

The Spectrum of Neurologic Phenotypes Associated With *NUS1* Pathogenic Variants: A Comprehensive Case Series

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Objective: A growing body of evidence indicates a strong genetic overlap between developmental and epileptic encephalopathies (DEEs) and movement disorders. De novo loss-of-function variants in *NUS1* have been recently identified in DEE cases. Herein, we report a large cohort of cases with pathogenic *NUS1* variants and describe their clinical presentation and the details of the associated epilepsy and movement disorders.

Methods: Cases with *NUS1*-related disorders were identified through a multicentric international collaboration made possible by the GeneMatcher platform. Clinical data were acquired through retrospective case-note review.

Results: We identified 41 subjects carrying 38 different pathogenic or likely pathogenic heterozygous *NUS1* variants. The majority of cases displayed developmental delays and intellectual disability of variable severity. Epilepsy was present in 68.3% of cases (28/41) with onset typically in early childhood. Strikingly, 87.8% of cases (36/41) presented with movement disorders and for 13 of these cases the movement disorder was not accompanied by epilepsy. The phenomenology of the movement disorders was complex with myoclonus observed in 68.3% of cases (28/41), either in isolation or in combination with dystonia, ataxia, and/or parkinsonism. Seven cases that otherwise did not have prominent movement disorders had mild incoordination and intention tremor, suggestive of cerebellar dysfunction. There was no observed genotype–phenotype correlation, suggesting that other genetic or acquired factors impact the clinical presentation.

Interpretation: Heterozygous *NUS1* pathogenic variants cause a complex neurological disorder, variably featuring developmental and epileptic encephalopathies and a broad spectrum of movement disorders, which represent the major source of neurological disability for most cases.

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Additional supporting information can be found in the online version of this article.

The *NUS1* gene (OMIM *610463), located on chromosome 6q22.1, encodes the transmembrane protein Nogo-B receptor (NgBR), which along with dehydrolipoyl diphosphate synthase (encoded by *DHDDS*; OMIM*608172) makes up the cis-prenyltransferase enzyme.¹ This enzyme is essential for biosynthesis of dolichol, a lipid critical for the process of protein N-glycosylation.^{2,3} Furthermore, the C-terminus of NgBR interacts with and stabilizes the NPC2 protein, which regulates intracellular cholesterol trafficking out of the lysosome.⁴

De novo *NUS1* heterozygous truncating variants and microdeletions have been recently identified in cases clinically diagnosed with developmental epileptic encephalopathies (DEEs)⁵ and in cases with progressive myoclonic epilepsies (PMEs).⁶ Importantly, an expanding spectrum of movement disorders, including myoclonus, dystonia, tremors, and chorea is increasingly recognized as a frequent feature of several genetically determined DEEs.⁷ Along these lines, a number of case reports and small case series have described the presence of movement disorders in cases with pathogenic *NUS1* variants, including tremor, myoclonus, dystonia, ataxia, and parkinsonism.^{8–14} However, only limited information regarding the frequency, spectrum of the movement disorders, and precise phenomenology in *NUS1*-mutated cases has been provided in the existing literature. Recent work from Galosi et al¹⁵ and Williams et al¹⁶ has expanded our knowledge of the neurologic phenotype associated with variants in *DHDDS*, demonstrating that pathogenic variants in the *NUS1* and *DHDDS* genes result in similar clinical features due to the shared pathway between these 2 genes.

Herein, we report the largest series to date of 41 cases harboring pathogenic variants in *NUS1* and describe in detail the presence and clinical characteristics of epilepsy and movement disorders. Importantly, movement disorders were identified in 87.8% of cases (36/41) and were a prominent feature in 29 of these cases, with myoclonus being commonly observed either in isolation or in association with other movement disorders, including ataxia, dystonia, and parkinsonism.

Methods

Cases

We collected clinical and molecular data of 41 cases with pathogenic or likely pathogenic variants in *NUS1* identified from different clinical centers in Europe, North America, South America, and Australia through international clinical collaborations and networking (GeneMatcher).¹⁷ Informed consent forms allowing for participation were signed by all study participants and/or their parents or guardians, and patient studies were approved by institutional review boards within the institutions in which the studies were performed.

All cases were unrelated except for case #26 and case #40 who are half siblings. Eight of the 41 cases were previously reported in the literature with 1 case being previously described by Wirth et al¹³ (case #7), 5 cases were reported in a recent case series by Williams et al (cases #22–26),¹⁶ one case was reported by Monfrini et al (case #35),¹⁸ and one case was reported by Riboldi et al (case #36).¹⁹ For those cases that were reported in the literature, previous reports were expanded upon by the collection of additional comprehensive data on movement disorders and epilepsy phenotypes when applicable, as well as the collection of any pertinent updates regarding clinical progression over time. Additionally, homemade and video-recorded examinations from 13 cases in our series, and 10 previously published cases, were collected and reviewed by the authors to better characterize the phenomenology of movement disorders.

