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Authors

Yang, Jennifer H
Friederich, Marisa W
Ellsworth, Katarzyna A
et al.

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Expanding the phenotypic and molecular spectrum of *NFS1*-related disorders that cause functional deficiencies in mitochondrial and cytosolic iron-sulfur cluster containing enzymes

Jennifer H. Yang[1,2,18], **Marisa W. Friederich**[3,4,18], **Katarzyna A. Ellsworth**[5], **Aliya Frederick**[1,2], **Emily Foreman**[6], **Denise Malicki**[7], **David Dimmock**[5], **Jerica Lenberg**[5], **Chitra Prasad**[8,9], **Andrea C. Yu**[10], **C. Anthony Rupar**[11,12], **Robert A. Hegele**[13,14], **Kandamurugu Manickam**[15], **Daniel C. Koboldt**[16], **Erin Crist**[16], **Samantha S. Choi**[16], **Sali M.K. Farhan**[17], **Helen Harvey**[6], **Shifteh Sattar**[1,2], **Natalya Karp**[8,9], **Terence Wong**[5], **Richard Haas**[1,2,19], **Johan L.K. Van Hove**[3,4,19], **Kristen Wigby**[5,6,19]

[1)]Department of Neurosciences, University of California San Diego, San Diego, CA 92093, USA

[2)]Division of Child Neurology, Rady Children's Hospital, San Diego, CA 92123, USA

[3)]Department of Pediatrics, Section of Clinical Genetics and Metabolism, University of Colorado, Aurora, CO 80045, USA

[4)]Department of Pathology and Laboratory Medicine, Children's Hospital Colorado, 13121 East 16th Avenue, Aurora, CO 80045, USA

[5)]Rady Children's Institute for Genomic Medicine, San Diego, CA 92123, USA

[6)]Division of Pediatrics, University of California San Diego, San Diego, CA 92093, USA

[7)]Department of Pathology, University of California San Diego, San Diego, CA 92093, USA

[8)]Schulich School of Medicine and Dentistry, Western University, London, Ontario N6A 5C1, Canada

[9)]Department of Pediatrics, Division of Medical Genetics, Schulich School of Medicine and Dentistry, Western University, London, Ontario N6A 5K8, Canada

Correspondence: Jennifer Yang, MD, jhy045@ucsd.edu.

Author's contributions:

JHY, MWF, RH, JHV and KW contributed to the study conception and design. All authors contributed to material preparation, data collection and analysis. MWF performed the laboratory experiments. The first draft of the manuscript was written by JHY and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Editorial Policies and Ethical Considerations

The authors affirm that all patients in this study underwent the appropriate consent for publication and all identifying information are removed from the manuscript.

Supplemental Data

Supplemental data include individual case reports, material and methods, and four figures.

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[10]Division of Metabolics and Newborn Screening, Department of Pediatrics, Children's Hospital of Eastern Ontario, Ottawa, ON, K1H 8L1, Canada

[11]Department of Pathology, London Health Science Centre, London, Ontario N6A 5A5, Canada

[12]London Health Sciences Centre, Children's Health Research Institute London, Ontario N6C 2V5, Canada

[13]Robarts Research Institute, Schulich School of Medicine and Dentistry, Western University, London, Ontario N6A 5K8, Canada

[14]Department of Biochemistry, Schulich School of Medicine and Dentistry, Western University, London, Ontario N6A 5C1, Canada

[15]Division of Genetics and Genomics, Nationwide Children's Hospital, Columbus, OH 43205 USA

[16]The Institute for Genomic Medicine, Nationwide Children's Hospital, Columbus, OH 43205, USA

[17]Departments of Neurology and Neurosurgery, and Human Genetics, the Montreal Neurological Institute and Hospital, McGill University, 3801 Rue University, Montréal, QC H3A 2B4, Canada

[18]These authors contributed equally to this work

[19]These authors contributed equally to this work

Abstract

Iron-sulfur cluster proteins are involved in critical functions for gene expression regulation and mitochondrial bioenergetics including the oxidative phosphorylation system. The c.215G>A p.(Arg72Gln) variant in *NFS1* has been previously reported to cause infantile mitochondrial complex II and III deficiency. We describe three additional unrelated patients with the same missense variant. Two infants with the same homozygous variant presented with hypotonia, weakness and lactic acidosis, and one patient with compound heterozygous p.(Arg72Gln) and p.(Arg412His) variants presented as a young adult with gastrointestinal symptoms and fatigue. Skeletal muscle biopsy from patients 1 and 3 showed abnormal mitochondrial morphology, and functional analyses demonstrated decreased activity in respiratory chain complex II and variably in complexes I and III. We found decreased mitochondrial and cytosolic aconitase activities but only mildly affected lipoylation of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase enzymes. Our studies expand the phenotypic spectrum and provide further evidence for the pathogenicity and functional sequelae of *NFS1*-related disorders with disturbances in both mitochondrial and cytosolic iron-sulfur cluster containing enzymes.

