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Expanding the *AFG3L2* Spectrum

A Link to Axonal Neuropathy

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Abstract

Objectives

The *AFG3L2* gene encodes a mitochondrial AAA-protease involved in inner mitochondrial membrane (IMM) proteostasis. Heterozygous variants in the AFG3L2 proteolytic domain cause Spinocerebellar Ataxia type 28, heterozygous variants in the AFG3L2 ATPase domain cause Optic Atrophy type 12, while biallelic variants lead to recessive Spastic Ataxia type 5. In this study, we aimed to investigate the link between *AFG3L2* haploinsufficiency and Charcot-Marie-Tooth (CMT) phenotypes.

Methods

We performed clinical evaluation, genetic analyses, electrophysiology, and functional studies in 1 patient's fibroblasts.

Results

In this study, we report a patient presenting with progressive symmetric distal muscle atrophy, weakness, *pes cavus*, and sensory deficits at lower limbs, in the absence of cerebellar or pyramidal signs. Electrophysiologic studies confirmed axonal sensorimotor neuropathy. After excluding common CMT-related genes, clinical exome sequencing revealed a heterozygous truncating variant in *AFG3L2* [NM_006793.3:c.121C > T; p.(Arg41*)]. In patient's fibroblasts, we observed ∼50% reduction in AFG3L2 protein levels, which is sufficient to hyperactivate the stress-sensitive IMM protease OMA1. Consequently, we detected increased OPA1 processing, mitochondrial shortening, and activation of the integrated stress response.

Discussion

These findings suggest that *AFG3L2* haploinsufficiency can underlie axonal CMT, expanding the clinical spectrum of *AFG3L2*-related diseases and emphasizing its potential inclusion in CMT diagnostic panels.

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Supplementary Material

Introduction

The *AFG3L2* gene encodes a mitochondrial AAA-protease localized in the inner mitochondrial membrane (IMM). AFG3L2 assembles into homo-oligomers or hetero-oligomers with paraplegin (*SPG7*), forming the *m*-AAA proteases, essential for protein quality control in the IMM.[1] Pathogenic variants in the *AFG3L2* gene cause distinct neurodegenerative disorders. Heterozygous variants in the AFG3L2 proteolytic domain lead to dominant Spinocerebellar Ataxia type 28 (SCA28),[2] while biallelic variants cause recessive Spastic Ataxia type 5 (SPAX5).[3,4]

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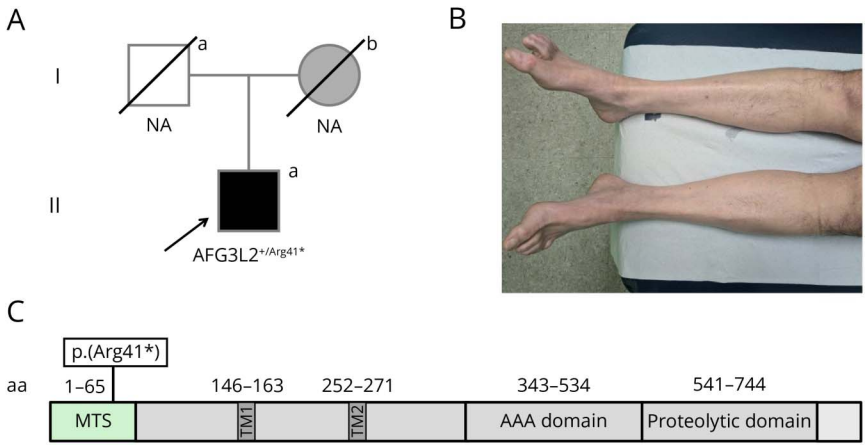
Figure 1 Genetic and Clinical Features of the Patient



(A) Pedigree of the proband. The proband's mother (individual I-b) reportedly presented with *pes cavus*, although her phenotype was not formally evaluated. + = Reference. (B) Clinical photograph of the proband showing bilateral *pes cavus* and distal lower-limb atrophy. (C) Location of the patient's variant in the *AFG3L2* gene.

