reported	Yes	Yes	Yes 3/5 reported cases	Only reported in one case		

TABLE 1 (Continued)

Features	Patients with defects (predicted or experimentally verified) in mitochondrial expression of NAXD				Patients with NAXE deficiency at the whole cell level	
	Case 11	Case 5	Case 7	Case 2	11 cases	23 cases
Cardiac presentation	No	No	Yes; tachycardia and dilated cardiomyopathy and impaired ventricular function	Yes; gross hypertrophic cardiomyopathy and acute myocarditis	Yes 2/10 reported cases	One case with heart failure
Neurodegeneration	No	No	Abnormal MRI scan	Yes	Yes 6/10 reported cases	Yes 13/14 cases (Note: 20/23 had brain MRI abnormality)
Gastroenteritis	No	Yes	No	Yes	Yes 4/6 reported cases	Not reported
Anaemia or pancytopenia	Not reported	Mild anaemia	Mild anaemia	Mild anaemia	Yes 5/7 reported cases (three pancytopenia, one leukopenia and one microcytic anaemia)	Not reported
Elevated creatine kinase levels	Yes	Not reported	Yes	Yes	Yes 4/4 reported cases	No 7/7 cases
Elevated lactate in cerebrospinal fluid or plasma	Not reported	Yes	Yes	Yes	Yes 5/9 reported cases	Yes 9/17 reported cases
Anaemia	Not reported	Mild	Mild	Mild	Yes 8/8 reported cases	Yes 1/1 reported case
Mitochondrial enzyme defect	Yes	Not reported	No	Yes	Yes 5/7 reported cases	Yes 1/8 reported cases

