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CASE REPORT

Case Report of Friedreich's Ataxia and *ALG1*-Related Biochemical Abnormalities in a Patient With Progressive Spastic Paraplegia

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ABSTRACT

Frataxin is an evolutionarily conserved mitochondrial protein responsible for iron homeostasis and metabolism. A deficiency of frataxin (encoded by *FXN*) leads to Friedreich's ataxia (FRDA), a progressive disorder that affects both the central and peripheral nervous systems, most commonly via a pathogenic GAA trinucleotide expansion. In contrast, pathogenic variants in *ALG1* in humans cause a form of congenital disorder of glycosylation. Here, we present a 15-year-old boy with a clinical presentation that raised concern for complex hereditary spastic paraplegia (HSP), with motor features including progressive spastic paraparesis, cervical dystonia, cerebellar dysfunction, and diminished lower extremity reflexes. The proband was initially found to have a novel compound heterozygous variant in *ALG1* on exome sequencing, along with N-glycan profiling revealing evidence of defective mannosylation and Western blot analysis demonstrating an 84% reduction in ALG1 expression. Although several of his clinical features could be explained by the *ALG1* variant specifically or considered as part of the presentation of CDGs in general, there were additional phenotypes that suggested an alternative, or additional, genetic diagnosis. Subsequently, he was found to have biallelic pathogenic GAA repeat expansions in *FXN* on genome sequencing, leading to a diagnosis of FRDA. Given that FRDA explained all his clinical features, the *ALG1* variant may have been a hypomorphic form and/or a biochemical phenotype. Our findings underscore the importance of considering FRDA as a differential diagnosis in cases of complex HSP and demonstrate the utility of unbiased genome sequencing approaches that include detection of trinucleotide repeat expansions for progressive motor disorders.

1 | Introduction

Frataxin is an evolutionarily conserved mitochondrial protein responsible for iron homeostasis and metabolism. Deficiency of frataxin, encoded by *FXN*, leads to the autosomal recessive condition Friedreich's ataxia (FRDA), a progressive disorder that affects the central and peripheral nervous systems. The most frequent pathogenic variant in *FXN* is a GAA trinucleotide repeat expansion, resulting in frataxin suppression (Cook and Giunti 2017). FRDA has an average onset between 10 and 15 years of age (Bidichandani and Delatycki 1993). Common features of the disorder include gait ataxia, spasticity, contractures, dysarthria, scoliosis, bowel and bladder dysfunction, and cardiomyopathy (Cook and Giunti 2017).

N-linked glycosylation is an evolutionarily conserved posttranslational modification process. In eukaryotes, the beginning of glycan assembly involves the stepwise addition of monosaccharides onto a dolichyl diphosphate carrier in the endoplasmic reticulum, catalyzed by genes in the asparagine-linked glycosylation (ALG) family (Larkin and Imperiali 2011). *ALG1* encodes the enzyme β-1,4 mannosyltransferase. ALG1 transfers mannose units onto a core oligosaccharide precursor that will undergo further modifications required for successful N-glycosylation (Ng et al. 2016; Xu et al. 2018).

Pathogenic variants in *ALG1* in humans cause a congenital disorder of glycosylation (ALG1-CDG). ALG1-CDG is associated with neurological and system effects, such as developmental delay, hypotonia, epilepsy, hearing loss, and vision changes (Morava et al. 2012; Xu et al. 2018).

Here, we present a 15-year-old boy with a presentation that raised concern for complex hereditary spastic paraplegia (HSP). On exome sequencing, he was found to have a novel compound heterozygous variant in *ALG1*, with functional studies suggesting biochemical disruption. Although several of his clinical features could be explained by the *ALG1* variant, there were additional phenotypes that suggested an alternative, or additional, genetic diagnosis. Subsequently, research genome sequencing identified biallelic pathogenic GAA repeat expansions in *FXN*, later confirmed through CLIA-certified testing, consistent with FRDA. Given that FRDA explained all his clinical features, the *ALG1* variant may have been a hypomorphic form and/or a biochemical phenotype. Thus, this case (1) demonstrates the importance of FRDA in the differential diagnosis of complex HSP and (2) underscores the need for broad sequencing approaches (i.e., genome sequencing) that include detection of trinucleotide repeat expansions for progressive motor disorders.