Genetic Analysis

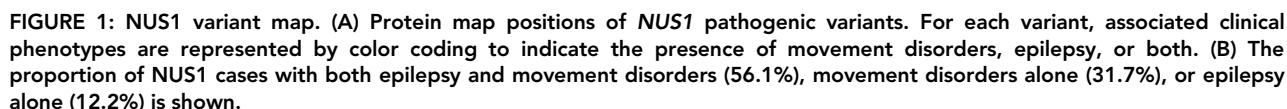
Molecular genetic diagnoses were made by whole-exome sequencing, whole-genome sequencing, gene panel testing, or chromosomal microarray. Sanger sequencing confirmation and segregation analysis of the variants was performed in all available family members when next-generation sequencing (NGS) had been performed in the proband only. Previously unreported genetic variants were assessed for pathogenicity following standard American College of Medical Genetics guidelines.²⁰

Results

We describe 41 cases (26 female cases and 15 male cases) ranging from 4 to 64 years of age at the most recent assessment who had pathogenic or likely pathogenic variants in *NUS1*. Most cases had no family history of similar symptoms, with the exception of cases #26 and #40 who are half-siblings and cases #4 and #32 who had positive family history consistent with an autosomal dominant pattern of inheritance.

Genetic Findings

We report a total of 38 different *NUS1* heterozygous variants, including 25 loss-of-function small indels or single nucleotide variants (6 stop-gain variants, 16 frameshift indels, 2 essential splice site variants, and 1 start codon loss), 7 whole gene deletions, 1 frameshift single exon deletion, and 5 missense variants (Fig 1 and Supplementary Table S1). One missense variant, c.868C>T (p.Arg290Cys), was present in the 2 unrelated cases #36 and #37 and an identical de novo frameshift duplication (c.128_141dupCCGCCTCTGCCGCG) was found in the 2 unrelated cases #23 and #33. An identical start codon loss mutation (c.3G>T) was identified in 2 half-siblings as well (cases #26 and #40).



In sporadic cases, *NUS1* variants were confirmed to be de novo in 28 cases, whereas, for 12 cases, genetic testing of both parents could not be completed. All identified small indels and single nucleotide variants are absent in the latest release of gnomAD (version 4.1.0; last accessed in July 2024) and all of these variants were classified as pathogenic or likely pathogenic according to American College of Medical Genetics and Genomics (ACMG) criteria. The only exception was the novel missense variant (c.622C>T; p.Cys208Arg) found in case #38. This variant is absent in gnomAD and has a Combined Annotation Dependent Depletion (CADD) score of 25 (top 1% of the most deleterious variants) and is predicted pathogenic by all in silico tools, but there was no available segregation data in the parents to help confirm the pathogenicity of this variant. Therefore, we pursued additional protein structural analysis of this variant as well as the other missense variants in our cohort.

clustering in any specific protein domain. To assess whether the *NUS1* missense variants that we identified in our cohort might contribute to loss of enzymatic function, we examined the position of these residues in a crystal structure of the NUS1-DHDDS complex as these 2 proteins constitute a heterodimeric cis-prenyltransferase (PDB: 7PAX).²¹ In this structure, 2 of the 5 identified mutations occur at residues on the interface between NUS1 and DHDDS (Fig 2). Residue R290 of NUS1 is part of a conserved RXG motif,²² which appears to engage with the substrates of the enzyme and possibly with a magnesium ion cofactor.²¹ Mutation of this residue has previously been shown to decrease substrate binding and the catalytic rate of the complex.²² Additionally, we found that residue C208 of NUS1 is part of a hydrophobic patch at the interface between NUS1 and DHDDS (Fig 2B). Mutation of this residue to arginine as in case #38 would both produce a steric clash and disrupt the hydrophobic interface, which may destabilize the association between NUS1 and DHDDS. With these data we were able to confirm that the c.622C>T; p.Cys208Arg variant meets ACMG classification criteria as a likely pathogenic variant.

Delays in the development of motor, cognitive, and/or language domains were observed in the majority of cases (see the Table and Supplementary Table S2). For a subset of cases, developmental milestones were normal until 1 to 2 years of age, at which time there was regression in both language and motor function (e.g. cases #1 and #2). The vast majority of cases had some degree of intellectual disability, which was most commonly classified as mild in severity (see the Table and Supplementary Table S2).

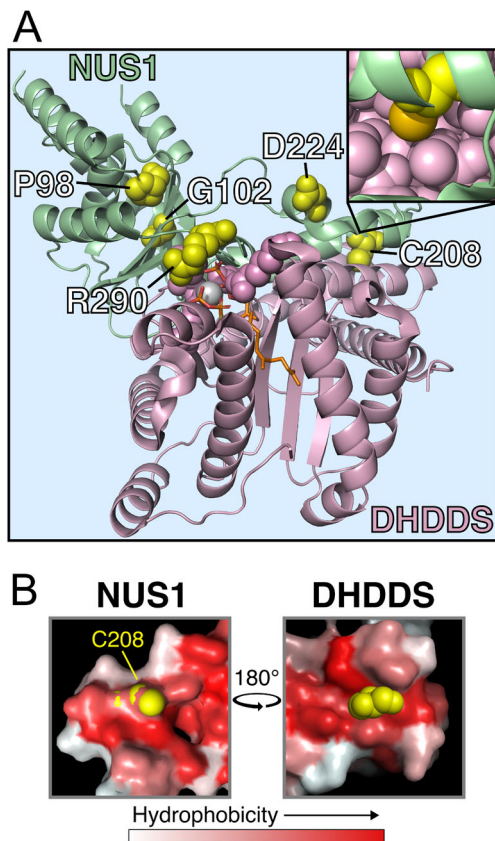


FIGURE 2: NUS1 and DHDDS protein structure. (A) The crystal structure (PDB: 7PAX) of the complex between NUS1 (green) and DHDDS (magenta). Substrates of the complex are shown in orange, whereas a magnesium cofactor is shown in gray. Residues with missense mutations identified in this study are shown in yellow. Inset: The residue C208 at the interface between NUS1 and DHDDS, with the sulfur atom colored darker. (B) Surface hydrophobicity map at the interface between NUS1 (left) and DHDDS (right) containing residue C208 (yellow).