Keywords

NFS1; iron-sulfur clusteropathies; mitochondrial; lactic acidosis; pediatric

Introduction

Iron-sulfur clusters are prosthetic groups that are integrated in proteins located in the mitochondria, cytosol and nucleus that are involved in a variety of cellular functions (Kispal,

Csere, Prohl, & Lill, 1999; Li, Tong, Hughes, & Rouault, 2006; Lill et al., 1999). Proteins with iron-sulfur clusters (ISC) and cytosolic iron-sulfur protein assembly proteins (CIA) are involved in critical regulatory functions or catalyze enzymatic reactions for gene expression regulation, DNA replication, DNA repair, regulation of telomere length, tRNA modification, ribosomal protein translation and iron homeostasis (Braymer & Lill, 2017; Cameron et al., 2011; Sheftel, Stehling, & Lill, 2010). Additionally, iron-sulfur clusters are incorporated as cofactors for apoproteins and electron carriers for complexes I, II and III of the oxidative phosphorylation system, Krebs cycle enzymes such as aconitase, and the biosynthesis of lipoate and biotin (Kispal et al., 1999; Sheftel et al., 2010; Stehling & Lill, 2013).

The biogenesis of iron-sulfur cluster proteins begins with NFS1, a cysteine desulfurase the function of which is to supply inorganic sulfur abstracted from cysteine for the assembly of iron-sulfur clusters (Land & Rouault, 1998; Rouault, 2012; Stehling & Lill, 2013; Stemmler, Lesuisse, Pain, & Dancis, 2010). NFS1 then forms a complex with iron-sulfur cluster biogenesis desulfurase interacting protein (ISD11) (Adam, Bornhövd, Prokisch, Neupert, & Hell, 2006; Lim et al., 2013; Wiedemann et al., 2006) to bind to the iron-sulfur cluster assembly enzyme (ISCU) scaffold protein, which also involves ferredoxin (FDX1 and FDX2) (Rouault, 2012; Shi et al., 2010). The newly formed complex is then chaperoned and bound to the ISC transfer protein glutaredoxin 5 (GLRX5) (Li et al., 2006), and inserted into various target proteins including respiratory chain complexes and catalytic enzymes (Braymer & Lill, 2017). The iron-sulfur cluster of lipoate synthase is essential for generating the cofactor of lipoate-bearing enzymes such as the glycine cleavage enzyme and the bioenergetic enzymes pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase (McCarthy & Booker, 2017). In addition, the incomplete iron-sulfur cluster charging of cytosolic aconitase has been associated with increased activation of iron-response elements (Wang et al., 2007). Single genes are often responsible for encoding proteins along the iron-sulfur cluster assembly pathway. Disruption of any of these genes can result in human disease secondary to downstream biochemical processes (Rouault & Tong, 2008).

We now recognize that disruption of iron-sulfur cluster biogenesis is associated with several neurodegenerative, metabolic and hematological diseases (Stehling & Lill, 2013; A. V. Vanlander & Van Coster, 2018). For example, iron-sulfur cluster dysfunction has been well delineated in the pathogenesis of Friedreich ataxia secondary to defective frataxin (FXN) resulting in cardiomyopathy, peripheral neuropathy and ataxia (Beilschmidt & Puccio, 2014). Pathogenic variants in *LYRM4*, which encodes for the NFS1-interacting protein ISD11, have been reported in a 2-month-old infant who died of severe neonatal lactic acidosis and an asymptomatic young adult patient with complex I, II and III deficiency (Lim et al., 2013). Various iron-sulfur clusteropathies resulting from pathogenic variants in *NFU1* (MIM# 605711), *BOLA3* (MIM# 614299), *IBA57* (MIM# 615330), *ISCA1* (MIM# 617613), *ISCA2* (MIM# 616370), *NUBPL* (MIM# 613621) *GLRX5* (MIM# 616859), and *PMPCB* (MIM# 617954) present with biochemical findings consistent with mitochondrial dysfunction along with variable neurodegenerative presentations including cavitating leukodystrophy, cardiomyopathy, optic neuropathy, and peripheral neuropathies (Alfadhel, 2019; Cameron et al., 2011; Rouault, 2012; Rouault & Tong, 2008; A. V. Vanlander & Van Coster, 2018; Vögtle et al., 2018). Biochemical findings for these diseases can be combinations of lactic acidosis, deficiency of complexes I, II, III of the respiratory

chain, low aconitase activity, and in some conditions deficient lipoylation with increased glycine levels.