2 | Methods

2.1 | Exome/Genome Sequencing

Following informed consent (IRB: P00032816), the proband and parents underwent research exome sequencing (ES), with results clinically validated at GeneDx, as part of an institutional pipeline for gene/variant discovery for different disease cohorts (Children's Rare Disease Cohorts Initiative) (Rockowitz et al. 2020). This patient and his variant were reported previously with limited details as part of analysis of the first 50 cases in a sequencing effort for cerebral palsy (CP) and CP masqueraders (Chopra et al. 2022). The proband subsequently underwent research trio whole-genome sequencing (WGS; NCT05354622, IRB: P00039630) including analysis using ExpansionHunter (Dolzhenko et al. 2019). Trio WGS analysis suggested homozygous GAA repeat of at least 119 units, which is above the threshold for most pathogenic *FXN* variants. Detection of pathogenic GAA repeat expansion of both *FXN* alleles from research genome sequencing was verified through CLIA-certified testing (University of Chicago, Ataxia Repeat Expansion Panel), though this panel did not specify precise repeat expansion length.

2.2 | ALG1 Expression

Proteins from controls (GM7492, GM1651) and patient cells (HNDS 0166-01) were isolated in solution by lysing the harvested fibroblast cells in RIPA buffer supplemented with protease inhibitors (SIGMA, USA) and 1 mM PMSF. Protein concentration was determined using Pierce BCA assay (Thermo Fisher Scientific, USA). Protein samples (20 μg) of controls and patient were first mixed with sample buffer (4x Laemmli buffer, 10% BME) and samples were denatured for 5 minutes at 95°C. Samples were then loaded onto the 10% NuPAGE Bis-Tris gel and electrophoretically separated at 200 V for 1 hour at room temperature (RT). Protein bands were transferred to NC membrane using iBlot transfer device (Thermo Fisher IB21001) at 23 V for 6 minutes. The membrane was blocked using BSA in 0.1% Tween-20 in TBS (TBST) for 1 hour at RT followed by incubation with primary antibodies at 4 °C for 24 hours (Rabbit ALG1 1:500 Cat#12872-1-AP Proteintech; Mouse β-Actin 1:20,000 Cat#AC001 ABclonal). The blot was then washed with TBST for three times 10 minutes each, followed by the incubation for 1 hour at 4°C with secondary antibodies (Donkey anti-Mouse Dy-light 800 Cat#SA510172 Invitrogen; Donkey anti-Rabbit Dy-light 680 Cat#SA5-10042 Invitrogen). Membrane was washed again with TBST for three times 10 minutes each. The bands were visualized and quantified in an Odyssey Fc system and Li-Cor Odyssey Image Studio version 3.1 using 700 and 800 channels (Li-Cor Biosciences, Lincoln, NE, USA). Quantified data were analyzed using Excel and GraphPad Prism software.

3 | Results

3.1 | Clinical Report

The proband is a 15-year-old boy. He was born at 42 weeks by vaginal delivery with unremarkable birth history.

He began walking at age 13 months. Toe walking was pronounced at age 2 years, and he started wearing braces at age 6 years. He received a diagnosis of attention deficit hyperactivity disorder (ADHD) and hypertrophic cardiomyopathy at age 7 years. Family history was noncontributory.

On examination at age 8.5 years, he had decreased range of motion and spasticity in his lower extremities, 1+ tendon reflexes, and impaired balance. MRI brain and spine at age 8 years was normal except for scoliosis.

He initially benefited from baclofen, night splints, stretching, and physical therapy. However, by age 11 years, his spasticity worsened following a growth spurt, and his gait appeared ataxic. At age 12 years, he began experiencing intermittent muscle stiffening and trialed dantrolene. He developed knee and hip flexion contractures. By age 13 years, he had significant lower extremity spasticity, mild cervical dystonia, cerebellar dysfunction (mild dysmetria, dysdiadochokinesia, slow horizontal saccades, and truncal ataxia), and absent reflexes in the lower extremities consistent with peripheral neuropathy. He had symptoms of neurogenic bladder dysfunction. He had bilateral high frequency hearing loss. These changes raised speculation of a potentially progressive disorder, namely HSP.

3.2 | Exome Sequencing, *ALG1* Variant Characterization

He underwent research ES, which identified a compound heterozygous variant in *ALG1*: NM_019109.4: c.123_126delCGACinsTGGTG, p.(Asp42GlyfsX34), maternally inherited, likely pathogenic; c.827G > A, p.(Arg276Gln), paternally inherited, variant of uncertain significance (VOUS). The frameshift variant is not observed at a significant frequency in large population cohorts (Lek et al. 2016) and has not been previously published as pathogenic or benign. The missense variant is observed in 0.0632% (177/280032 alleles) in large population cohorts (Lek et al. 2016). In silico analysis supports a deleterious effect of this missense variant on protein structure/function. There are no previous publications detailing this variant as pathogenic or benign, although a different missense variant at the same residue (R276W) has been published as likely pathogenic and implicated in ALG1-CDG (Ng et al. 2016).