Nine cases had a moderate degree of intellectual disability, 4 cases had a severe intellectual disability, and for 3 cases the degree of intellectual disability was not formally characterized. Full-Scale Intelligence Quotient (FSIQ) testing was available for 7 cases and ranged from 32 to 74.

Movement Disorders

Movement disorders were observed in 36 of 41 cases and there was notably a wide spectrum of movement phenomenologies across the cohort (see the Table and Supplementary Table S3). The age of onset of movement disorders was typically in early childhood (median age of onset = 3 years, and range birth to 20 years) with 18 cases presenting with movement disorders at 2 years of age or younger. The most commonly observed movement disorders were myoclonus (Supplementary Videos S1–S5 and S7–S13), ataxia (see Supplementary Videos S1, S2, S6, and S9–S12), and tremor (see Supplementary Videos S1,

S3, S5–S7, S10, and S11, also see the Table) and the majority of cases (27/41) displayed a complex phenotype involving multiple prominent movement disorders, including myoclonus and tremor accompanied by variable involvement of dystonia, ataxia, and parkinsonism (see Supplementary Table S3). Among those cases with combined movement disorders, 11 cases had predominantly myoclonus dystonia (see Supplementary Videos S2–S4, S7, and S10) and for 11 cases myoclonus and ataxia were the principal movement features (see Supplementary Videos S1, S11, and S12). In contrast, in 7 cases the movement disorder phenotype was mild with isolated mild gait ataxia and intention tremor without other overt movement disorders (see Supplementary Video S6).

A review of additional videos from the literature revealed multiple hyperkinetic movement disorders consistent with myoclonus dystonia syndrome in 4 cases, with ataxia observed in 3 of them.^{8,10,23}

In our case series, myoclonus was typically generalized affecting the limbs, face, and trunk, but was most prominent in the upper extremities. Myoclonus tended to be present both at rest and with maintenance of posture and was worsened by action in a subset of cases. In 4 cases, myoclonus was noted to be alcohol responsive. Across the cohort, medications that were effective in improving myoclonus in at least 1 patient included clonazepam, brivaracetam, levetiracetam, zonisamide, and propranolol.

Tremors varied in qualitative description with some cases having a low amplitude, high frequency postural myoclonic tremor, whereas others had either an intention tremor or a jerky irregular tremor. Tremors were likely variable in etiology, occurring as a feature of myoclonus, dystonia, or cerebellar dysfunction. Propranolol and clonazepam were both effective at partially alleviating tremor in 2 cases, respectively.

Ataxia was a frequent feature across the cohort (26/41) with common occurrence of gait ataxia as well as variable involvement of limb dysmetria. Ataxia was often mild in severity, and for a subset of cases, gait ataxia and mild coordination difficulties were the sole movement disorder. Explosive speech indicative of cerebellar dysfunction was observed in 2 cases as well.

Dystonia was reported in 13 of 41 cases and when present always involved the limbs, predominantly the upper extremities (see Supplementary Videos S2–S4, S7, S10, and S11). In a subset of these cases, cervical or truncal dystonia was described as well.

Parkinsonism, including rigidity, bradykinesia, rest tremor, and shuffling gait, was observed in 6 of 41 cases in combination with other movement disorders (2 cases had myoclonus-dystonia syndrome plus parkinsonism, 3 cases had a combination of myoclonus, ataxia, and

TABLE. Frequency of Clinical Features Observed in NUS1 Cases

Clinical Feature	Number of Cases (percentage of total cases)
Neurodevelopmental features	
Developmental delays	34 (82.9%)
Intellectual disability	36 (87.8%)
Movement disorders	
Any movement disorder	36 (87.8%)
Myoclonus	28 (68.3%)
Ataxia	26 (63.4%)
Tremor	26 (63.4%)
Dystonia	13 (31.7%)
Parkinsonism	6 (14.6%)
Paroxysmal movement disorders	4 (9.8%)
Tics	2 (4.9%)
Chorea	1 (2.4%)
Epilepsy	
Epilepsy diagnosis	28 (68.3%)
Tonic-clonic seizures	18 (43.9%)
Febrile seizures	9 (22.0%)
Myoclonic seizures	8 (19.5%)
Absence or myoclonic absence seizures	8 (19.5%)
Eyelid myoclonia	6 (14.6%)
Myoclonic astatic seizures	3 (7.3%)
Focal seizures	4 (9.8%)
Non-convulsive status epilepticus	2 (4.9%)
Other relevant features	
Dysarthria	13 (31.7%)
Anxiety	12 (29.3%)
Pyramidal tract signs	6 (14.6%)
Axial hypotonia	4 (9.8%)
Attention deficits	4 (9.8%)
Asymmetric hemiparesis	2 (4.9%)
Restricted upgaze	4 (9.8%)
Oculomotor apraxia	4 (9.8%)
Sensorineural hearing loss	3 (7.3%)
Disease course	
Slowly progressive course	20 (48.8%)
Static course	18 (43.9%)

parkinsonism, and 1 case had ataxia and dystonia along with parkinsonism; see Supplementary Videos S1 and S4). Bradykinesia when present could be either asymmetric or symmetric. Carbidopa-levodopa was trialed in 2 cases with parkinsonism and was effective in 1 of these cases.

