Phenotypic continuum of NFU1-related disorders

Rauan Kaiyrzhanov1, Maha S. Zaki2, Tracy Lau1, Sambuddha Sen3, Reza Azizimalamiri4, Mina Zamani5, Gözde Yeşil Sayın6, Taru Hilander7, Stephanie Ethymiou1, Viorica Chelban1, Ruth Brown8, Kyle Thompson9, Maria Irene Scarano10, Jaya Ganesh11, Kairgali Koneev12, Ismail Musab Gulaçar6,13, Richard Person14, Dinara Sadykova15, Yerdan Maidyrov12, Tahereh Seifi5, Aizhan Zadagali16, Geneviève Bernard17,18,19, Katrina Allis14, Houda Zghal Elloumi14, Amanda Lindy14, Ehsan Taghiabadi20, Sumit Verma21, Rachel Logan22, Brian Kirmse23, Renkui Bai14, Shaimaa M. Khalaf24, Mohamed S. Abdel-Hamid25, Alireza Sedaghat26, Gholamreza Shariati27,28, Mahmoud Issa2,13, Jawaher Zeighami28, Hasnna M. Elbendary2, Garry Brown8, Robert W. Taylor9,29, Hamid Galehdari7, Joseph J. Gleeson30,31, Christopher J. Carroll7, James A. Cowan3, Andres Moreno-De-Luca32, Henry Houlden1 & Reza Maroofian1

1Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK
2Human Genetics and Genome Research Division, Clinical Genetics Department, National Research Centre, Cairo, Egypt
3Department of Chemistry and Biochemistry, The Ohio State University, 100 West 18th Avenue, Columbus, Ohio, 43210, USA
4Department of Paediatric Neurology, Golestan, Medical, Educational, and Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
6Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, 34098, Turkey
7Genetics Section, Molecular and Clinical Sciences, St George’s, University of London, London, UK
8Oxford Medical Genetics Laboratories, The Churchill Hospital, Oxford, OX3 7LJ, UK
9Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Faculty of Medical Sciences Newcastle University, Newcastle upon Tyne, NE2 4HH, UK
10Division of Genetics, Cooper Health System, Children’s Regional Hospital, Sheridan Pavilion Camden, New Jersey, 08103, USA
11Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, USA
12Department of Neurology and Neurosurgery, Asfendiyarov Kazakh National Medical University, Almaty, 050000, Kazakhstan
13Department of Genetics, Institute of Graduate Studies in Health Sciences, Istanbul University, Istanbul, 34098, Turkey
14GeneDx, Gaithersburg, Maryland, 20877, USA
15Astan Medical University, Nur-Sultan, Kazakhstan
16L.N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan
17Departments of Neurology and Neurosurgery, Pediatrics and Human Genetics, McGill University, Montreal, Canada
18Division of Medical Genetics, Department Specialized Medicine, McGill University Health Centre, Montreal, Canada
19Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, Canada
20Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran
21Department of Neurology, Emory University School of Medicine, Georgia, Atlanta, USA
22Division of Neurosciences, Children’s Healthcare of Atlanta, Atlanta, Georgia, USA
23Division of Genetics, University of Mississippi Medical Center, Jackson, Mississippi, USA
24Pediatrics Department, Assiut University, Assiut, Egypt
25Medical Molecular Genetics Department, Human Genetics and Genome Research Institute, National Research Centre, Cairo, Egypt
26Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
27Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
28Narges Medical Genetics and Prenatal Diagnosis Laboratory, East Mihan Ave., Kianpars, Ahvaz, Iran
29NHS Highly Specialised Service for Rare Mitochondrial Disorders, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, NE1 4LP, UK
30Department of Neurosciences, University of California, San Diego, La Jolla, California, 92093, USA
31Rady Children’s Institute for Genomic Medicine, San Diego, California, 92025, USA
32Department of Radiology, Autism & Developmental Medicine Institute, Genomic Medicine Institute, Geisinger, Danville, Pennsylvania, 17822, USA

Correspondence
Reza Maroofian, Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK. Tel: +44 (0)20 3448 4069; Fax: +44 (0) 20 7278 5616; E-mail: r.maroofian@ucl.ac.uk