The Nitrogen Fixation 1 Homolog gene (*NFS1*; MIM# 603485]) is located on chromosome 20 and encodes for the NFS1 protein. The cysteine desulfurases were first described in nitrogen-fixing bacteria that utilized pyridoxal 5'-phosphate (PLP) as a co-factor to form alanine and elemental sulfur from cysteine (Zheng, White, Cash, Jack, & Dean, 1993). While NFS1 localizes mainly to the mitochondria (Land & Rouault, 1998), it also performs other functions in the eukaryotic cytosol including thiolation of tRNA (Mühlenhoff et al., 2004). Several studies have demonstrated pathogenicity of NFS1 deficiency in murine and *in vitro* models. Murine fibroblasts with depletion in NFS1 mRNA showed reduction in complex I, II, and aconitase (Fosset et al., 2006). In addition, NFS1 silencing in HeLa cells demonstrated mitochondrial structural changes and decreased aconitase and complex II enzyme activity, which were restored with the addition of mitochondrially targeted murine-NFS1 (Biederbick et al., 2006).

Despite the growing evidence of pathogenicity of derangements in NSF1 from animal models and *in vitro* studies, the association of this gene with human disease remains limited. The first case report described three patients from a consanguineous family from the Old Order Mennonite community with a homozygous variant NM_021100.4: c.215G >A p.(Arg72Gln) in *NFS1* that implicated the role of NFS1 in human disease (Farhan et al., 2014). In the case series, two siblings died at 7 months of age, one with lethargy, myocardial failure, and seizures and the other with hypotonia and feeding problems progressing to multiorgan failure. A third sibling was started on coenzyme Q^{10}, carnitine, vitamin C, vitamin D and creatine at 6 months of age and is currently still alive at 21 years old with mild developmental delay and occasional exercise fatigue. Biochemical findings for these siblings included elevated lactate with decreased activity of complexes II and III in skeletal muscle and liver. More recently, the same variant was reported in a consanguineous Christian-Arab family with intra-familial phenotypic variability and decreased activities in complexes I, II, and III (Hershkovitz et al., 2021). In this family, two infants presented with symptoms of progressive hypotonia, lactic acidosis and respiratory failure. The first child presented at 40 days old and subsequently died three days later, and the second child presented at 7 months and died of cardiac arrest. The third child had a single episode at one month of age with lactic acidosis, elevated liver enzymes and creatine kinase during an acute infection without further exacerbations.

Results

Herein we report three additional, unrelated patients with variants in *NFS1* who were identified through the GeneMatcher platform (Sobreira, Schiettecatte, Valle, & Hamosh, 2015). Two of these new cases harboring the homozygous c.215G>A p.(Arg72Gln) variant presented similarly to the previously reported family (Farhan et al., 2014) with lactic acidemia and multi-system disease, whereas the third patient is compound heterozygous for a new variant and presented at 18 years old with gastrointestinal symptoms, fatigue and lactic acidosis. The clinical features of all three patients are summarized in Table 1, and the individual case reports are included in the supplemental materials.

Patient 1 presented at 6 months of age with hypotonia, fatigue and poor feeding. He developed multi-organ failure the day after receiving propofol for a sedated magnetic resonance imaging study with respiratory failure, dilated cardiomyopathy, inflammatory myositis, acute hepatitis, systemic hypotension requiring multiple vasopressors and continuous renal replacement therapy for severe lactic acidosis. Metabolic testing raised a concern for underlying mitochondrial disorder. Trio whole genome sequencing, performed as previously described (Kingsmore et al., 2019), revealed a homozygous p.(Arg72Gln) variant in *NFS1* (Supp. Figure S1). Cascade testing in the unaffected sibling did not show the p.(Arg72Gln) variant on either allele. A muscle biopsy of the lateral thigh showed histologic evidence of abnormal mitochondrial organization and lipid accumulation (Supp. Figure S1).