Movement disorders that were observed in only rare cases included chorea and tics.

Paroxysmal exacerbations of movement disorders were described in 4 cases. Case #27 demonstrated paroxysmal episodes of lower extremity myoclonus provoked while walking. Case #21 had episodic exacerbations of left hemi-dystonia accompanied by orofacial choreiform movements (see Supplementary Video S10). Case #8 had episodic exacerbations of myoclonus and dystonia. Finally, case #10 had intermittent exacerbations of ataxia accompanied by esotropia (see Supplementary Video S6).

Epilepsy

Epilepsy was a common feature of the disorder and was reported in 28 of 41 cases (see the Table and Supplementary Table S4). The seizure onset was typically in early childhood (median age of seizure onset = 2.5 years, range birth to 29 years). Fourteen cases had seizure onset at 2 years of age or younger, 11 had seizure onset between 2 and 18 years of age, and only 3 cases had adult onset of seizures with generalized tonic-clonic seizures starting at age 23 years in case #20, there were 2 isolated myoclonic seizures at age 29 years in case #38, and tonic-clonic seizures starting at age 22 years in case #39.

Most cases with epilepsy had multiple seizure semiologies (see Supplementary Table S4). Tonic-clonic seizures were the most common seizure type. The tonic-clonic seizures were known or presumed to be of generalized onset in 11 cases and of unknown or focal onset in 7 cases. Febrile convulsions were reported in 9 cases. Eyelid myoclonia was a notable early feature in both of our index cases (cases #1 and #2), noted by the parents at age 2 years. Eyelid myoclonia was noted as a clinical feature in 4 additional cases and 1 case without a diagnosis of epilepsy had electroencephalogram (EEG) findings that were characteristics of eyelid myoclonia with activation of epileptiform activity with eye closure (case #10). Myoclonic seizures were only diagnosed in 8 cases, however, this may be due to challenges in classifying cortical myoclonus as a movement disorder versus seizure. Other generalized seizure types were also commonly noted, including absence, myoclonic-absence, and myoclonic astatic seizures. Focal seizures were rare across the cohort but were reported in 4 cases (cases #5, #34, #36, and #41). Nine cases were considered to have DEE. Two cases of non-convulsive