Abstract
Bi-allelic variants in Iron–Sulfur Cluster Scaffold (NFU1) have previously been associated with multiple mitochondrial dysfunctions syndrome 1 (MMDS1) characterized by early-onset rapidly fatal leukoencephalopathy. We report 19 affected individuals from 10 independent families with ultra-rare bi-allelic...
NFU1 missense variants associated with a spectrum of early-onset pure to complex hereditary spastic paraplegia (HSP) phenotype with a longer survival (16/19) on one end and neurodevelopmental delay with severe hypotonia (3/19) on the other. Reversible or irreversible neurological decompensation after a febrile illness was common in the cohort, and there were invariable white matter abnormalities on neuroimaging. The study suggests that MMDS1 and HSP could be the two ends of the NFU1-related phenotypic continuum.

Introduction

Iron–sulfur [Fe-S] clusters are important cofactors that play a role in various cellular functions, including electron transfer along the respiratory chain, citric acid cycle, heme biosynthesis, DNA replication and repair, as well as iron homeostasis.1–3 Iron–Sulfur Cluster Scaffold (NFU1) (MIM: 608100) is an [Fe-S] cluster biosynthesis factor involved in the last steps of maturation and transfer of [4Fe-4S] clusters into target proteins, including lipoic acid synthetase, mitochondrial aconitase and some subunits of respiratory chain complexes I and II.4,5 Bi-allelic variants in NFU1 have previously been associated with multiple mitochondrial dysfunctions syndrome 1 (MMDS1)6 characterized by early-onset leukoencephalopathy leading to a fatal outcome, typically before the age of 15 months.6

Recently, bi-allelic variants in NFU1 have been reported in two individuals with a milder phenotype, presenting with slowly progressive spastic paraplegia with a relapsing–remitting course, long survival, and intact cognition or mild intellectual disability (ID).7,8 Here, we report 19 affected individuals from 10 independent families with ultra-rare bi-allelic NFU1 missense variants associated with a phenotype ranging from early-onset pure to complex hereditary spastic paraplegia (HSP) characterized by a longer survival (16/19) and neurodevelopmental delay (NDD) with severe hypotonia (3/19).

Methods

Participants and clinical investigations

Exome sequencing (ES) and genome sequencing (GS), data sharing between international genetic centers, and the GeneMatcher platform9 were employed to identify the 10 families reported here. Clinical data were collected via a uniform proforma. Parents/legal guardians of all affected individuals consented to the publication of genetic and clinical information. The study was approved by The Research Ethics Committee Institute of Neurology University College London (IoN UCL) (07/Q0512/26) and the local Ethics Committees of each participating center.

Exome sequencing and genome sequencing

Research/diagnostic solo or trio-ES/GS, variant filtering, and variant confirmation by Sanger sequencing and segregation analysis were performed on genomic DNA extracted from blood in different genetic laboratories following the methods previously described (Table 1). Family 10 had a mitochondrial panel test performed for the proband and targeted genotyping for familial variants for the sibling (further details in Table 1 and Supplementary Table S1).

SDS-PAGE and western blot analysis

Human fibroblasts from an affected individual (F1-II:1) were obtained and lysed and samples were subjected to SDS-PAGE and western blotting as described previously15 and a list of primary antibodies is provided (Supplementary Methods).

Protein modeling and characterization, Fe-S cluster transfer monitoring, and enzymatic assays

Protein characterization by variable temperature circular dichroism (CD) and analytical ultracentrifugation, Fe-S cluster transfer monitored by circular dichroism, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase activity assay studies were performed (Supplementary Methods).