Patient 2 presented at 6 months with biphasic stridor and progressive lethargy requiring intubation and vasopressor support. Laboratory evaluation was significant for lactic acidemia, elevated liver enzymes, and urine organic acids concerning for a mitochondrial disorder. The clinical team was familiar with this case presentation due to their prior experience with the first reported family with *NFS1* related disease (Farhan et al., 2014). Targeted analysis was able to identify a homozygous c.215G>A p.(Arg72Gln) variant in *NFS1.*

Patient 3 presented at 18 years old with gastrointestinal symptoms, fatigue, and exercise intolerance without any preceding childhood symptoms. She had lactic acidemia, elevated creatine kinase, elevated serum glycine and abnormal urine organic acids including elevated lactate, moderate ketonuria and minor elevations in 3-methylglutaconic acid. Whole exome sequencing identified a paternally inherited c.215G>A p.(Arg72Gln) variant and maternally inherited c.1235G>A p.(Arg412His) variant in *NFS1.* The NM_021100.4: c.1235G>A p.(Arg412His) variant was presumed to be rare (MAF= 0.000015, 4 heterozygotes and no homozygotes in gnomAD), affected a highly conserved amino acid, and was predicted to be damaging by 10/10 *in silico* tools. The c.1235G>A p.(Arg412His) variant had not been reported as a disease-causing variant but mapped to the same three protein domains: aminotransferase, class V/cysteine desulfurase; cysteine desulfurase, NifS. Skeletal muscle biopsy from patient 3 showed COX-negative fibers, diffuse markedly decreased succinate dehydrogenase (SDH) staining, abnormal lipid content with mildly increased droplet quantity and size, and increased type 2 fibers.

After enrollment in an IRB approved study (COMIRB# 16–0146) to better characterize the biochemical dysfunction, we performed bioenergetic enzymes assays in the skeletal muscle tissue of patients 1 and 3 with methods outlined in the Supplemental section. Our studies showed a severe decrease in the activity of respiratory chain complex II in both patients, a mild decrease in complex III activity and reduced combined II/III activity in patient 1, and a mild decrease in complex I activity in patient 3 (Table 2). Blue native polyacrylamide gel electrophoresis with in-gel activity staining were performed in patient 3, which showed strongly reduced complex II activity in muscle (Supp. Figure S2). In fibroblasts from patient 1, the respiratory chain enzyme activities were normal, including complex II (184 nmol.min^{-1}.mg protein^{-1}, controls 131–364, 79% of normal −0.6 SD). Cytosolic and mitochondrial aconitase enzymes, which are very sensitive to iron-sulfur

cluster homeostasis, were also measured. In skeletal muscle tissue, both the mitochondrial aconitase activity and the cytosolic aconitase activities were lower in patients 1 and 3 compared to 10 control tissues ($p<0.01$) (Table 2 and Supp. Figure S3). In fibroblasts of patient 1, the aconitase activity was also decreased: mitochondrial aconitase for the patient was 5.13 ± 0.78 nmol. min^{-1}.mg $protein^{-1}$ compared to controls 8 .52 ± 1.78 nmol. min^{-1}.mg $protein^{-1}$, and cytosolic aconitase for the patient was 1.21±0.18 nmol. min^{-1}.mg $protein^{-1}$ compared to controls 3.35 ± 1.00 nmol. min^{-1}.mg $protein^{-1}$, $p<0.01$ (Supp. Figure S3), demonstrating greater sensitivity to the iron-sulfur cluster dysfunction than the respiratory chain enzyme activities. Finally, since deficiency in iron-sulfur clusters often affects lipoate synthase activity, we measured pyruvate dehydrogenase activity which was normal in fibroblasts of patient 1 (1.60 nmol.min^{-1}.mg $protein^{-1}$ compared to controls - 1.66 nmol.min^{-1}.mg $protein^{-1}$, range 0.96 – 3.03 nmol.min^{-1}.mg $protein^{-1}$). Western blot analysis of lipoylated pyruvate dehydrogenase and α-ketoglutarate dehydrogenase enzymes showed a mild decrease of lipoylation in muscle from patient 3, and no clear decrease in either muscle or fibroblasts from patient 1, thus surprisingly providing limited evidence for the impairment of lipoate synthesis (Supp. Figure S4).