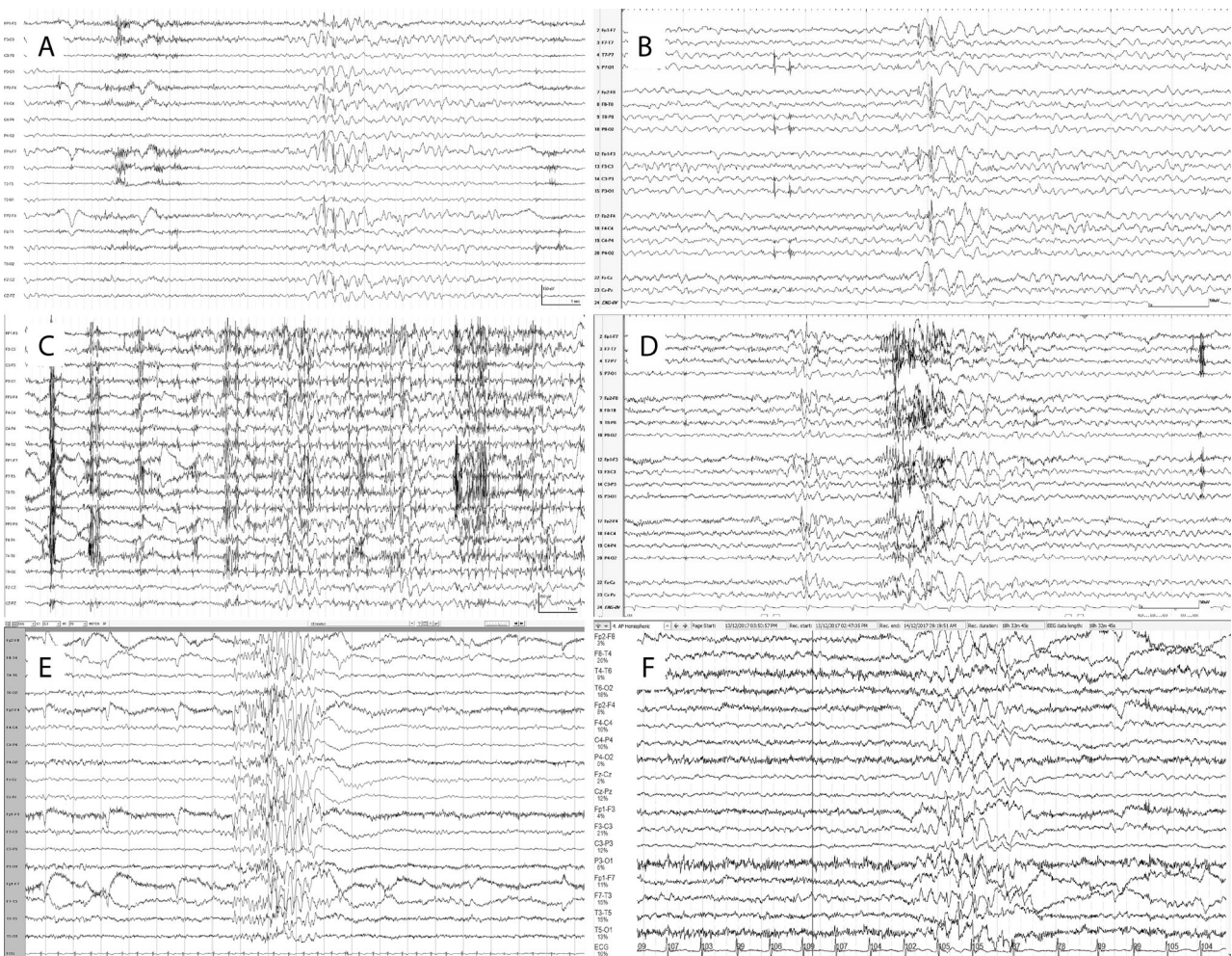


FIGURE 3: Generalized epileptiform discharges on electroencephalogram. (A, B) Case 2 ambulatory EEG at age 32 years demonstrating interictal 4 to 6 Hz generalized, bifrontally predominant spike-wave activity (A) and increased EMG with and without corresponding spike-wave activity during a period of patient-reported “Head jerking, eyelid fluttering and shoulder jerking” (B). EEG settings = 10 μ V/mm and 10 s/page. (C, D) Case 2 routine EEG at age 38 years demonstrating interictal 4 to 6 Hz generalized bifrontally predominant spike-wave activity during drowsiness (C) and paroxysmal fast and spike-wave activity during an axial myoclonic jerk (D). EEG settings = 10 μ V/mm and 10 s/page. (E, F) Case 9 EEG at age 9 years demonstrating photoparoxysmal response (E). Photoparoxysmal response was seen for all frequencies 5 to 25 Hz. EEG settings = 10 μ V/mm and 20 s/page. EEG at age 12 years demonstrating 4 to 5 Hz, generalized, bifrontally maximal spike-wave activity without clinical correlate (F). EEG settings = 10 μ V/mm and 10 s/page. EEG = electroencephalogram; EMG = electromyography; Hz = hertz.

status (absence status in 1 case) were reported (cases #3 and #15).

Of the 13 cases who did not have a clear clinical diagnosis of epilepsy, 6 had epileptiform abnormalities on EEG (cases #10, #14, #19, #21, #23, and #26) and 7 cases were treated with anti-seizure medications, including clonazepam, levetiracetam, brivaracetam, or carbamazepine, for treatment of movement disorders.

EEGs were performed in 36 cases (see Supplementary Table S4). Eighteen cases had EEGs with generalized epileptiform activity (Fig 3), most typically spike-wave complexes that were 2.5 to 3 hertz (Hz) or 3 to 3.5 Hz. Focal or multifocal EEG features were noted in 3 cases with co-existing generalized abnormalities and in 5 cases

without generalized abnormalities. A slow background was noted in several cases. In a few cases, changes to the EEG were noted over time. For example, for case #3, generalized spike-wave activity was initially 2 Hz and increased to 3 to 3.5 Hz with subsequent EEGs. In another case (case #29), focal occipital sharp-wave activity was noted at age 4 years and generalized spike-wave complexes at age 12 years.

Seizure control was achieved for the majority of cases with epilepsy. Data on anti-seizure medication effectiveness was available for 24 cases. Of these cases, just under half (42%) had complete seizure control, approximately one third of cases (37.5%) had greater than 75% improvement in seizure frequency, and only 3 cases (12%) had

less than 25% improvement in seizure frequency. Anti-seizure medications that were reported to be effective at decreasing seizure frequency in at least a subset of cases included clobazam, clonazepam, ethosuximide, lamotrigine, levetiracetam, sultiame, topiramate, and valproate. Carbamazepine and lacosamide were effective at treating focal-onset seizures in cases #34 and #41, respectively.

Other Neurologic and Psychiatric Features

Other commonly observed neurologic features across the cohort included dysarthria and pyramidal tract signs, including spasticity or hyperreflexia and asymmetric hemiparesis (see the Table and Supplementary Table S2). Reported eye movement abnormalities included restricted upgaze and oculomotor apraxia. Other neurologic features reported included axial hypotonia and mild sensorineural hearing loss.

Psychiatric features were fairly common across the cohort, particularly anxiety. Other observed neuropsychiatric features included autism spectrum disorder and attention deficits.