Results

Clinical phenotype

The cohort comprises 19 affected individuals from 10 independent families. There were 8 females and 11 males, 13 of whom are currently alive with a mean age of 9.8 ± 5.7 years (range 2.1–25) at the study recruitment (Fig. 1A). Six affected individuals succumbed to their rapidly progressive disease course triggered by a febrile illness between the ages of 6–36 months. Detailed clinical information is provided in Supplementary Table S1 and Supplemental Case Reports.
| Family | Reported in | Ethnic origin | Consanguinity | Genetic investigation | WES/WGS and variant filtering method | Variant at cDNA level* | Variant at protein level* | Sex | Age at last examination | Age of onset | Disease duration | Family history | Mortality | Mortality Mortality | Onset with delayed walking/progressive spastic paraplegia | Mild ID/Cognitive impairment | Lower limb spasticity |
|--------|-------------|---------------|---------------|----------------------|------------------------------------|-----------------------|--------------------------|-----|------------------------|-------------|------------------|---------------|-----------|---------------------|-----------------------------|---------------------------|-------------------------|
| F1 (F1-II:1, F1-II:2) | This study | Iranian-Arab | Yes | ES | Makrythanasis et al., 2018 | c.721G>C | p.(Val241Leu) | 2F | 10y and 17 y | 6–9 m and 9–10 m | 9 y and 11 y | Yes | Alive (2) | Yes (2) | No | Yes (2) |
| F2 (F2-II:1) | This study | American | No | ES | Makrythanasis et al., 2018 | c.298G>C | p.(Ala100Pro); p.(Arg101Gly) | 1M | 25 y | 1 y | 8 y and 11 y | No | No | Yes (2) | No (2) | Yes (2) |
| F3 (F3-II:1, F3-II:2) | This study | Egyptian | Yes | ES | Guillen Sacoto et al., 2020 | c.362T>C | p.(Val121Ala) | 3F | 2 y and 6 y and 12 y | 0.9 y and 1.2 y | 6 m and 0.5 y | Yes | Alive (2) | Yes (2) | No (2) |
| F4 (F4-II:4, F4-II:5, F4-II:6) | This study | Egyptian | Yes | ES | Guillen Sacoto et al., 2018 | c.295C>G | p.(Leu99Val) | 1M | 4.5 m and 6 m | 1 y and 0.9 m | 2 y and 1.6 y | Yes | Alive (2) | Yes (2) | No (2) |
| F5 (F5-II:1, F5-II:2) | This study | Kazakh | Yes | ES | Lindy et al., 2018 | c.398T>C | p.(Phe88Ser) | 2M | 3 y and 2.2 y | 3–4 m (2) | 6 y and 5 y | Yes | Alive (2) | Yes (2) | No (2) |
| F6 (F6-II:2, F6-II) | This study | Turkish | No | ES | Tonduti et al., 2015 | c.629G>T | p.(Cys210Phe) | 1M | 7.5 and 6.5 y | 18 m (2) | 28.5 y and 25 m | Yes | Alive (2) | Yes (2) | No (2) |
| F7 (F7-II:1, F7-II:2) | This study | American | Yes | ES | Tonduti et al., 2015 | c.548C>G | p.(Pro183Arg); p.(Leu133Pro) | 1M | 12 y | 2 y | Died at 13 m and 25 m | No | No | Yes (1) | Alive (2) |
| F8 (F8-II:5, F8-II) | This study | European-American | No | ES | Uzunhan et al., 2020 | c.629G>T | p.(Phe189Arg) | 1M | 1 y and 2 y | 6 m and 3 y | Died after febrile illness (2) | Yes | Alive | Yes |
| F9 (F9-II:1) | This study | Not reported | Yes | No | Not reported | c.565G>A | p.(Gly189Arg) | 1 M | 30 y | 23 m | Yes | No | Alive |
| F10 (F10-II:1, F10-II:2) | This study | Not reported | No | Yes | Not reported | c.146delC | p.(Pro49LeuTer8); p.(Gly189Arg) | 1 F | 3.5 y | 23 m | Yes | No | Alive |