After the results of ES, the proband underwent N-glycan profiling, which showed mildly increased Hex1GlcNA at 0.14% of total glycans (normal ≤ 0.10%) and NeuAc1Hex1HexNax2 at 0.21% (normal < 0.06%), indicating possible mild mannosylation deficiency. Carbohydrate-deficient transferrin testing and urine oligosaccharides were normal. Evaluation by hematology showed mild iron deficiency anemia and normalization of previously noted anomalies (prolonged prothrombin time and low protein C level) with no evidence of coagulation or thrombophilia defect. Functional studies in fibroblasts from the proband revealed an 84% reduction in ALG1 expression (Figure 1).

3.3 | Genome Sequencing, *FXN* Variant Characterization

The *ALG1* variant or a diagnosis of a CDG could have explained several of his clinical features, including cardiomyopathy, scoliosis, spasticity, and progressive symptoms. However, he had additional clinical findings—ataxia, diminished reflexes, and lack of intellectual disability—that suggested an alternative, or additional, genetic diagnosis. He underwent research genome sequencing, revealing biallelic pathogenic GAA repeat expansions in *FXN* consistent with a diagnosis of FRDA, later confirmed through CLIA-certified testing. Research analysis using ExpansionHunter estimated at least 119/119 GAA repeat units in *FXN*. The proband's blood frataxin level was 3 ng/mL (normal range > 19).

4 | Discussion

The diagnosis of FRDA explains all of our patient's clinical features. Specifically, elements of our patient's presentation common to previous reports of FRDA include progressive cerebellar dysfunction/ataxia, progressive lower extremity spasticity, peripheral neuropathy, hearing loss, cardiomyopathy, scoliosis, and neurogenic bladder dysfunction (Bidichandani and Delatycki 1993).

This case underscores the importance of FRDA in the differential diagnosis of complex HSP, particularly in the presence of ataxia, absent/diminished lower extremity reflexes, and cardiomyopathy. The diagnosis of FRDA has significant clinical implications for treatment, given the availability of a disease-modifying therapy (omaveloxolone). Our patient presented with progressive lower extremity spasticity as the dominant clinical feature. A case series of three siblings with FRDA described spastic paraplegia in one of the patients with FRDA. This case series summarized an additional 21 patients from the literature

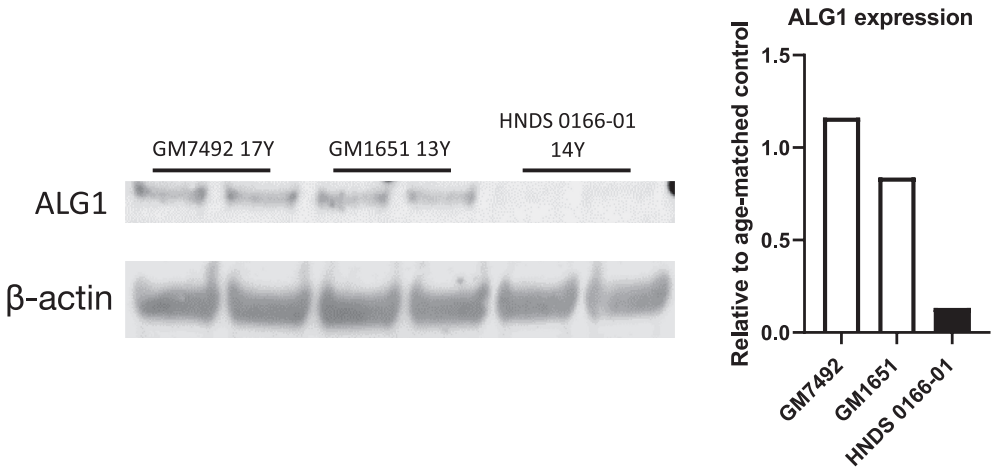

FIGURE 1 | Western blot showing reduced expression of ALG1 in the patient (HNDS 0166-01) versus two age-matched controls. Patient was 14 years at the time of Western blot analysis.

with FRDA and spasticity, reported as presentations of FRDA, FRDA with spastic ataxia, FRDA with spastic paraparesis, FRDA with spastic tetraparesis, and spastic ataxia-Acadian FRDA (Badhwar et al. 2004). Although spastic paraparesis without ataxia as the initial presentation of FRDA is less common, *FXN* is one of the genes included on some commercial spastic paraplegia gene panels.