Disease Course

The disease course was either static or slowly progressive over time, with a progressive course reported in 20 of 41 cases. For those cases who had progression of symptoms over time, the main change observed was a gradual worsening of movement disorders, including tremor, myoclonus, or ataxia. The rate of change in movement disorders tended to be very slow. For example, case #23 displayed worsening myoclonus and parkinsonism as well as slow deterioration in ataxia over the course of decades. For several cases, the progression in movement disorders was significant enough to lead to impaired ambulation (cases #8, #16, and #25) but for others (eg, case #26) gait was preserved despite worsening myoclonus. For some cases, the course was initially slowly progressive in childhood but subsequently became static. For instance, for case #18, there was a gradual progression of myoclonus throughout childhood which subsequently plateaued upon reaching adulthood. Development of new movement disorders in adulthood was uncommon, but for case #1 involuntary movements began in early childhood and then there was subsequent development of parkinsonism at approximately age 50 years with shuffling gait and worsening tremor. For case #9 and case #22, although there was a gradual progression in involuntary movements over time, both cases displayed improvements in cognitive and behavioral functions.

Neuroimaging

Across the cohort, brain magnetic resonance imaging (MRI) did not consistently demonstrate significant abnormalities. In a small subset of cases, diffuse cortical atrophy (cases #8 and #20) or cerebellar atrophy (cases #1, #16, #35, and #36) was observed. Other likely incidental abnormalities noted in individual cases included structural abnormalities in the corpus callosum (cases #5 and #30), unilateral hippocampus hypoplasia (case #3), a cavernous malformation (case #1), an arachnoid cyst (case #29), and small vessel ischemic changes (case #8).

A DaTscan was performed in only 5 cases. Case #1 had parkinsonism with hypomimia and shuffling gait and the DaTscan showed reduced uptake in the right putamen demonstrating evidence of dysfunction in the nigrostriatal pathway. Case #17 had bradykinesia and rigidity on examination but had normal DaTscan imaging. A normal DaTscan was also observed in 3 cases who did not have clinical evidence of parkinsonism.

Magnetic resonance spectroscopy (MRS) was performed in 5 cases. MRS was normal in 4 cases and in case #23 MRS showed elevated choline on long echo time (TE) spectroscopy and elevated glutamine-glutamate on short TE spectroscopy.

Discussion

This case series provides a comprehensive description of the phenotypes associated with pathogenic variants in *NUS1*. We found that heterozygous pathogenic variants in *NUS1* are associated with complex neurologic symptoms, including developmental delays and variable degree of intellectual disability, as well as frequent occurrence of epilepsy and a broad spectrum of both hyperkinetic and hypokinetic movement disorders. Neurologic symptoms typically manifested in infancy or early childhood and clinical follow-up demonstrated either a static or very slowly progressive disease course across the cohort. Among the movement disorders observed, myoclonus was particularly prominent and was typically generalized but with a propensity for the upper extremities and face. Patients commonly exhibited multiple movement disorders including frequent co-occurrence of myoclonus-dystonia and myoclonus-ataxia.

Homozygous pathogenic variants of *NUS1* were initially reported by Park et al who described a congenital disorder of glycosylation in 2 siblings with a homozygous missense variant in the *NUS1* gene.²⁴ The siblings presented with a severe phenotype with axial hypotonia and acral spasticity, refractory seizures, and developmental delay. No movement disorders were described in these cases. Attempts at modeling homozygous loss of function

of *NUS1* demonstrated that complete *NUS1* knockout is embryonic lethal in animal models.²⁴

In humans, initial information about the effects of heterozygous loss of function of *NUS1* came from analysis of cases harboring chromosomal deletions in the chromosome 6q region involving the *NUS1* gene. Previously described 6q deletion syndromes have included neurodevelopmental disorders and epilepsy, often with a severe phenotype, and there have been reports of associated movement disorders in a significant subset of these cases.^{25–28} Five 6q deletion cases described by Rosenfeld et al included the *NUS1* gene, 3 of which had movement disorders including (1) ataxic gait, (2) late onset myoclonus with ataxia and fine tremors with associated cerebellar vermian atrophy, and (3) progressive tremors with incoordination, respectively.²⁵ Szafranski et al thereafter reported 6 cases involving microdeletion in the 6q21q22.31 region, 5 of which included the *NUS1* gene.²⁶ All cases had neurodevelopmental delay with epilepsy and 3 of the 6 cases had movement disorders, including 2 with tremors alone and 1 with polyminimyoclonus with mild intention tremors. The critical region for the neurodevelopmental phenotype in these cases was narrowed down to the *NUS1* and *SLC35F1* genes.²⁶ More recently, Canafoglia et al reported 3 cases of 6q22.1 deletions, all of whom had intellectual disability and prominent myoclonic jerks which were demonstrated to be cortical in origin.²⁹