(Continued)
| F1 (F1-II:1, F1-II:2) | F2 (F2-II:1) | F3 (F3-II:1, F3-II:2) | F4 (F4-II:4, F4-II:5, F4-II:6) | F5 (F5-II:1, F5-II:2) | F6 (F6-II, F6-II) | F7 (F7-II:1, F7-II:2) | F8 (F8-II:5, F8-II) | F9 (F9-II:1) | F10 (F10-II:1, F10-II:2) | Previously published reports with HSP phenotype |
|----------------------|--------------|-----------------------|--------------------------------|-----------------------|------------------|---------------------|-------------------|---------------|-------------------------|-----------------------------------------------|
| Deterioration with loss of milestones with intercurrent viral/febrile illness | Yes | Yes | No (2) | Yes (2), No (1) | Yes (2) | Yes (2) | No (2) | Yes (2) | Yes | Yes (2) | Yes |
| Recovery of loss of milestones/previous status after recovery from illness | Yes (1), NA (1) | Yes | NA (2) | No (2), NA (1) and Yes (1) | Yes (2) | NAP | No (1), NA (1) | Yes | No (2) | Yes | Yes |
| Mild WMHs in T2WI | Yes (2) | No** | No (2) | Yes (2) | Yes | Yes (1), NA (1) | Yes (1)**, NA (1) | No** | Yes (2) | Yes | Yes |
| Mild T2 hyperintensity in BG | Yes (2) | No** | No (2) | Yes (2) | Yes (1), NA (1) | Yes (1)**, NA (1) | Yes (1)**, NA (1) | No** | Yes (2) | No | No |
| CC abnormalities | Yes (2) | No** | No (2) | Yes (2) | Yes (1), No (1) | Yes (1), NA (1) | Yes (1), NA (1) | No (1), NA (1) | No** | Yes (2) | Yes |
| Areas of cystic degeneration/leukomalacia | No (2) | No** | No (2) | No (2) | No | No (1), NA (1) | No (1), NA (1) | No** | Yes (1), No (1) | No | Yes |

ES, exome sequencing; GS, genome sequencing; WES, Whole exome sequencing; WGS, Whole genome sequencing; NGS, next generation sequencing; F, female; M, male; y, year(s); m, month(s); NAP (no applicable); T2WI, T2 weighted images; BG, basal ganglia; CC, corpus callosum.

*Based on the transcripts NM_001002755.4 & NP_001002755.1 respectively.

**Reported as normal. Study not independently reviewed.

***Questionable, please refer to Supplementary Table S1.

Allele frequencies of the variants, in silico predictions, and ACMG classifications are provided in Supplementary Table S1.
Prenatal features and neonatal periods were unremarkable in all subjects. No delay in early developmental milestones including head control, crawling, sitting, and speech was reported in 14/19 individuals. Cognitive function was impaired in 5/18 individuals. Gait acquisition was delayed due to either the insidious onset of limb spasticity in 13/19 individuals or spasticity precipitated by a deterioration in the context of a febrile illness in 4/19 individuals (Fig. 1B). The lower limb spasticity was detected at a mean age of 12 ± 6 months in the cohort. Spasticity was progressive leading to contractures in 13/19 persons and necessitating Achilles’ tendon repair surgery in 4/19 affected individuals.

Remarkably, febrile illness leading to metabolic decompensation played a significant role in the disease course of 13/19 individuals. Thus, in F1-II:1 it led to febrile seizures followed by the onset of lower limb spasticity and reversible cognitive and motor regression, and truncal hypotonia. Similarly, F5-I:1, F6-II:3, and F8-II:5 developed reversible truncal hypotonia and/or progressive lower limb spasticity following several episodes of febrile illness. Intermittent and reversible gait deterioration associated with episodes of febrile illness was reported in F2-II:1 and F9-II:1, along the course of their disease, which was described as waxing and waning. While the episodes of febrile illness had not been fatal for these persons, the other six affected individuals had regression and developed severe muscular hypotonia leading to early mortality after a febrile illness.

The mean disease duration at the most recent examination for the living individuals was 8.8 ± 5.7 years (range 1.5–24). Neuromuscular examination revealed lower limb spasticity in all persons with increased deep tendon reflexes, upgoing plantar reflexes, and mild symmetrical muscle wasting. Muscle tone from the upper limbs was uniformly intact. While two individuals were wheelchair-bound, eight could ambulate using crouches or rollator walker, and three could walk independently with spastic gait. Ataxic gait was reported in 2/12 individuals, which was intermittent in one person (F2-II:1). The oldest individual in the cohort was aged 25 years and had a normal cognitive function and could perform most activities of daily living despite his slowly progressive lower limb spasticity. Seven persons had results available of metabolic screening, which were unremarkable in all.