Conversely, heterozygous variants in the AFG3L2 ATPase domain are associated with dominant Optic Atrophy type 12 (OPA12).[5,6]

We previously demonstrated in *Afg3l2*$^{-/-}$ murine fibroblasts and in fibroblasts from patients carrying *AFG3L2* pathogenic variants the accumulation of nonassembled mtDNA-encoded proteins, resulting in proteotoxic stress within the IMM. This stress triggers the hyperactivation of the IMM-resident protease OMA1, which in turn overprocesses the key regulator of IMM fusion OPA1, thereby promoting mitochondrial fragmentation.[6,7] We have also recently documented the activation of the OMA1-DELE1-HRI axis (the mitochondrial ISR[8]) in preclinical SPAX5 models. By suppressing global protein translation while promoting the expression of cytoprotective genes such as ATF4, we found that this pathway is a protective mechanism to survive mitochondrial proteotoxicity caused by AFG3L2 depletion.[4]

AFG3L2 variants have not previously been associated with isolated peripheral neuropathy. This is particularly relevant given the genetic heterogeneity of CMT, where mitochondrial dysfunction and altered proteostasis are increasingly recognized as pathogenic mechanisms.[9]

In this study, we report the first case of a patient carrying a heterozygous truncating variant in *AFG3L2* [NM_006793.3: c.121C > T; p.(Arg41*)], presenting with chronic axonal polyneuropathy in the absence of cerebellar, pyramidal, or optic involvement.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

Written informed consent to disclose in agreement with regulations of Ospedale San Raffaele was obtained from the patient for publication of clinical details and images; identifying information has been removed, and the signed consent form is on file.

Details on experimental protocols are reported in eMethods.

Data Availability

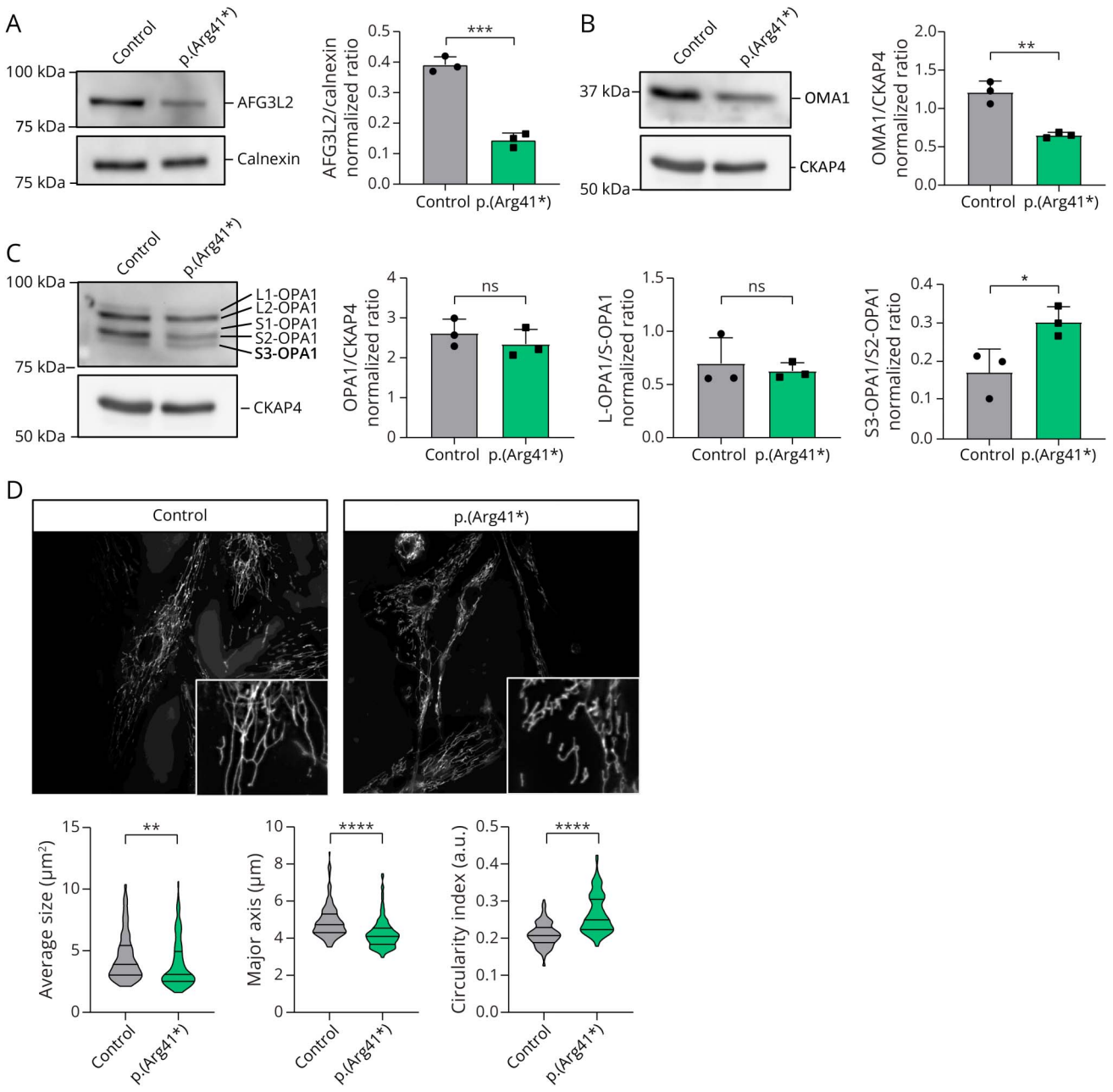
Anonymized data not published within this article will be made available by request from any qualified investigator.