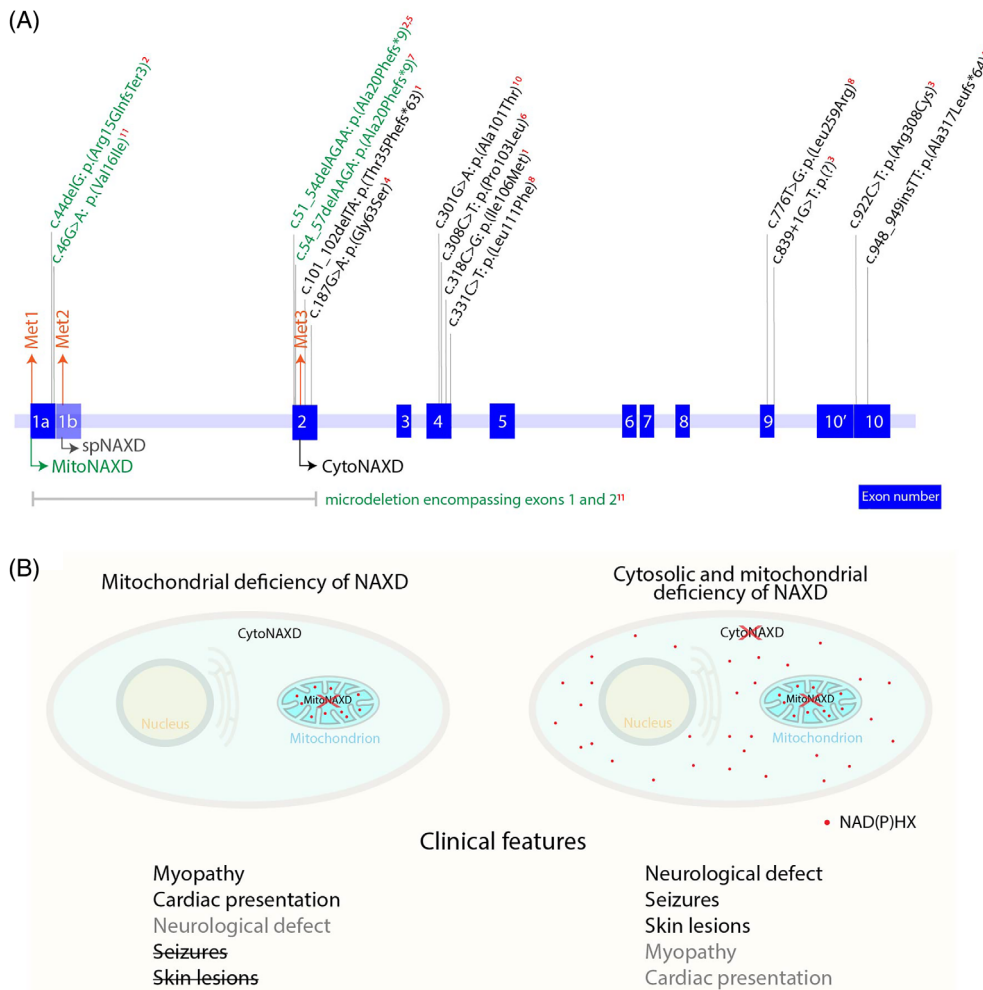


FIGURE 2 Clinical features reveal distinct patterns for mitochondrial or whole cell deficiency of NAXD. (A) Graphical presentation of the location of pathogenic NAXD variants from patients. MitoNAXD variants are in green. The three initiating methionines (Met) are indicated in red. Numbers in red superscripts next to the variants map them to the corresponding NAXD variant in Supplemental Table 1. (B) The clinical features of mitochondrial or whole-cell deficiency of NAXD show distinct differences (detailed in Table 1 and Supplemental Table 1). MitoNAXD deficiency is marked with absence of skin lesions and seizures, with milder neurological features. CytoNAXD, cytosolic isoform of NAXD; MitoNAXD, mitochondrial isoform of NAXD; spNAXD, ER isoform of NAXD

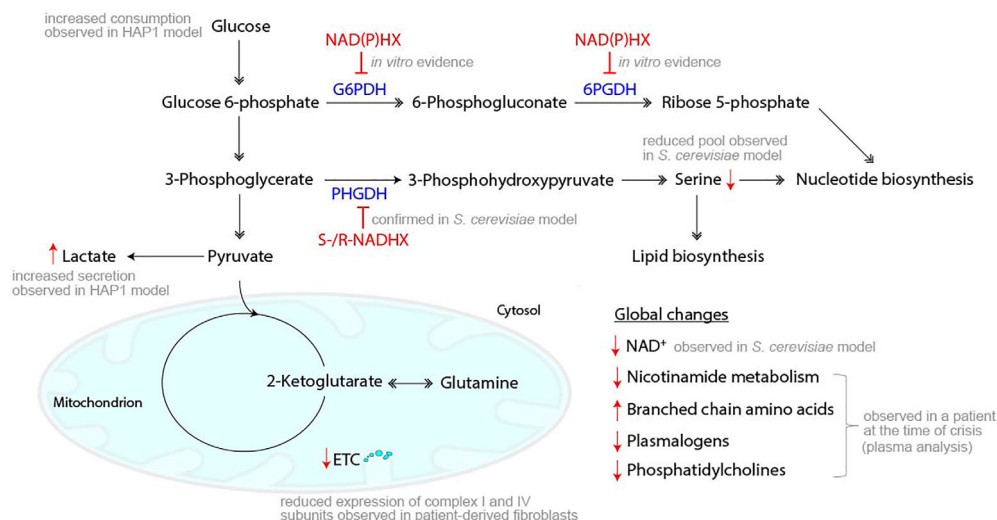
of NADHX. NADHX levels increased in stationary phase cultures and during cultivation at higher temperature. The most striking secondary metabolic perturbation detected in the NAXD deficient yeast cells was inhibition of the serine biosynthetic pathway. It could be shown that NADHX inhibits the transhydrogenase activity of yeast phosphoglycerate dehydrogenase (encoded by the *SER3* and *SER33* genes), the first enzyme in the serine biosynthesis pathway. The resulting reduction in serine pools was detected by metabolomics measurements. The low expression of *CHA1*, which encodes the catabolic serine/threonine deaminase, in the NAXD deficient yeast model was also coherent with the decreased serine levels as *CHA1* is a serine-inducible gene. Finally, NAXD deficient yeast cells also showed a reduced NAD⁺ pool. Preliminary analysis of NAXD deficient HAP1 cells (where a mutated NAXD gene encodes a truncated protein containing the first 126 amino acids out of the 329 amino acids of the native mitochondrial isoform followed by nine non-native amino acids), showed decreased viability as well as increased glucose consumption and lactate secretion suggesting mitochondrial impairment) compared to control

cells.²⁷ Additionally, fibroblasts from NAXD deficiency patients displayed mitochondrial dysfunction (decreased levels of complex I and IV subunits) and decreased viability in “mitochondrial stress-galactose” media.⁹