Discussion

Our report expands upon the phenotypic and biochemical profile that further establishes biallelic variants in *NSF1* as a causal gene of multi-system mitochondrial disease. The two infant patients (patients 1 and 2) had the same homozygous c.215G>A p.(Arg72Gln) variant described in the case report by Farhan et al (2014). The authors of that paper identified the novel variant through exome sequencing and demonstrated pathogenicity of the variant by showing reductions in NFS1 protein levels compared to controls through Western blot analysis and deficiency in respiratory chain complex II and III on muscle and liver tissue through enzymology assays. In addition, co-immunoprecipitation with antibodies against NFS1 from patient fibroblast cells and wild-type cells showed reduction in co-precipitation with NFS1 and ISD11 suggesting that the first steps of iron-sulfur cluster synthesis were compromised, which likely explained the phenotypic similarity between patients with NFS1 and ISD11 disease (Lim et al., 2013).

After combining the data of our cohort and all previously published cases of NFS1 variants, we found that eight of the nine total individuals were homozygous for the p.Arg72Gln variant and presented in infancy (Table 1). Most presented at less than 7 months of age with acute clinical and metabolic decompensation without prior history of developmental delay. Presenting symptoms included fatigue, lethargy, poor feeding, and varying degrees of hypotonia with extremity weakness followed by rapidly progressive clinical decompensation with lactic acidemia, elevated liver enzymes, and multi-organ system failure. Six of the eight individuals were critically ill requiring intensive care unit management though not all patients were able to survive the initial course. Reported complications included respiratory failure, dilated or hypertrophic cardiomyopathy, myocardial ischemia, hemorrhagic pancreatitis, pulmonary hemorrhage, adrenal insufficiency, and seizures. In contrast to the original report by Farhan and colleagues (Farhan et al., 2014), none of the newly reported patients had seizures, which likely reflects the clinical heterogeneity for this variant. It is also possible that the seizures were secondary to critical illness and metabolic

derangements rather than inherent epileptogenicity of the brain. While four infants died from their initial presentation, one infant from Family 2 and the two additional infants described in this report (patients 1 and 2) survived their initial hospital course and none had further relapses requiring intensive care at the time of this publication.

With respect to the biochemical profile, core features in individuals with biallelic variants in *NFS1* include lactic acidemia, varying levels of creatine kinase and transaminase elevations, elevated glycine, and evidence of complex II and variably I and III dysfunction on respiratory chain analysis. All patients had abnormal urine organic acids with peaks in different Krebs cycle intermediates though there were no specific biochemical signatures. The infants in Family 2 as well as patients 1 and 3 in our cohort had elevated serum glycine which has been described in patients with variant non-ketotic hyperglycinemia in other disorders of iron-sulfur cluster biogenesis (*BOLA3*, *GLRX5*) as a result of lipoate synthase deficiency (Baker et al., 2014). Our respiratory chain findings are consistent with prior respiratory chain enzyme analyses and similar to other iron-sulfur clusteropathies where complex II seems to be the most sensitive to iron-sulfur cluster defects with complex I and III variably affected (A. V. Vanlander & Van Coster, 2018; Vögtle et al., 2018). Our functional analysis also suggests decreased aconitase activity in both the cytosol and mitochondria which was previously only demonstrated in cell models (Fosset et al., 2006). The normal lipoylation of proteins in both muscle and fibroblasts from patient 1 probably reflects the variability of the biochemical presentation of an incomplete defect.

The spectrum of the phenotypic severity among the various infants with the same biallelic variant could be explained by genetic heterogeneity and the circumstances of the triggering event. In Family 2, it remains unclear why the third infant survived her initial course while her siblings did not. However, it should be noted that the deceased siblings were found to have an additional homozygous variant in *NDUFAF5* while the surviving sibling had a heterozygous variant in that gene. This finding confounds the reported clinical variability as the significance of the *NDUFAF5* gene is unknown. Furthermore, enzymatic analyses were not available for all three siblings to compare potential functional variabilities. The later onset presentation in patient 3 at age 18 years with hypotonia, extremity weakness and lactic acidemia but without multi-organ system failure further expands the clinical spectrum of *NFS1*-related disease. The COX negative fibers observed in muscle biopsy are likely secondary to mitochondrial dysfunction. A primary COX deficiency is less likely given concurrent SDH deficiency. Additionally, most primary COX deficiencies are due to mutations in nuclear DNA which was not reported in the whole exome analysis for patient 3 (Rak et al., 2016). To our knowledge, this is the first reported patient with compound heterozygous variants in *NFS1*, and suggests that the homozygous p.(Arg72Gln) variant causes a more severe presentation due to a worsening respiratory chain function.