More recently, the effects of specific *NUS1* variants, including single-nucleotide variants or small indels, on neurologic phenotypes have begun to be characterized. Hamdan et al found *de novo* *NUS1* heterozygous loss-of-function variants in 3 cases with DEE.⁵ The clinical picture across the cohort consisted of developmental delay and refractory seizures. Movement disorders reported included tremors in 2 cases and ataxia in 1 of the cases.⁵ In addition, 2 unrelated Japanese cases with the same novel heterozygous *NUS1* variant presented with childhood-onset epilepsy, scoliosis, and movement disorders, including ataxia and myoclonic jerks in both cases.⁸ EEG with back averaging in one of these cases confirmed a cortical origin of myoclonic jerks.⁸ In the last several years, additional case reports or small case series of heterozygous *NUS1* variants have been reported,^{6,9–14,18,19,30–32} and in a recent comprehensive literature review of *NUS1* variants by Williams et al, it was found that across 21 reported cases of *NUS1* heterozygous pathogenic variants movement disorders were described in all cases with multifocal myoclonus, tremor, and mild ataxia being commonly observed.¹⁶ Similarly, movement disorders were reported in 80% of previously described 6q deletion syndromes.¹⁶

Given the complex and heterogeneous neurologic phenotype observed in our series, *NUS1* should be broadly considered on the differential diagnosis of myoclonus syndromes, for which the clinical picture involves myoclonus in isolation or in combination with other movement disorders and/or epilepsy. *NUS1*-related disorders can present with childhood onset of myoclonus and dystonia, and thus may have overlapping clinical features with classic myoclonus dystonia syndrome (MDS) due to variants in the epsilon sarcoglycan (*SGCE*) gene or MDS caused by variants in other genes, such as *ADCY5*, *KCTD17*, and *KCNN2*.^{33,34} There are notable differences between classic MDS and the neurologic phenotypes observed in our *NUS1* cohort, however, particularly the frequent presence of intellectual disability and epilepsy. *NUS1* mutations should be considered when a patient with myoclonus and dystonia presents with additional features not typically associated with MDS, including developmental delays, intellectual disability, seizures, pyramidal tract signs, or other movement disorders, such as ataxia and parkinsonism. Furthermore, myoclonus caused by *SGCE* mutations is thought to be subcortical in origin.^{33,34} Conversely, in our *NUS1* cohort, there is evidence to suggest that myoclonus may be predominantly of cortical origin, including the tendency for myoclonus to localize to the distal upper limbs and face, as well as the demonstration of EEG correlate to myoclonic jerks in a subset of cases. In addition to myoclonus and dystonia phenotypes, a combination of myoclonus and cerebellar ataxia was commonly observed in our cohort, and thus *NUS1*-related disorders may also share clinical features with progressive myoclonus ataxia (PMA) syndromes related to variants in *CSTB*, *GOSR2*, *KCNK1*, among many others.³⁵

Given the frequent combination of developmental delays and intellectual disability in combination with epilepsy in our cohort, *NUS1* variants should be considered in the differential diagnosis of individuals presenting with DEE. Epilepsy in *NUS1*-related neurologic disorder may present similarly to a variety of DEE syndromes including Lennox–Gastaut and Myoclonic-atonic epilepsy. *NUS1*-related disorders can also present as a PME syndrome and thus may mimic PME due to Unverricht–Lundborg disease, Lafora disease, and neuronal ceroid lipofuscinoses. Given the broad spectrum of neurologic phenotypes, testing for a *NUS1*-related neurologic disorder should be considered in any patient with intellectual disability and childhood onset epilepsy and/or movement disorders with a static or slowly progressive course. *NUS1* whole gene deletions can be identified via chromosomal microarray but whole exome or whole genome sequencing is

indicated to evaluate for pathogenic truncating, splice site, or missense variants.

Recently, Williams et al reported on overlapping neurologic phenotypes observed in cases with *DHDDS* and *NUS1* variants, highlighting the converging mechanism of loss of function of these genes.¹⁶ The 5 *NUS1* cases from this series are included in our cohort (cases #22–26) and the 3 *DHDDS* cases described by Williams et al notably had a comparable phenotype, including intellectual disability, epilepsy in 2 of the 3 cases, and multiple movement disorders, with myoclonus present in all 3 cases.¹⁶ Despite the similarities between these disorders, there are some distinctive traits between *DHDDS* and *NUS1*-related disorders. For instance, *DHDDS* cases described by Galosi et al,¹⁵ seem to experience higher frequency of refractory seizures compared with *NUS1* cases and a more pronounced progressive course compared to patients with *NUS1*. Furthermore, facial dysmorphisms reported in the *DHDDS* group were only observed in a small number of *NUS1* cases. *DHDDS* and the Nogo-B receptor, the protein encoded by *NUS1*, together constitute the cis-prenyltransferase enzyme which is located on the endoplasmic reticulum and mediates dolichol biosynthesis, a lipid critical for the process of protein N-glycosylation. Therefore, the synergistic activity of the Nogo-B receptor and *DHDDS* in mediating dolichol biosynthesis provides a clear mechanistic explanation for the overlapping phenotypes seen in *DHDDS*- and *NUS1*-linked neurodevelopmental disorders. Given the role of *NUS1* in regulating protein N-glycosylation, the pathogenic effects of variants in this protein likely involve impaired glycosylation of substrate proteins and therefore identification of key substrates mediating pathogenesis is an important area of further investigation.