Several *in vitro* studies demonstrated that NAD(P)HX is a potent inhibitor of multiple cytosolic dehydrogenases dependent on NAD or NADPH (Figure 3). The inhibited enzymes include the pentose phosphate pathway enzymes glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, glycerol 3-phosphate dehydrogenase, and the serine biosynthesis enzyme 3-phosphoglycerate dehydrogenase (at least the yeast enzyme).^{27–29} It is likely that NAD(P)HX also inhibits other NAD(P)H-dependent dehydrogenases, for example, enzymes feeding into the mitochondrial respiratory chain, or key enzymes in other organelles, although this remains to be proven.

A recent study provides interesting preliminary indicators of metabolic perturbations due to NAXD deficiency occurring in a patient at the time of clinical crisis.²² Plasma metabolomic analyses showed significant depletion of nicotinamide metabolites, as may be expected due to NAD(P)HX repair deficiency, as well as alterations in

FIGURE 3 Metabolic perturbations linked to NAXD deficiency. The figure summarises the targets of NADHX and pertinent metabolic blocks in different models studied to date. G6PDH, glucose 6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; PHGDH, 3-phosphoglycerate dehydrogenase; ETC, electron transport chain



tricarboxylic acid cycle intermediates and increased branched-chain amino acid levels, indicative of mitochondrial dysfunction. Plasma metabolomics also provided indirect evidence of disturbances of serine metabolism in the patient, reminiscent of the previously described metabolic perturbations in the yeast NAXD deficient model.²⁷ Finally, Manor et al²² reported that the NAXD deficiency patient exhibited reduced plasma levels of plasmalogens, phosphatidylcholines, and certain sphingomyelins, which depend on metabolic inputs from many subcellular compartments, including peroxisomes and the endoplasmic reticulum, for their synthesis.²² It is plausible that perturbation of serine metabolism, along with NAD depletion (exacerbated by febrile illness) and energy crisis due to mitochondrial dysfunction, may all contribute to impaired lipid biosynthesis. Excitingly, niacin treatment in the NAXD deficiency patient reported by Manor et al restored the plasma levels of the reported differential metabolites,²² indicating that brute-force increase in overall NAD levels through niacin treatment may compensate for an absence of NAXD function in mitochondria, and probably in other compartments as well.¹³

Modelling of two patient missense variants in recombinant NAXD protein showed impaired dehydratase activity, particularly at high temperatures.⁹ This observed thermo-instability of the NAXD enzyme with pathogenic missense variants clearly provides a credible causal link to the fever-induced deterioration of patients. The above data strongly suggest that accumulation of NAD(P)HX, consequent to pathogenic variants in *NAXD* or *NAXE*, renders cells highly sensitive to temperature and metabolic stress, which in turn can cause impaired cell growth or viability, consistent with the observed rapid progression of the disease.⁹

4 | CURRENT THERAPEUTIC APPROACHES FOR NAXD DEFICIENCY

Rapid deterioration after an otherwise trivial fever, infection or illness is the commonest feature of the reported NAXD deficiency patients,^{9,10–14} and the rapid and severe clinical escalation limits opportunities for therapeutic interventions. Since the rapid regression of patients typically follows illness, infection or fever, careful management to reduce fever and avoid illness in known cases whenever possible is recommended to potentially manage clinical relapses. This approach is also recommended to possibly prevent acute decompensation in siblings with biallelic *NAXD* variants who are not yet clinically affected. To date, these episodes of fever or illness have triggered an irreversible clinical progression which has been lethal in all NAXD deficiency cases,^{9,10–12} but two.^{13,14}