Infections and other clinical circumstances leading to the initial metabolic crisis are external factors that can affect the severity of presentation. In our cohort, patient 1 had a particularly severe course with very elevated creatine kinase (41,475 U/L) compared to the other cases and a severe cardiomyopathy. He was found to have concurrent non-SARS2 coronavirus and mycoplasma infections that may have caused an infectious myositis in addition to the significantly decreased activity in complex II and complex II/III, which was seen other

NFS1 patients particularly in the muscle (Farhan et al., 2014). He decompensated one day after receiving propofol for a sedated MRI study, further complicating his clinical course. Propofol infusion syndrome is a well-recognized phenomenon in patients with mitochondrial disorders as propofol has been shown to interact with coenzyme Q to impede respiratory chain function by decreasing complex II+III activity (A. V. Vanlander et al., 2012; Arnaud Vincent Vanlander et al., 2015).

Defects in iron-sulfur cluster protein synthesis leading to mitochondrial dysfunction disproportionately affect highly metabolic tissues including the central nervous system, cardiac muscle, skeletal muscle, renal tubules, and the liver (Nordin, Larsson, Thornell, & Holmberg, 2011; A. V. Vanlander & Van Coster, 2018). The milder phenotype in fibroblasts could relate to tissue specific differences such as differential stability of the mutant protein but could also relate to higher concentrations of the substrate cysteine and the cofactor pyridoxal-phosphate present in the tissue culture medium. Dysfunction in the iron-sulfur cluster pathway can have fatal outcomes due to the potential for rapid multi-organ deterioration especially in young infants. Although many patients with iron-sulfur clusteropathies have poor clinical prognosis, early identification of *NFS1* variants may lead to more favorable outcomes. Patient 1 had a striking recovery of his cardiac and renal function after surviving his initial illness. The clinical trajectories and phenotypic variations seen in our patients with *NFS1*-associated disease are similar to the patients with *LYRM4*-associated disease where the infants were more severely affected compared to the milder phenotypes in the adolescent and young adult patients. The mechanism behind this phenotypic difference may be explained by the low levels of endogenous cysteine in early infancy (Lim et al., 2013; A. V. Vanlander & Van Coster, 2018). Abnormalities in the iron-sulfur cluster biosynthesis are thought to affect young infants due to low activity of hepatic cystathionase, which is essential for the endogenous synthesis of cysteine in the neonatal period with gradually increasing activity after the first few months of life (Zlotkin & Anderson, 1982). Hepatic metallothionein degradation may provide an additional source of cysteine, and its level is highest in the newborn period but drops rapidly by four months (Zlotkin & Cherian, 1988). The inverse relationship between cystathionase and metallothionein may lead to further decreased iron-sulfur cluster production which is already impaired in patients with *NFS1* or *LYRM4* associated disease. Exogenous sources of cysteine should also be considered as formula tends to have higher amounts of cysteine compared to breast milk and can affect total cysteine levels. This is especially relevant for infants and less so for older patients. For this reason, patient 1 was started early on N-acetylcysteine which theoretically provided additional sources of cysteine as well as restoration of glutathione levels. In addition to ubiquinol and B vitamins, pyridoxal-5-phosphate was also added since it is a co-factor for cysteine desulfurase, and for some PLP-containing enzymes that can have a chaperone-like function (Cellini, Montioli, Oppici, Astegno, & Voltattorni, 2014).

In conclusion, we present two additional unrelated patients with the homozygous p.(Arg72Gln) variant and one patient with compound heterozygous variants in *NFS1* that add to the existing literature for the spectrum of phenotypes seen in *NFS1*-related disorders. The biochemical analyses that were only previously studied in cell models further add evidence for the functional sequelae to establish *NFS1* as a disease-causing gene. Based

on the clinical courses so far, patients with the homozygous variant seem to have severe clinical decompensations in the setting of an external stressor but can recover well and improve clinically once they are past the critical first year of life. However, there is a potential for complications from mitochondrial dysfunction to present later in adolescence and early adulthood as reflected in the case of patient 3 with heterozygous variants. These cases highlight the importance of utilizing precision medicine to rapidly identify these patients as early as possible to provide appropriate supportive management and long-term prognostication.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

All data that support the findings of this study are present in the manuscript and in the supplemental materials.