Brain MRI DICOM files were available for six individuals (F1-II:1, F1-II:2, F7-II:1, F8-II:5, F10-II:1, and F10-II:2), low-resolution photos of brain MRI studies were available for four individuals (F4-II:5, F4-II:6, F5-II:2, and F6-II:2), and photos of a head CT in one person (F5-II:1). All available neuroimaging studies were independently reviewed by a board-certified neuroradiologist. Four additional individuals had neuroimaging studies that were reported as normal but not independently reviewed (F2-II:1, F3-II:1, F3-II:2, and F9-II:1). Shared neuroimaging findings included T2 hyperintense signal involving the bilateral posterior centrum semiovale, corona radiata, and periretal regions (9/9, 100%); T2 hyperintense signal involving the bilateral thalami (9/9, 100%), basal ganglia (7/9, 78%), and pons and cerebellum (7/8, 88%), hypoplastic corpus callosum (9/10, 90%), bilateral cerebral white matter volume loss (8/11, 73%), mega cisterna magna (9/11, 82%), mild prominence of the lateral ventricles and frontoparietal sulci (7/11, 64%), and scattered areas of restricted diffusion which may represent active demyelination, metabolic encephalopathy, or acute/subacute ischemia (3/4, 75%). A single individual (1/11, 9%) had areas of cystic degeneration/leukomalacia in the white matter of the bilateral frontal lobes and another (1/11, 9%) had vermian hypoplasia. Figure 1D shows shared neuroimaging findings and the Supplemental information has case-based descriptions of the brain MRI/CT interpretations.

Genetic analysis reveals ultra-rare bi-allelic variants in NFU1

A total of 9 different NFU1 missense variants were identified in this study (Figs. 2A to C). Exome sequencing carried out in the proband F1-II:1 revealed a homozygous likely pathogenic NFU1 variant: NM_001002755.4: c.721G>C, p.(Val241Leu). In the third affected individual F2-II:1, trio-ES identified compound heterozygous NFU1 variants: a variant of uncertain significance (VUS), c.298G>C, p.(Ala100Pro) inherited from the father and a VUS, c.301A>G, p.(Arg101Gly) inherited from the mother.

Exome sequencing of four unrelated Egyptian families, F3 to F6, led to the identification of the same pathogenic homozygous NFU1 variant: c.362T>C, p.(Val121Ala). A homozygous VUS, c.295C>G, p.(Leu99Val) was also identified by ES of the proband of family 7. In family 8, a homozygous VUS, c.263T>C, p.(Phe88Ser) was identified by ES of the proband. Trio-ES in family 9 found compound heterozygous NFU1 variants: a maternally inherited likely pathogenic variant, c.629G>T, p.(Cys210Phe) and a paternally inherited VUS, c.548C>G, p.(Pro183Arg). In family 10, the combination of a mitochondrial gene panel and Sanger sequencing revealed potential compound heterozygous NFU1 variants: a VUS, c.629G>T, p.(Cys210Phe) and a VUS, c.398T>C, p.(Leu133Pro). Apart from, p.(Leu99Val), all the homozygous variants are residing within sizeable regions of homozygosity. All the variants are either absent or observed in extremely low allele frequencies in numerous population variant frequency databases (~2 mln alleles). They affect highly
Phenotypic continuum of NFU1-related disorders

(A) Age distribution of affected individuals.

(B) Delayed gait acquisition.

(C) Neurological regression/decompensation.

(D) MRI images showing various manifestations of NFU1-related disorders.

© 2022 The Authors. Annals of Clinical and Translational Neurology published by Wiley Periodicals LLC on behalf of American Neurological Association.
conserved residues across different species (Fig. 2D) and segregated with the disease phenotype in all the families (Fig. 2A). No other relevant variants associated with neurological or neurodevelopmental disorders were identified in the currently known monogenic disease-causing genes in the ES data.

The characteristics of the variants are further summarized in Table 1 and Supplementary Table S1.

Steady-state levels of OXPHOS complexes I, II, III, and IV are decreased in homozygous p.(Val241Leu) fibroblasts

Western blot analysis on fibroblast samples showed that subunits of each complex of the electron transport chain (CI-CIV) were decreased in the affected individual F1-II:1 compared to the heterozygous mother (F1-I:1) and a pediatric control, whereas levels of complex V subunit ATP5A remained relatively unchanged (Fig. 2E).