The outstanding feature of the NAXD deficiency patients who did not succumb after illness is likely due to the application of niacin (also referred to as vitamin B3 or nicotinic acid or nicotinamide)-based therapies early in their clinical presentation. High-dose vitamin B3 (500 mg/d) was reported to alleviate the skin manifestations and stabilise neurological symptoms.¹⁴ A more recent report²² describes the second NAXD deficiency patient who received niacin supplementation (100 mg/d). Here, reduction of creatine kinase (CK) levels towards normal and a significant improvement in the clinical outcome were reported 11 months after intervention. The CK levels were sustained at lower, though still slightly elevated, levels 17 months post-crisis. Additionally, a similar therapeutic approach seems to benefit *NAXE* deficiency patients. A patient with *NAXE* deficiency was

treated with Coenzyme Q10 and niacin (40–80 mg/d) and showed improved clinical outcomes after treatment including dramatic improvements in severe spasticity, and continuous motor and cognitive improvement.²⁰ Another patient with NAXE deficiency received vitamin B complex (thiamine 60 mg, riboflavin 3 mg, nicotinamide 30 mg and pyridoxine 3 mg per day) and coenzyme Q10, and this patient showed progressive neurological improvement, recovery of muscle power and at the time of the report, remained alive.¹⁵

From the first reports of pathogenic variants in *NAXE*, niacin therapy was speculated to be of benefit for disorders of the nicotinamide nucleotide repair system, as it is a precursor that feeds into *de novo* NAD synthesis.¹⁷ Now, it is exciting to see potential treatments being tested in patients and providing a degree of protection against severe clinical outcomes. The outcomes reported so far for the two treated NAXD deficiency patients^{13,14} and two treated NAXE deficiency cases^{15,20} support the use of niacin-based therapies for stabilising the clinical progression and furnish some evidence that these disorders may be treatable. However, more specific targeted therapies could be of even greater benefit, particularly if applied early in the clinical course, and so a systematic approach to identification of novel therapies is of utmost priority.

5 | FUTURE RESEARCH REQUIREMENTS

Gaining a deep molecular understanding of rare genetic disorders can be challenging as there is often limited published data, and precious patient-derived samples can be difficult to obtain. Premature death in disorders such as NAXD and NAXE deficiency further compounds the challenge. To date, the only known models for NAXD deficiency are yeast and HAP1 cells knocked out for *NAXD*²⁷ and NAXD deficiency patient-derived fibroblasts.^{9,27} Studies of these models gave the first insights into the metabolic pathways and processes that are affected by NAXD deficiency at the cellular level. All NAXE deficiency patients and the majority of NAXD deficiency cases showed primarily neurological symptoms combined with multi-systemic impairments such as skin lesions, cardiac dysfunction and respiratory insufficiency. These observations underline the complexity of NAD(P)HX repair disorders, which could be more specifically addressed in highly vulnerable cell types derived from induced pluripotent stem cells (iPSCs), or more comprehensively studied in whole organism models. Recent advances in gene editing and iPSC-derived models provide a solid basis for deeper investigations into

cell-compartment and cell-type specific effects of different NAXD pathogenic variants.

Rodents are widely used to study neurodegeneration in common metabolic disorders.³⁰ They can also be used to investigate neurological impairment in rare inherited metabolic diseases, as exemplified by a study in a mouse model for the metabolite repair disorder L-2-hydroxyglutaric aciduria,³¹ which allowed the elucidation of how L-2-hydroxyglutarate accumulation leads to the leukodystrophy observed in this disease. The similarities in the clinical presentation between patients with NAXE or NAXD deficiency provide robust evidence in support of NAD(P)HX accumulation and/or NAD(P)H depletion being the common causal factor involved in the pathogenesis and of a minor implication, if any, for a secondary role reported for NAXE as a secreted Apolipoprotein A-1 binding protein that is involved in cholesterol efflux, angiogenesis, and atherosclerosis. *Naxe* knockout mice have so far been analysed mostly with a focus on these latter secondary functions,^{7,8,32} and it is unclear whether these mice have any neurological impairment. Given the moonlighting functions of NAXE and the essential function of NAXD in NAD(P)HX repair, developing a *Naxd* deficient mouse model seems like an inevitable next step to progress in whole organism disease modelling of NAD(P)HX repair deficiency and ongoing work is under way to reach this goal. As a complementary approach, *NAXD* and *NAXE* deficient models are currently being generated in zebrafish, a model organism that is also widely used for the study of neurodegenerative, developmental and metabolic disorders.^{33,34} The external development and transparency of zebrafish embryos make them an ideal model to study an early-onset disorder. Based on the observations presented in the current review, it is apparent that refining the whole organism disease models to achieve compartment-specific NAXD deficiency could be valuable in the future for developing tailor-made, variant-specific treatments.