Results

Case Report

The patient is in his seventh decade with no relevant medical history, born to nonconsanguineous parents. At the time of evaluation, both parents were deceased because of unrelated causes. However, the mother had bilateral *pes cavus* (Figure 1A). Symptoms began in the fifth decade with nonprogressive distal lower-limb paresthesias. Electrodiagnostic study performed 8 years after onset showed absent sensory and motor responses in the lower limbs, normal conduction in the upper limbs, and needle EMG with chronic neurogenic changes in distal leg muscles. Examination revealed distal lower-limb atrophy and bilateral *pes cavus* (Figure 1B), mild stocking-distribution hypoesthesia and hypopallesthesia, and weakness of tibialis anterior (MRC 4/5), extensor hallucis longus, extensor digitorum longus, and peroneals (MRC 3+/5). Lower-limb reflexes were absent; upper-limb reflexes were preserved. The CMT Neuropathy Score was 6/36 (higher scores reflect greater severity).[10] Mild parkinsonian signs (hypomimia, generalized hypokinesia/bradykinesia, and a coarse tremor of all limbs) were present, but the patient declined Dopamine Transporter SPECT. No pyramidal or cerebellar signs were observed. Blood tests excluded acquired neuropathies, and brain MRI and optical coherence tomography were normal.

Figure 2 Halved AFG3L2 Protein Levels and OMA1 Activation in Patient Fibroblasts



(A–C) WB analysis performed on patient and control's cell lysates, showing levels of (A) AFG3L2, (B) OMA1, and (C) OPA1, with relative quantification; calnexin and CKAP4 were used to verify equal loading. Bars represent means ± SEM; n = 3. (D) Representative images of the patient's and control's human primary fibroblasts' mitochondrial network highlighted by MitoTracker staining, along with a relative quantification of the major axis, average size, and circularity index. An unpaired Student's t-test was used to quantify all the experiments: ns = not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