Functional study of the p.(Val241Leu) variant

Protein modeling revealed all nine variants were located in the NFU1 protein domains. Six variants, p.(Phe88Ser), p.(Leu99Val), p.(Ala100Pro), p.(Arg101Gly), p.(Val121Ala), and p.(Leu133Pro), were found in the NiF U N-terminal domain while the remaining three, p.(Pro183Arg), p.(Cys210Phe), and p.(Val241Leu), lie in the NiFU C-terminal [Fe-S] cluster binding domain (Figs. 2C,F, Supplementary Results). The p.(Val241Leu) variant was further investigated via in vitro [Fe-S] cluster reconstitution experiments, however, the results showed no significant change in the protein’s secondary structure (Supplementary Fig. S1C, Supplementary Results).

Additional discussion of the structural and functional biochemistry of other variants is provided in the Supplementary Material. As patient fibroblasts were available only for the p.(Val241Leu) variant, the impact of the variant on the enzymatic activities of PDH and KGDHC was measured only for this variant. Enzymatic activity assays in fibroblasts derived from F1-II:1, homozygous for p.(Val241Leu), showed decreased activity for PDH (0.50 nmol/mg protein/min; normal range 0.6–0.9 nmol/mg protein/min), while the mean activity in the heterozygous mother was normal (0.82 nmol/mg protein/min; normal range 0.6–0.9 nmol/mg protein/min). The KGDHC activity was not impaired (Supplementary Fig. S2, Supplementary Results).

Discussion

The bioenergetic function of the mitochondrion is dependent on Fe-S cluster-containing proteins. Three distinctly organized biosynthetic pathways are involved in the maturation of Fe-S clusters in mammals with the last step involving NUBPL, NFU1, BOLA3, IBA57, ISCA2, and ISCA1. Bi-allelic pathogenic variants in five of them, starting from NFU1 in the above-mentioned list, are currently associated with MMDs types 1 to 5 respectively, and typically present with severe and fatal early onset encephalopathy with multiple biochemical abnormalities.6 An atypical presentation of MMDS has been reported in several individuals, mainly encompassing a slightly milder disease course with a longer survival for NFU1 and ISCA2, or a complex HSP phenotype for NFU1 and IBA57.7,8,20 Our report expands the number of individuals with bi-allelic NFU1 variants presenting with complex HSP and also describes previously unreported pure HSP phenotype, thereby suggesting that MMDS1 and HSP could be the two ends of the NFU1-associated phenotypic continuum.

Pyramidal symptoms in the form of spastic tetraparesis have frequently been reported in individuals with typical MMDS1 presentation, highlighting the role of mitochondria and axonal transport in the corticospinal tract function.6 Currently, due to a large overlap between HSP and other inherited neurological disorders, at least 28/81 genetic forms of HSP are assigned alternative phenotypes on the Online Mendelian Inheritance in Man (OMIM) database, resulting in a diagnostic challenge.21 Therefore, delineating the full phenotypic spectrum of disease-associated genes and accurate clinical classification are important for diagnostic rates.

Another remarkable aspect of the present cohort is the presence of neurological decompensation after a febrile
Phenotypic continuum of NFU1-related disorders

R. Kaiyrzhanov et al.
illness. Interestingly, three distinct responses to episodes of febrile illness were observed in our cohort: (1) fatal outcome after the first or consecutive episode in six individuals; (2) onset of spasticity with variably reversible motor and cognitive regression in three individuals; and (3) intermittent and fully reversible gait deterioration in four other individuals. The episodes of neurological deterioration, most likely caused by acute illness-induced metabolic decompensation, is common in mitochondrial diseases, but have rarely been reported in the mitochondrial causes of HSP.

A remarkable inter-and intrafamilial phenotypic variability was observed in the present cohort and the same amino acid substitutions of the NFU1 protein have been observed in both MMDS1 and HSP cases (Supplementary discussion for details). The relatively milder course and phenotypic variability of the most commonly reported MMDS1-associated NFU1 variant, c.565G>A, p.(Gly189Arg), was further supported in reports by Uzunhan et al. (2020) and Tonduti et al. (2015), who described individuals with the same NFU1 variant and presenting with HSP, with one individual reaching the age of 30 years. Defects in NFU1 seem to present with a range of phenotype severities suggesting the clinical spectrum of NFU1-associated disease (Fig. 1C). The constellation of shared neuroimaging findings noted in individuals for whom images were available is similar to the findings reported by Tonduti et al. (2015), including white matter abnormalities of the periventricular regions and thinning of the corpus callosum. Similarly, neuroimaging features present in our cohort overlap with some of those reported by Uzunhan et al. (2020). These include white matter hyperintensity, which was present in most of the individuals in the present series, cystic degeneration and cavitation in the frontal regions, which was noted in individual F10-II.2, and areas of restricted diffusion involving the bilateral subcortical and periventricular white matter, noted in three out of four individuals with available diffusion-weighted imaging.