6 | THE FUTURE OF TARGETED THERAPEUTIC APPROACHES FOR NAXD DEFICIENCY

Although research into metabolite repair genes has been neglected until recently,³⁵ the demonstration of their causal implication in severe disease development clearly shows that they should by no means be discarded as metabolic curiosities without much interest for human health.^{9,17,36,37} The NAXE and NAXD deficiency disorders strikingly revealed the critical importance of these repair enzymes in preserving neuronal health, but also other key organs such as the skin, the heart, and skeletal

muscle. Many fundamental questions remain regarding the biology of NAXD and NAXE deficiency and a better understanding of their consequences will be essential for the development of more efficient and targeted therapies. Despite the early success of the preliminary niacin-based n-of-1 interventions for NAXD and NAXE deficiency cases,^{13,14,15,20} the precise pathways critically impacted during crises and disease progression remain to be identified. It would therefore be of great interest to probe the molecular and cellular consequences of both NAXD and NAXE deficiency in appropriate disease models. This research will provide new and unique insights into the NAD(P)H damage and repair processes in most vulnerable tissues such as the brain. These studies will also highlight the interplay between neuronal and glial metabolism, neuronal and glial function, and the external environment (e.g., inflammatory triggers) during disease development.

A deeper understanding of metabolite repair disorders such as NAXD and NAXE deficiency may inform new or more specific compounds as targeted treatments. Additionally, high throughput drug discovery may be used to identify compounds that may be of even greater therapeutic efficacy for these severe and often fatal disorders. Two major interventions may prevent neurodegeneration in children during these febrile episodes. The first intervention could be focused on reducing the burden of damaged metabolites and maintaining the pool of healthy NAD(H) and NADP(H) cofactors, and the second intervention could be based on bypassing the major metabolic blocks induced by NAD(P)HX accumulation (Figure 3).

We anticipate that, in addition to directly helping patients with NAXD and NAXE pathogenic variants, these studies could potentially have a profound impact on our understanding of other more common neurodegenerative disorders, with broader implications notably for other mitochondrial and metabolic disorders where NAD(H)/NADP(H) deficiency is a critical contributing factor.

ACKNOWLEDGMENTS

The research conducted at the Murdoch Children's Research Institute was supported by the State Government of Victoria's Operational Infrastructure Support Program. The work was supported by funding from the Mito Foundation to NVB and J.C, the Murdoch Children's Research Institute (MCRI) Near Miss grant to NVB and the MCRI Strategic Pilot Project in Stem Cell and Genomics Medicine grant to NVB and J.C. The Chair in Genomic Medicine awarded to J.C is generously supported by The Royal Children's Hospital Foundation.

The research performed at the Luxembourg Centre for Systems Biomedicine was supported by a CORE grant from the Luxembourg National Research Fund (FNR) under project code C18/BM/12661133 and a donation from the Junclair Foundation to CLL.

The work was independent from the sponsors and the content was not influenced by the sponsors. Open access publishing facilitated by The University of Melbourne, as part of the Wiley - The University of Melbourne agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

We declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supporting data is available in Supplemental Table 1.