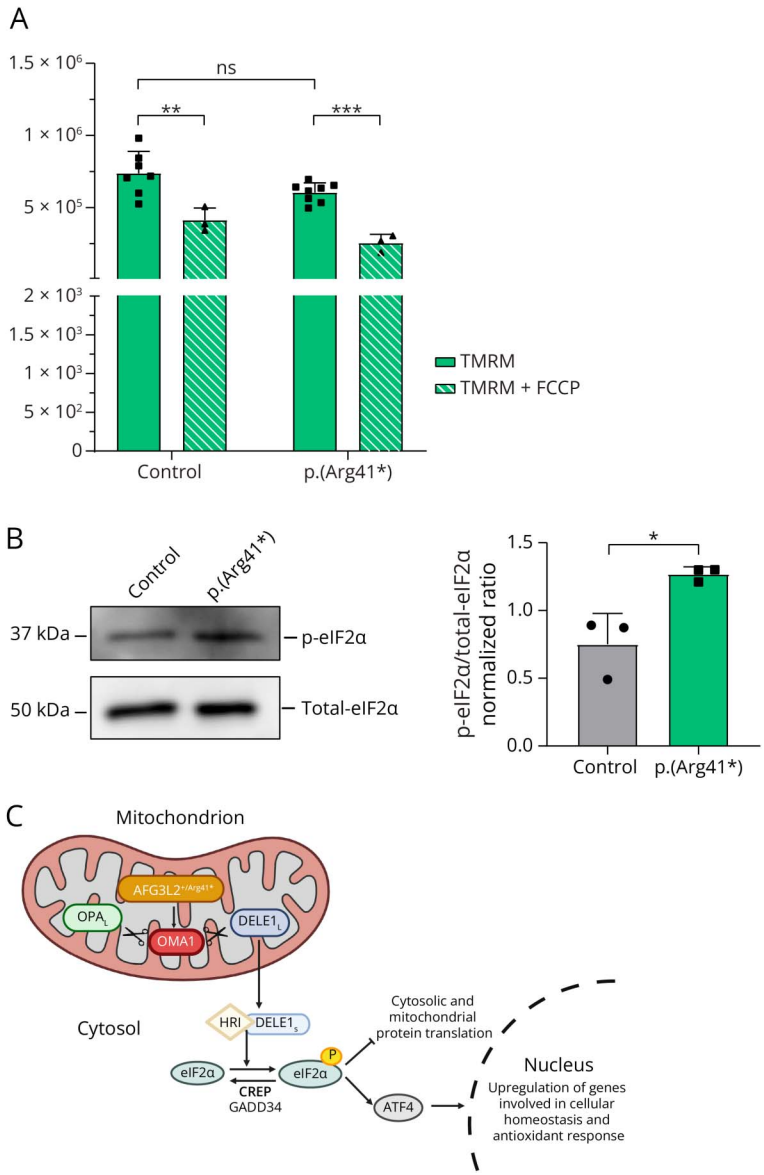
Genetic Testing

A previous analysis excluded PMP22 duplication. Clinical exome sequencing (CES) identified a heterozygous variant in *AFG3L2* (NM_006796.3:c.121C > T, p.Arg41*) resulting in a premature stop codon within the mitochondrial targeting sequence (Figure 1C). The variant is extremely rare (gnomAD 2.1.1 hg37: 0.0035%, European excluding Finnish: 0%; gnomAD 34.1.1 hg38: 0.0015%, European excluding Finnish: 0.00025%) and predicted pathogenic due to likely nonsense-mediated mRNA decay. According to ACMG criteria, it was classified as a Variant of Uncertain Significance. No additional variants with pathogenic relevance were detected (see eMethods), and segregation analysis could not be performed because of the unavailability of family members.

Functional Studies

We first evaluated the effect of the p.(Arg41*) variant on AFG3L2 protein levels by western blot on primary skin

Figure 3 Activation of the Integrated Stress Response in Patient Fibroblasts



(A) Analysis of ΔΨm by flow cytometry of TMRM fluorescence intensity in patient and control's primary fibroblasts. Bars represent mean ± SEM; n = at least 3. Two-way ANOVA with Tukey correction: ns = not significant, $^{**}p < 0.01$, $^{***}p < 0.001$. (B) WB performed on patient and control's cells lysates, showing levels of P-eIF2α and Total-eIF2α, with relative quantification. Bars represent means ± SEM; n = 3. An unpaired Student's t-test was used: ns = not significant, $^{*}p < 0.05$. (C) Schematic representation of the pathogenic cascade based on the results obtained. Created with BioRender.com.