Apart from the possible epigenetic factors, we suspect that the variants observed in the present study might be causing milder defects for the NFU1 structure and function compared to the MMDS1-linked variants. The different variants might affect, for example, partner protein binding in a distinct way leading to high phenotypic variability connected to NFU1 variants (Supplementary Discussion for further details). The residual PDH activity and the normal KGDHC activity in F1-II:1 might be consistent with a less severe form of the disease with prolonged survival. The decreased OXPHOS protein levels and the only slightly affected PDH and normal KGDHC activity results in F1-II:1 fibroblasts might be explained by the changed affinity of the mutated NFU1 for the different partner proteins (Supplementary Discussion for further details). The disparity between plasma and CSF metabolites levels has been shown in an individual with MMDS, suggesting the manifestation of biallelic NFU1 variants with tissue-specific phenotypes. In summary, we report 16 affected individuals with an HSP presentation of bi-allelic NFU1 variants and 3 affected individuals with GDD, hypotonia, longer survival, and fatal response to metabolic decompensation, which contrasts with the typical MMDS1 presentation of NFU1 deficiency and highlights the NFU1-associated disease continuum.

Acknowledgments

The authors would like to thank the participants and their families for their essential help with this work. We are grateful to The Spastic Paraplegia Foundation, The Brain Research Trust (BRT), The UK HSP Society, The Medical Research Council (MRC UK MR/J004758/1, G0802760, G1001253), The Wellcome Trust (equipment and the Synaptopathies strategic award (104033)) and the EU FP7/2007-2013 under grant agreement number 2012-305121 (NEUROMICS), The Muscular Dystrophy Association (MDA) USA, Muscular Dystrophy UK, Rosetrees Trust, Ataxia UK, and British Neurological Surveillance Unit (BNSU). R.K. was supported by the Research Training Fellowship grant from the European Academy of Neurologists. V.C. is supported by the Association of British Neurologists/MSA Trust Clinical Research Training.
Conflict of Interest

G.B. is/was a consultant for Passage Bio Inc (2020–2021) and Ionis (2019). She is/was a site investigator for the Alexander’s disease trial of Ionis (2021), Metachromatic leukodystrophy of Shire/Takeda (2020–2021), Krabbe and GM1 gene therapy trials of Passage Bio, and Adrenoleukodystrophy/Hematopoietic stem cell transplantation natural history study of Bluebird Bio (2019) and a site sub-investigator for the MPS II gene therapy trial of Regenxbio (2021). She has received an unrestricted educational grant from Takeda (2021–2022). She serves on the scientific advisory board of the Pelizaeus-Merzbacher Foundation and is the Chair of the Medical and Scientific Advisory Board of the United Leukodystrophy Foundation. She is on the editorial boards of Neurology Genetics, Frontiers in Neurology – Neurogenetics, and Journal of Medical Genetics. RP, KA, HZE, AL and RB are employees of GeneDx, LLC.