Pathogenic variants in *NUS1* have previously been demonstrated to reduce lysosomal protein NPC2 levels, likely due to abnormal glycosylation of NPC leading to increased intracellular cholesterol accumulation.⁴ Notably, in their cohort of cases with pathogenic *DHDDS* variants, Galosi et al¹⁵ demonstrated pathological evidence of lysosomal dysfunction of lipid processing with abundant lipid-like material in cutaneous myelinated fibers and Schwann cells.¹⁵ Furthermore, functional investigation of *NUS1* loss of function in a zebrafish model revealed lysosomal cholesterol accumulation and lysosomal proteolytic deficits,¹⁴ and a loss of function variant of the *NUS1* homolog in drosophila similarly showed cholesterol accumulation particularly in dopaminergic neurons.³⁶ These findings suggest that therapies targeting cholesterol accumulation could potentially be beneficial in *NUS1*-related neurologic disorders.

Lysosomal dysfunction is also an important component of disease pathogenesis in Parkinson's disease (PD) and other neurodegenerative forms of parkinsonism. Interestingly, 6 of our cases demonstrated parkinsonism and in 1 of these cases there was confirmed evidence of nigrostriatal degeneration on DaTScan. The *NUS1* gene has recently been identified as a potential candidate gene for early onset PD with functional studies in drosophila demonstrating a significant reduction in dopaminergic neurons and total dopamine levels in the brain.^{37,38} However other studies have found contradictory results with analysis of *NUS1* variants in Chinese cases finding no association between *NUS1* and PD in 2 studies.^{39,40} Similarly, analysis of European populations did not demonstrate an enrichment of rare damaging *NUS1* variants in PD cases.⁴¹ Interestingly, one recent study found reduced levels of soluble Nogo-B in the serum of PD cases compared with controls.⁴²

In our case series, *NUS1* missense mutations did not cluster in any specific protein domain and no genotype–phenotype correlations were identified. Notably, even cases with identical pathogenic variants demonstrated significant variability in phenotype. For example, cases #23 and #33 had an identical *NUS1* duplication variant but had divergent phenotypes with case #23 displaying mild intellectual disability and multiple prominent movement disorders (myoclonus, dystonia, and parkinsonism) but no seizures, whereas case #33 had normal cognition, myoclonus, and epilepsy. Similarly, cases #36 and #37 had the same *NUS1* c.868C>T missense variant with case #37 having severe intellectual disability, whereas case #36 only had mild intellectual impairments. Of the 7 cases in our series with *NUS1* whole gene deletions, some had epilepsy whereas others did not. The reason for the high degree of phenotypic variability across the cohort, even between individuals with identical variants, is unknown, but because loss of function of *NUS1* likely leads to impaired protein glycosylation there may be additional genetic modifiers that can influence the impact of impaired glycosylation of different protein substrates, perhaps in a cell-type-dependent manner. More specifically, given the evidence that *NUS1* variants may result in impairments in lysosomal lipid processing,³⁶ individuals who harbor additional genetic variants linked to lysosomal dysfunction may be theorized to be prone to a more severe phenotype.

Although the *NUS1* gene is expressed equally throughout the central nervous system,⁴³ brain imaging and histopathology have demonstrated diffuse cortical and/or cerebellar involvement, particularly involving the vermis.^{24,25} Mild global cortical atrophy or cerebellar vermis atrophy were also noted in a subset of our cases.

Notably ataxia was a common clinical feature, particularly gait ataxia with impairment in tandem gait, consistent with cerebellar vermal dysfunction. We hypothesize that myoclonus in *NUS1* variants is likely to be cortical in origin, as highlighted by recent neurophysiologic characterization of *NUS1*-related myoclonus,^{8,29} and the clinical characteristics of myoclonus in our series were consistent with cortical myoclonus as well. Ataxia and intention tremors on the other hand are likely to be cerebellar in origin.

Conclusions

Our findings confirm that heterozygous *NUS1* variants are a cause of epilepsy and DEE and provide a comprehensive overview of the complex neurological phenotypes observed in individuals with *NUS1* variants. We observed a broad spectrum of movement disorders, including frequent occurrence of myoclonus, tremors, and ataxia with variable involvement of dystonia and parkinsonism. Future functional studies are indicated to explore whether *NUS1* variants may lead to abnormal lysosomal function due to abnormal glycosylation and maturation of NPC2.

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Author Contributions

S.M.B., M.N., E.G., and N.E.M. contributed to the conception and design of the study. S.M.B., M.N., N.P., W.A.K., M.A.-G., M.A., G.B., T.B., F.B., S.B., B.B., M.B., T.C., N.C., B.C., H.S.D., J.-M.D., C.A.E., K.M.E., C.F., S.J.F., M.C.G., D.G., A.G., R.H., D.H.-S., P.H., M.H., I.U.I., L.J., C.L., T.L., A.L., G.L., M.L.-S., L.M., C.M., H.C.M., B.A.M., S.M.-A., C.M., S.S.M., F.M., S.N., T.O., M.Pad., M.Pau., G.P., A.P., F.R., G.M.R., C.R.-J., F.S.-S., I.E.S., N.S., C.M.S., A.S., S.T., C.T.-R., M.T., C.Tra., C.Tro., T.F.T., O.V., P.V., M.L.W., U.W., L.J.W., T.W., M.Z., H.Z., E.R., V.L., S.G., V.S.C.F., G.C., D.K., E.G., and N.E.M. contributed to the acquisition and/or analysis of the data. S.M.B., M.N., R.C., E.G., and N.E.M. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

The data supporting the findings of this study are available within the article and its supporting information.

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