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Web Resources

OMIM, http://www.omim.org/

gnomAD, https://gnomad.broadinstitute.org/

GeneMatcher, https://genematcher.org/

Table 1:

Clinical features, diagnostic evaluations, and treatment

	Farhan et al			*Hershikovitz et al*			*Current paper*		
	Family 1			**Family 2**			**Family 3**	**Family 4**	**Family 5**
	Patient IV-I	**Patient IV-II**	**Patient IV-III**	**Patient II-2**	**Patient II-4**	**Patient II-5**	**Patient 1**	**Patient 2**	**Patient 3**
Current Age	Deceased	Deceased	21 years	Deceased	Deceased	6 years	1.5 years	2.5 years	21 years
Age at Presentation	7 months	6 weeks	6 months	40 days	7 months	1 month	6 months	6 months	18 years
Sex	Female	Male	Male	Male	Male	Female	Male	Male	Female
Ethnicity/ Heritage	Old Order Mennonite	Old Order Mennonite	Old Order Mennonite	Christian Arab	Christian Arab	Christian Arab	Mixed European[†]	Old Order Mennonite[‡]	Mixed European[§]
NFS1 variants	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln)	c.215G>A p.(Arg72Gln), c.1235G>A p.(Arg412His)
Zygosity	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Homozygous	Compound heterozygous
Method of mutation detection	Whole exome sequencing	Whole exome sequencing	Whole exome sequencing	Whole exome sequencing	Whole exome sequencing	Whole exome sequencing	Whole genome sequencing	Targeted variant analysis	Whole exome sequencing
Clinical Features									
Developmental delays prior to presentation	−	−	−	−	−	−	−	−	−
Hypotonia	+	+	+	+	+	+	+	+	+
Bulbar weakness	−	−	−				+	−	−
Extremity weakness	+	+	+	+	+	+	+	+	+
Dilated or hypertrophic Cardiomyopathy, EF%	+	+	−	+	+	−	Dilated, EF <25%, now 61%	Mild dilatation of ascending aorta Normal EF	Normal EF 60–65%
Elevated CK		+	+	+	+	+	+	+	−
Lactic acidosis	+	+	+	+	+	+	+	+	+
Respiratory failure	+	+	−	+	+	−	+	+	−
Elevated liver enzymes	+	+	+	+	+	+	+	+	+
Seizures	+	+	−	−	−	−	−	−	−

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	Farhan et al			*Hershikovitz et al*			*Current paper*		
	Family 1			**Family 2**			**Family 3**	**Family 4**	**Family 5**
	Patient IV-I	**Patient IV-II**	**Patient IV-III**	**Patient II-2**	**Patient II-4**	**Patient II-5**	**Patient 1**	**Patient 2**	**Patient 3**
Other exam findings	Hemorrhagic pancreatitis	Myocardial ischemic injury	Mild tremor	Adrenal insufficiency			Poland syndrome[¶] areflexia	Hepatomegaly Pulmonary hemorrhage	Mild strabismus
Diagnostic evaluations									
Peak lactate (mmol/L)							12.5	3.9	12.1
Peak CK (U/L)							41,475	752	1,456
Elevated serum glycine				+	+	+	+		+
Abnormal urine organic acids	+	−	+	+	+	+	+	+	+
CSF amino acids							Normal		
Muscle biopsy	+	+					+		+
Histological		Abnormal[††]					Abnormal[‡‡]		Abnormal[§§]
ETC enzyme analysis	↓ complex II, III	↓complex II, III		↓complex I, II, III			↓ complex II, III		↓ complex II
Abnormal neuroimaging (MRI/CT)		Cerebral infarction (CT)					−		−
EEG							Normal		
EMG/ NCS							↓ conduction amplitudes		
Clinical Course									
Required ICU admission	+	+	−	+	+		+	+	−
Survived initial course	−	+	−	−	+		+	+	
Relapses (#)		1			0		0	2	0
Readmission to ICU		+					−	−	
Treatment									
N-Acetylcysteine Current dose mg/kg/day							60mg/kg/day		
Coenzyme Q10 Current dose mg/kg/day		+	5mg/kg/day				15 mg/kg/day	15 mg/kg/day	15 mg/kg/day
B vitamins Current dose mg/kg/day		+					B-complex 10 mg/kg/day	Thiamine, 15 mg/kg/day	B2, 4 mg/kg/day