References

1. Yoon T, Cowan JA. Frataxin-mediated iron delivery to ferrochelatase in the final step of heme biosynthesis. J Biol Chem. 2004;279(25):25943-25946.
2. Tong WH, Rouault TA. Metabolic regulation of citrate and iron by aconitases: role of iron-sulfur cluster biogenesis. Biometals. 2007;20(3–4):549-564.
3. White MF, Dillingham MS. Iron-Sulphur clusters in nucleic acid processing enzymes. Curr Opin Struct Biol. 2012;22(1):94-100.
4. Lill R, Dutkiewicz R, Freibert SA, et al. The role of mitochondria and the CIA machinery in the maturation of cystolic and nuclear iron-sulfur proteins. Eur J Cell Biol. 2015;94(7–9):280-291.
5. Vanlander AV, Van Coster R. Clinical and genetic aspects of defects in the mitochondrial iron-sulfur cluster synthesis pathway. J Biol Inorg Chem. 2018;23(4):495-506.
6. Lebignet E, Schiff M, Golimelli-Cohen MP. A review of multiple mitochondrial dysfunction syndromes, syndromes associated with defective Fe-S protein maturation. Biomedicine. 2021;9(8):989.
7. Tondu D, Dorboz I, Imbard A, et al. New spastic paraplegia phenotype associated to mutation of NFU1. Orphanet J Rare Dis. 2015;10:13.
8. Uzunhan TA, Çakar NE, Seyhan S, Aydin K. A genetic mimic of cerebral palsy: homozygous NFU1 mutation with marked intrafamilial phenotypic variation. Brain Dev. 2020;42(10):756-761.
9. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36(10):928-930.
10. Makrythanasis P, Maroofian R, Stray-Pedersen A, et al. Biallelic variants in KIF14 cause intellectual disability with microcephaly. Eur J Hum Genet. 2018;26(3):330-339.
11. Guillen Sacoto MJ, Tchasovnikarova IA, Torti E, et al. De novo variants in the ATPase module of MORC2 cause a neurodevelopmental disorder with growth retardation and variable craniofacial dysmorphism. Am J Hum Genet. 2020;107(2):352-363.
12. Jalali Sefid Dashti M, Gamieldien J. A practical guide to filtering and prioritizing genetic variants. Biotechniques. 2017;62(1):18-30.
13. Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet. 2010;11(6):415-425.
14. Lindy AS, Stosser MB, Butler E, et al. Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. Epilepsia. 2018;59(5):1062-1071.
15. Thompson K, Majd H, Dallabona C, et al. Recurrent De novo dominant mutations in SLC25A4 cause severe early-onset mitochondrial disease and loss of mitochondrial DNA copy number. Am J Hum Genet. 2016;99(4):860-876.
16. Liu W, Xie Y, Ma J, et al. IBS: an illustrator for the presentation and visualization of biological sequences. Bioinformatics. 2015;31(20):3359-3361.
17. Nizon M, Bouteron A, Boddaert N, et al. Leukoencephalopathy with cysts and hyperglycemia may
result from NFU1 deficiency. Mitochondrion. 2014;15:59-64.

18. Ahting U, Mayr JA, Vanlander AV, et al. Clinical, biochemical, and genetic spectrum of seven patients with NFU1 deficiency. Front Genet. 2015;6:123.

19. Hartman TG, Yosovich K, Michaeli HG, et al. Expanding the genotype-phenotype spectrum of ISCA2-related multiple mitochondrial dysfunction syndrome-cavitating leukoencephalopathy and prolonged survival. Neurogenetics. 2020;21(4):243-249.

20. Lossos A, Stümpfig C, Stevanin G, et al. Fe/S protein assembly gene IBA57 mutation causes hereditary spastic paraplegia. Neurology. 2015;84(7):659-667.

21. Saputra L, Kumar KR. Challenges and controversies in the genetic diagnosis of hereditary spastic paraplegia. Curr Neurol Neurosci Rep. 2021;21(4):15.

22. Parikh S, Goldstein A, Karaa A, et al. Patient care standards for primary mitochondrial disease: a consensus statement from the mitochondrial medicine society. Genet Med. 2017;19(12):7-8. doi:10.1038/gim.2017.107

23. Casari G, Marconi R. Spastic Paraplegia 7. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. GeneReviews®. University of Washington, Seattle; 2006.

24. Baple E, Crosby A. Troyer Syndrome. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. GeneReviews®. University of Washington, Seattle; 2004.

25. Ames EG, McFadden K, Keegan CE, et al. Metabolomic profiling of plasma and cerebrospinal fluid highlight differences and diagnostic utility in multiple mitochondrial dysfunctions syndrome. In: McCabe E, McCabe L, Beitner R, et al., eds. Society for Inherited Metabolic Disorders 41th Annual Meeting. Molecular Genetics and Metabolism; 2019:219-320.

Supporting Information

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

Data S1: Supplemental information including case reports, supplemental methods, supplemental experimental results and discussion

Table S1: Supplemental clinical-genetic table