ORCID

Nicole J. Van Bergen  <https://orcid.org/0000-0002-6768-3665>

REFERENCES

1. Van Bergen NJ, Guo Y, Rankin J, et al. NAD(P)HX dehydratase (NAXD) deficiency: a novel neurodegenerative disorder exacerbated by febrile illnesses. *Brain*. 2019;142:50-58. doi:10.1093/brain/awy310
2. Linster CL, Van Schaftingen E, Hanson AD. Metabolite damage and its repair or pre-emption. *Nat Chem Biol*. 2013;9:72-80. doi:10.1038/nchembio.1141
3. Marbaix AY, Noel G, Detroux AM, et al. Extremely conserved ATP- or ADP-dependent enzymatic system for nicotinamide nucleotide repair. *J Biol Chem*. 2011;286:41246-41252. doi:10.1074/jbc.C111.310847
4. Niehaus TD, Richardson LG, Gidda SK, et al. Plants utilize a highly conserved system for repair of NADH and NADPH hydrates. *Plant Physiol*. 2014;165:52-61. doi:10.1104/pp.114.236539
5. Marbaix AY, Tyteca D, Niehaus TD, Hanson AD, Linster CL, Van Schaftingen E. Occurrence and subcellular distribution of the NAD(P)HX repair system in mammals. *Biochem J*. 2014;460:49-60. doi:10.1042/BJ20131482
6. Qiu X, Luo J, Fang L. AIBP, angiogenesis, hematopoiesis, and atherogenesis. *Curr Atheroscler Rep*. 2021;23(1):1. doi: 10.1007/s11883-020-00899-9.
7. Choi SH, Kim KY, Perkins GA, et al. AIBP protects retinal ganglion cells against neuroinflammation and mitochondrial dysfunction in glaucomatous neurodegeneration. *Redox Biol*. 2020;37:101703. doi:10.1016/j.redox.2020.101703
8. Mao R, Meng S, Gu Q, et al. AIBP limits angiogenesis through γ -secretase-mediated upregulation of notch signaling. *Circ Res*. 2017;120:1727-1739. doi:10.1161/CIRCRESAHA.116.309754
9. Schneider DA, Choi S-HH, Agatista-Boyle C, et al. AIBP protects against metabolic abnormalities and atherosclerosis. *J Lipid Res*. 2018;59:854-863. doi:10.1194/jlr.M083618