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	Farhan et al			*Hershikovitz et al*			*Current paper*		
	Family 1			**Family 2**			**Family 3**	**Family 4**	**Family 5**
	Patient IV-I	**Patient IV-II**	**Patient IV-III**	**Patient II-2**	**Patient II-4**	**Patient II-5**	**Patient 1**	**Patient 2**	**Patient 3**
Folinic acid Current dose mg/kg/day							1 mg/kg/day		
Levocarnitine Current dose mg/kg/day			30mg/kg/day				81 mg/kg/day	100 mg/kg/day	50mg/kg/day
Pyridoxial-5-phosphate Current dose mg/kg/day							5 mg/kg/day		
Alpha-lipoic acid Current dose mg/kg/day							10 mg/kg/day	15mg/kg/day	
Creatine monohydrate Current dose mg/kg/day			100mg/kg/day				50 mg/kg/day		

Abbreviations: +, present/yes; −, not present/no; ↓, decreased; CK, creatine kinase; MRI, magnetic resonance imaging; CT, computed tomography; EEG, electroencephalogram; EMG/NCS, electromyogram/nerve conduction study; EF, ejection fraction; CSF, cerebrospinal fluid; ICU, intensive care unit. Blank cells indicate not applicable or no available information

†Limited heritage. Mixed European, parents unrelated

‡Within same community as family 1 but not closely related

§Limited heritage. Mixed European, German descent on maternal side

¶Hypoplasia of the left pectoralis major muscle consistent with prior diagnosis of Poland syndrome

††Basophilic granules, decreased SDH, focally prominent subsarcolemmal collections of PAS-positive glycogen

‡‡Ragged red fibers, increased lipids, abnormal mitochondrial morphology

§§Markedly decreased/deficient SDH, abnormal lipid droplets, increased type 2 fibers

Table 2:

Skeletal muscle respiratory chain complex enzyme activity of patients 1 and 3

	Enzyme activity[a] (Reference Range)	SD[b]	Patient % of control mean	Enzyme activity/ Citrate Synthase (Reference Range)	SD
Patient 1					
Complex I	40.8 (22.4 – 95.9)	−0.1	90%	127 (84.3 – 288.8)	−0.7
Complex II	16.2 (46.2 – 175.9)	**−4.9**	**18%**	**50** (187.3 – 861.9)	**−6.1**
Complex III	5.6 (2.15 – 63.65)	−1.4	32%	17.4 (8.64 – 234.16)	−1.6
Complex II+III	27.0 (24 – 124.4)	**−2.1**	**43%**	**84** (151 – 638.4)	**−3.2**
Complex IV	1.4 (0.90 – 6.7)	−1.3	53%	4.3 (4.1 – 26.9)	−1.3
Citrate synthase	322.0 (152.7 – 434)	1.0	125%	---	--
Aconitase mito[c]	**9.35** (18.3–42.2)		**32%**		
Aconitase cyto[c]	**1.92** (3.15–8.05)		**40%**		
Patient 3					
Complex I	**20.7** (22.4 – 95.9)	**−2.0**	46%	**72** (84.3 – 288.8)	**−2.7**
Complex II	11.1 (46.2 – 175.9)	**−6.1**	**12%**	**39** (187.3 – 861.9)	**−7.2**
Complex III	7.1 (2.15 – 63.65)	−1.1	41%	25 (8.64 – 234.16)	−1.2
Complex II+III	**19.6** (24 – 124.4)	**−2.9**	**31%**	**68** (151 – 638.4)	**−3.9**
Complex IV	1.4 (0.90 – 6.7)	−1.4	51%	5.0 (4.1 – 26.9)	−1.4
Citrate synthase	287.0 (152.7 – 434)	0.5	112%	---	--
Aconitase mito	**0.57** (18.3–42.2)		**1.9%**		
Aconitase cytosol	**0.22** (3.15–8.05)		**4.5%**		

a. The activities in skeletal muscle tissue of each respiratory chain complex and of the combined complex II-III are shown expressed as nmol.min^{-1}.mg protein^{-1} and as the ratio over the activity of citrate synthase. The patient's values are followed by normal values in parentheses.

b. The values are also expressed as standard deviations (Z-score) of the log transformed values of controls, which are normally distributed, and a Z-score of <−2 is considered significant.

c. Aconitase activities, mitochondrial (mito) and cytosolic (cyto) are provided as nmol.min^{-1}.mg protein^{-1} and the percentage of the average of controls. The patient's values are followed by normal values of 10 controls in parentheses. Activities that are reduced are highlighted in bold.