fibroblasts comparing with 1 age- and sex-matched healthy control. A ~50% reduction in AFG3L2 was observed in patient's cells (Figure 2A), consistent with haploinsufficiency. OMA1 protein levels were reduced in patient vs control cells (Figure 2B), reflecting OMA1 autocatalytic degradation on activation[7] in the disease condition. Consequently, we analyzed the post-translational processing of OPA1. Human OPA1 has 8 isoforms from alternative splicing, which retain 1 or 2 cleavage sites (CS1 and CS2). These isoforms are further processed by the IMM proteases YME1L (at CS2) and OMA1 (at CS1), generating 5 distinguishable forms in western blot: 2 long (L-OPA1: L1, L2) and 3 short (S-OPA1: S1, S2, S3) (Scheme in eFigure 1), which are normally maintained in balance under physiologic conditions.[11] Although we did not detect any difference in OPA1 amount or in the L-OPA1/S-OPA1 ratio, we found a significant increase of the OPA1 S3 band relative to S2 in the patient, consistent with the selective activation of OMA1 (Figure 2C) (eFigure 1). In agreement, live imaging revealed that patient fibroblasts have shorter, although still tubular, mitochondria compared with the elongated, interconnected network seen in control (Figure 2D).

We assessed mitochondrial membrane potential (ΔΨm), which was preserved in p.(Arg41*) fibroblasts, indicating intact mitochondrial cristae integrity and function (Figure 3A).

Finally, we examined whether the activation of OMA1 induced the mitochondrial integrated stress response (ISR) in

patient cells via the DELE1-HRI pathway.[8] Western blot analysis revealed increased phosphorylation of eIF2α in patient fibroblasts compared with controls (Figure 3B), indicating ISR activation (Scheme in 3C).

Discussion

In this study, we describe the first case of a patient carrying a heterozygous truncating variant in *AFG3L2* [NM_006793.3:c.121C > T; p.(Arg41*)], presenting with a chronic axonal polyneuropathy without cerebellar, pyramidal, or optical nerve involvement. The clinical phenotype was consistent with a CMT-like neuropathy, characterized by distal muscle atrophy, sensory loss, and *pes cavus*. Functional studies on patient-derived fibroblasts revealed halved AFG3L2 protein levels, consistent with haploinsufficiency. The downstream cascade involves OMA1 hyperactivation, which on the one hand enhances OPA1 processing in CS1 leading to a mild but significant mitochondrial shortening, and on the other hand triggers the mitochondrial ISR. This molecular cascade is consistent with mechanisms previously described in *AFG3L2*-related disorders, especially in SPAX5,[4] and strongly supports the pathogenic role of the identified variant in a mitochondrial axonopathy phenotype.

Notably, the ISR and the closely related unfolded protein response have been increasingly implicated in various forms of CMT disease,[12,13] suggesting a converging mechanism with significant therapeutic implications.

The novelty of our report lies in linking AFG3L2 haploinsufficiency to an isolated axonal neuropathy, thereby expanding the clinical spectrum of *AFG3L2*-related disorders. To date, pathogenic variants in *AFG3L2* have been associated with SCA28, SPAX5, and OPA12, with peripheral neuropathy reported only in a few patients within syndromic contexts.[3,14] The identified AFG3L2 p.(Arg41*) variant has previously been reported as causing SCA28, although no clinical description of the patient was provided.[5] The same patient was later included in a cohort with metabolic presentations, again without detailed clinical information,[15] thus preventing assessment of potential peripheral nervous system involvement. Our findings indicate that AFG3L2 should be considered in the differential diagnosis of CMT-like presentations, particularly when common CMT genes are negative.

It is increasingly evident that several genes classically associated with complex syndromes can manifest primarily—or even exclusively—with peripheral neuropathy.[9] This phenotypic variability may result from partial, rather than complete, loss of gene function, leading to milder or more restricted clinical presentations. Additional factors, including genetic or environmental modifiers, may further influence the disease phenotype.

Nevertheless, this study presents some limitations, including the absence of segregation data and the fact that the patient was evaluated using CES rather than whole-genome sequencing.

In conclusion, our findings identify AFG3L2 haploinsufficiency as a novel cause of isolated axonal neuropathy and expand the spectrum of *AFG3L2*-associated disorders.

Author Contributions

A. Rocco: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. C. Laurini: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. Y.M. Falzone: major role in the acquisition of data; analysis or interpretation of data. S. Calzavara: major role in the acquisition of data; analysis or interpretation of data. U. Del Carro: major role in the acquisition of data; analysis or interpretation of data. S.C. Previtali: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. F. Maltecca: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data.