10. Borna N, Kishita Y, Abe J, et al. NAD(P)HX dehydratase protein-truncating mutations are associated with neurodevelopmental disorder exacerbated by acute illness. *Brain*. 2020;143:e54. doi:10.1093/brain/awaa130
11. Majethia P, Mishra S, Rao LP, et al. NAD(P)HX dehydratase (NAXD) deficiency due to a novel biallelic missense variant and review of literature. *Eur J Med Genet*. 2021;64:104266. doi:10.1016/j.ejmg.2021.104266
12. Malik MUU, Nadir H, Jessop ZMM, Cubitt JJJ. Cutaneous manifestations of NAXD deficiency - a case report. *Ann Med Surg*. 2020;60:352-355. doi:10.1016/j.amsu.2020.11.026
13. Van Bergen NJ, Walvekar AS, Linster CL, et al. Reply: niacin therapy improves outcome and normalizes metabolic abnormalities in a NAXD-deficient patient. *Brain*. 2022;145:e41-e42. doi:10.1093/brain/awac066
14. Zhou J, Li J, Stenton SL, et al. NAD(P)HX dehydratase (NAXD) deficiency: a novel neurodegenerative disorder exacerbated by febrile illnesses. *Brain*. 2020;143:e8. doi:10.1093/brain/awz375
15. Manor J, Calame D, Gijavanekar C, et al. Niacin therapy improves clinical outcome with normalization of metabolic abnormalities in a patient with NAXD deficiency. *Brain*. 2022;145:e36-e40.
16. Chiu L, Lin S, Chen C, et al. NAXE gene mutation-related progressive encephalopathy: a case report and literature review. *Medicine (Baltimore)*. 2021;100:e27548. doi:10.1097/MD.00000000000027548
17. Incecik F, Ceylaner S. Early-onset progressive encephalopathy associated with NAXE gene variants: a case report of a Turkish child. *Acta Neurol Belg*. 2020;120:733-735. doi:10.1007/s13760-019-01242-z
18. Kremer LS, Danhauser K, Herebian D, et al. NAXE mutations disrupt the cellular NAD(P)HX repair system and cause a lethal Neurometabolic disorder of early childhood. *Am J Hum Genet*. 2016;99:894-902. doi:10.1016/j.ajhg.2016.07.018
19. Mohammadi P, Heidari M, Ashrafi MR, Mahdiah N, Garshasbi M. A novel homozygous missense variant in the NAXE gene in an Iranian family with progressive encephalopathy with brain edema and leukoencephalopathy. *Acta Neurol Belg*. 2021. [Online ahead of print]. doi:10.1007/s13760-021-01717-y
20. Spiegel R, Shaag A, Shalev S, Elpeleg O. Homozygous mutation in the APOA1BP is associated with a lethal infantile leukoencephalopathy. *Neurogenetics*. 2016;17:187-190. doi:10.1007/s10048-016-0483-3
21. Trinh J, Imhoff S, Dulovic-Mahlow M, et al. Novel NAXE variants as a cause for neurometabolic disorder: implications for treatment. *J Neurol*. 2020;267:770-782. doi:10.1007/s00415-019-09640-2
22. Yu D, Zhao F-M, Cai X-T, Zhou H, Cheng Y. Clinical and genetic features of early-onset progressive encephalopathy associated with NAXE gene mutations. *Zhongguo Dang Dai Er Ke Za Zhi*. 2018;20:254-258.
23. Van Bergen NJ, Linster CL, Christodoulou J. Reply: NAD(P)HX dehydratase protein-truncating mutations are associated with neurodevelopmental disorder exacerbated by acute illness. *Brain*. 2020;143:e55.
24. Pieper A, McKnight SL. Benefits of enhancing nicotinamide adenine dinucleotide levels in damaged or diseased nerve cells. *Cold Spring Harb Symp Quant Biol*. 2018;83:207-217. doi:10.1101/sqb.2018.83.037622
25. Strömland Ø, Niere M, Nikiforov AA, VanLinden MR, Heiland I, Ziegler M. Keeping the balance in NAD metabolism. *Biochem Soc Trans*. 2019;47:119-130. doi:10.1042/BST20180417
26. Radenkovic D, Reason VE. Clinical evidence for targeting NAD therapeutically. *Pharmaceuticals*. 2020;13:247. doi:10.3390/ph13090247
27. Becker-Ketter J, Paczia N, Conrotte JF, et al. NAD(P)HX repair deficiency causes central metabolic perturbations in yeast and human cells. *FEBS J*. 2018;285:3376-3401. doi:10.1111/febs.14631
28. Prabhakar P, Laboy JI, Wang J, et al. Effect of NADH-X on cytosolic glycerol-3-phosphate dehydrogenase. *Arch Biochem Biophys*. 1998;360:195-205. doi:10.1006/abbi.1998.0939
29. Yoshida A, Dave V. Inhibition of NADP-dependent dehydrogenases by modified products of NADPH. *Arch Biochem Biophys*. 1975;169:298-303. doi:10.1016/0003-9861(75)90344-6
30. de Bem AF, Krolow R, Farias HR, et al. Animal models of metabolic disorders in the study of neurodegenerative diseases: an overview. *Front Neurosci*. 2021;14:604150. doi:10.3389/fnins.2020.604150
31. Rzem R, Achouri Y, Marbaix E, et al. A mouse model of L-2-Hydroxyglutaric aciduria, a disorder of metabolite repair. *PLoS One*. 2015;10:e0119540. doi:10.1371/journal.pone.0119540
32. Kim J-D, Zhu L, Sun Q, Fang L. Systemic metabolite profiling reveals sexual dimorphism of AIBP control of metabolism in mice. *PLoS One*. 2021;16:e0248964. doi:10.1371/journal.pone.0248964
33. Choi T-Y, Choi T-I, Lee Y-R, Choe SK, Kim CH. Zebrafish as an animal model for biomedical research. *Exp Mol Med*. 2021;53:310-317. doi:10.1038/s12276-021-00571-5
34. Kodera K, Matsui H. Zebrafish, Medaka and turquoise killifish for understanding human neurodegenerative/neurodevelopmental disorders. *Int J Mol Sci*. 2022;23:1399. doi:10.3390/ijms23031399
35. Van Schaftingen E, Rzem R, Marbaix A, et al. Metabolite proof-reading, a neglected aspect of intermediary metabolism. *J Inherit Metab Dis*. 2013;36:427-434. doi:10.1007/s10545-012-9571-1
36. Veiga-da-Cunha M, Chevalier N, Stephenne X, et al. Failure to eliminate a phosphorylated glucose analog leads to neutropenia in patients with G6PT and G6PC3 deficiency. *Proc Natl Acad Sci U S A*. 2019;116:1241-1250. doi:10.1073/pnas.1816143116
37. Veiga-da-Cunha M, Van Schaftingen E, Bommer GT. Inborn errors of metabolite repair. *J Inherit Metab Dis*. 2020;43:14-24. doi:10.1002/jimd.12187

SUPPORTING INFORMATION

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

How to cite this article: Van Bergen NJ, Walvekar AS, Patraskaki M, Sikora T, Linster CL, Christodoulou J. Clinical and biochemical distinctions for a metabolite repair disorder caused by NAXD or NAXE deficiency. *J Inherit Metab Dis*. 2022;45(6):1028-1038. doi:10.1002/jimd.12541