The relationship of the *ALG1* variant to our patient's presentation is unclear, and we presently do not have enough evidence to say this variant is causative of clinical symptoms in our patient. On one hand, functional analysis of the participant's cell line and biochemical testing from the patient suggest there could be disrupted *ALG1* production. Features of our patient's presentation common to previously reported phenotypes of ALG1-CDG include cardiomyopathy, scoliosis, and contractures, and hearing impairment (Morava et al. 2012; Ng et al. 2016; Öncül, Kose, and Eminoğlu 2022; Rohlfing et al. 2014; Schwarz et al. 2004). On the other hand, if our patient had ALG1-CDG, his presentation would be mild compared to the typical severity of ALG1-CDG. For example, he does not have intellectual disability, seizures, visual involvement, facial dysmorphisms, or gastrointestinal concerns, which are reported in ALG1-CDG (Ng et al. 2016). Moreover, with respect to the *ALG1* c.827G > A (p.Arg276Gln) variant, it is present in healthy population databases, including Genome Aggregation Database and Exome Aggregation Consortium. There are conflicting variant-phenotype assertions about this variant in ClinVar (accession RCV000555504.16). One of the ClinVar submissions for this variant has deemed the clinical significance as likely benign, two have deemed it as a likely pathogenic variant, and one has deemed it of uncertain significance. In the latter instance in which the clinical significance was deemed of uncertain significance (ClinVar submission access SCV003823171), the variant was detected as heterozygous in a newborn with cerebellar hypoplasia, microcephaly, intrauterine grown restriction, and premature birth, and a second reportable variant was not detected in the *ALG1* gene in this individual [personal communication with the submitter, Revvity Omics]. Thus, while there is evidence of reduced ALG1 protein expression in our patient, the *functional* consequence of this remains to be determined.

Given that the FRDA explained all of his clinical features, the *ALG1* variant may be a hypomorphic form and/or a biochemical phenotype. One possible explanation may be that the residual enzyme activity is sufficient to prevent severe clinical disease. As an example, individuals with so-called "biochemical variant galactosemia" have ~15% residual enzyme activity of GALT (galactose-1-phosphate uridylyltransferase) yet do not have clinical disease (Berry 1993, 2012). Further analysis is needed to clarify the exact functional role this *ALG1* variant is playing.

This case underscores the need for broad sequencing approaches that include detection of trinucleotide repeat expansions and congenital disorders of glycosylation for progressive motor disorders. Specifically, when broad, non-biased sequencing is being considered for cerebral palsy (CP) or its mimics, spastic-ataxia, and other genetic motor disorders, we suggest considering genome sequencing as a first-line test (Srivastava et al. 2022), due to its ability to detect a broad range of variant types. This suggestion parallels approaches for other neurodevelopmental conditions: for example, genome sequencing as a first-line or second-line test is currently the recommendation by the American College of Medical Genetics and Genomics for intellectual disability (Manickam et al. 2021). One downside of this approach is that it may detect presymptomatic genetic diagnoses unrelated to these motor symptoms, including trinucleotide repeat expansion disorders other than FRDA, such as Huntington disease. Genetic counseling, therefore, is an essential component of this process that would include discussion of this scenario. Moreover, when metabolic screening approaches are conducted in parallel or addition to molecular sequencing approaches, CDT analysis and N-glycan profiling could be considered in the metabolic evaluation of patients with complex HSP.

In summary, this case report suggests that FRDA should be considered in the differential diagnosis of complex HSP, particularly in the presence of peripheral neuropathy, ataxia, and cardiomyopathy. Unbiased genome sequencing should be considered for progressive motor disorders, particularly for phenotypes with significant genetic heterogeneity, particularly given the increasing availability of specific disease-modifying therapies.

Author Contributions

The authors confirm contribution to the paper as follows: Study conception and design: Siddharth Srivastava. Data collection: Aisling Quinlan, Lance Rodan, Elizabeth Barkoudah, Ibrahim Shammas, Wasantha Ranatunga, and Eva Morava-Kozicz. Analysis and interpretation of results: Amy Tam, Afshin Saffari, Devin Oglesbee, Gerald Berry, Darius Ebrahimi-Fakhari, and Siddharth Srivastava. Draft manuscript preparation: Aisling Quinlan and Siddharth Srivastava. All authors reviewed the results and approved the final version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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