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Disclosure

C. Laurini, Y.M. Falzone, and S.C. Previtali are members of the ERN-NMD Network. Go to Neurology.org/NG for all disclosures.

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References

1. Quiros PM, Langer T, Lopez-Otin C. New roles for mitochondrial proteases in health, ageing and disease. *Nat Rev Mol Cell Biol.* 2015;16(6):345-359. doi:10.1038/nrm3984
2. Di Bella D, Lazzaro F, Brusco A, et al. Mutations in the mitochondrial protease gene AFG3L2 cause dominant hereditary ataxia SCA28. *Nat Genet.* 2010;42(4):313-321. doi:10.1038/ng.544
3. Pierson TM, Adams D, Bonn F, et al. Whole-exome sequencing identifies homozygous AFG3L2 mutations in a spastic ataxia-neuropathy syndrome linked to mitochondrial m-AAA proteases. *PLoS Genet.* 2011;7(10):e1002325. doi:10.1371/journal.pgen.1002325
4. Franchino CA, Brughera M, Baderna V, et al. Sustained OMA1-mediated integrated stress response is beneficial for spastic ataxia type 5. *Brain.* 2024;147(3):1043-1056. doi:10.1093/brain/awad340
5. Caporali L, Magri S, Legati A, et al. ATPase domain AFG3L2 mutations alter OPA1 processing and cause optic neuropathy. *Ann Neurol.* 2020;88(1):18-32. doi:10.1002/ana.25723
6. Baderna V, Schultz J, Kearns LS, et al. A novel AFG3L2 mutation close to AAA domain leads to aberrant OMA1 and OPA1 processing in a family with optic atrophy. *Acta Neuropathol Commun.* 2020;8(1):93. doi:10.1186/s40478-020-00975-w
7. Tulli S, Del Bondio A, Baderna V, et al. Pathogenic variants in the AFG3L2 proteolytic domain cause SCA28 through haploinsufficiency and proteostatic stress-driven OMA1 activation. *J Med Genet.* 2019;56(8):499-511. doi:10.1136/jmedgenet-2018-105766

8. Guo X, Aviles G, Liu Y, et al. Mitochondrial stress is relayed to the cytosol by an OMA1-DELE1-HRI pathway. *Nature* 2020;579(7799):427-432. doi:10.1038/s41586-020-2078-2
9. Rossor AM, Haddad S, Reilly MM. The evolving spectrum of complex inherited neuropathies. *Curr Opin Neurol.* 2024;37(5):427-444. doi:10.1097/WCO.0000000000001307
10. Shy ME, Blake J, Krajewski K, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. *Neurology.* 2005;64(7):1209-1214. doi:10.1212/01.WNL.0000156517.00615.A3
11. Song Z, Chen H, Fiket M, Alexander C, Chan DC. OPA1 processing controls mitochondrial fusion and is regulated by mRNA splicing, membrane potential, and Yme1L. *J Cell Biol.* 2007;178(5):749-755. doi:10.1083/jcb.200704110
12. Clayton BLL, Popko B. Endoplasmic reticulum stress and the unfolded protein response in disorders of myelinating glia. *Brain Res.* 2016;1648(Pt B):594-602. doi:10.1016/j.brainres.2016.03.046
13. Spaulding EL, Hines TJ, Bais P, et al. The integrated stress response contributes to tRNA synthetase-associated peripheral neuropathy. *Science.* 2021;373(6559):1156-1161. doi:10.1126/science.abb3414
14. Musova Z, Kaiserova M, Kriegova E, et al. A novel frameshift mutation in the AFG3L2 gene in a patient with spinocerebellar ataxia. *Cerebellum.* 2014;13(3):331-337. doi:10.1007/s12311-013-0538-z
15. Coutelier M, Hammer MB, Stevanin G, et al.; Spastic Paraplegia and Ataxia Network. Efficacy of exome-targeted capture sequencing to detect mutations in known cerebellar ataxia genes. *JAMA Neurol.* 2018;75(5):591-599. doi:10.1001/jamaneurol.2017.5121