BRIEF COMMUNICATION

Expansion of the genetic landscape of *ERLIN2*-related disorders

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Abstract

ERLIN2-related disorders are rare conditions of the motor system and clinical details are limited to a small number of prior descriptions. We here presented clinical and genetic details in five individuals (four different families) where three subjects carried a common homozygous p.Asn292ArgfsX26, associated also with sensorineural hearing loss in one child. One further subject had a de novo p.Gln63Lys and one harbors the homozygous p.Val136Gly because of maternal isodisomy of chromosome 8. Overall, we expanded the clinical and genetic spectrum of *ERLIN2*-related disorders and we reiterate that autosomal-dominant transmission is a potential mode of inheritance. Future research will elucidate disease mechanisms.

Introduction

ERLIN2 (ER lipid raft-associated 2) is an endoplasmic reticulum protein that mediates intracellular calcium signaling through its action on inositol 1,4,5-trisphosphate receptors, making it an important regulator of several cellular processes important for neurodevelopment and neurotransmission.[1–3]

Defects in *ERLIN2* are a known cause of several neurodegenerative conditions. Biallelic pathogenic variants in *ERLIN2* are responsible for autosomal-recessive hereditary spastic paraplegia (HSP) 18 (SPG18; MIM 611225), a progressive disorder characterized by intellectual disability (ID), multiple joint contractures, and gradual development of spasticity and weakness primarily affecting the lower extremities. Affected individuals often present during infancy or childhood with delayed motor milestones and loss of motor abilities.[4] Recently, an autosomal-dominant form was described in two unrelated families with affected members carrying the same heterozygous missense variant in *ERLIN2* (c.386G> C; p.Ser129Thr).[5] Finally, a homozygous splice junction variant causing reduced *ERLIN2* transcript was the cause of juvenile primary lateral sclerosis in a consanguineous family with several members affected by language regression, pseudobulbar palsy, reduced muscle bulk, distal greater than proximal weakness, and kyphosis/scoliosis.[6]

In this report, we expand the clinical and genetic spectra of *ERLIN2*-related disorders by presenting five individuals from four different families with varying presentations of developmental regression, spasticity, motor impairment, and in one case, epileptic seizures.

Three families (four affected individuals) demonstrate autosomal-recessive inheritance with biallelic variants, whereas the fifth affected individual has a heterozygous de novo variant consistent with an autosomal-dominant form of *ERLIN2*-related disorder.

Patients and Methods

Participants in this study underwent clinical evaluations at Boston Children's Hospital, Kennedy Krieger Institute, IRCCS Fondazione Stella Maris, and IRCCS Istituto delle Scienze Neurologiche di Bologna. Two participants underwent clinical whole exome sequencing through Gene-Dx, and three participants had multigene panel testing using methodologies and a bioinformatic pipelines already reported elsewhere.[7] No other pathogenic or likely pathogenic variants were identified that would explain the clinical presentation.

Results

Demographic and genetic features

The five individuals (two males and three females) in this cohort had heterogenous demographic, clinical, and genetic characteristics (Table 1, Table S1). The age at last follow-up ranged from 3 to 51 years, and two were male. Three individuals were of South Asian descent (Pakistan and Sri Lanka), one case was from Italy, and one case was of Ghanese descent.

The types of *ERLIN2* alterations represented in this cohort included a common homozygous frameshift variant found in two unrelated consanguineous families (p.Asn292ArgfsX26) and a de novo heterozygous missense variant (p.Gln63Lys) (Figure 1). The remaining individual was homozygous for a c.407T> G variant (p.Val136Gly) due to maternal uniparental isodisomy (UPD) of chromosome 8, with the unaffected mother heterozygous for this variant. Evidence for pathogenicity of the missense variants derives from scores in multiple in silico prediction tools and a high evolutionary conservation of mutated residues across species (see Figure 1).

The three different *ERLIN2* variants found in the five participants in our cohort are likely pathogenic changes. Patient 1, patient 3, and patient 4 harbored the same homozygous frameshift variants expected to cause loss of function, a known mechanism of disease for *ERLIN2*-related disorders, although this was not proved at the cDNA level. The missense variants identified in patient 2 and patient 5 were novel but several lines of evidence support that they are disease-causing. The variants are absent in large population databases (ExAC, 1000 Genomes) and predicted to be deleterious by in silico tools (CADD scores> 23).

Symptom onset

Age of onset of motor delay and/or spasticity spanned infancy/early childhood in the four individuals with homozygous *ERLIN2* variants to adulthood in one individual with heterozygous de novo missense. Developmental regression was notable in three patients (patient 1, patient 2, patient 3), characterized by loss of ambulatory abilities. In patient 1, regression occurred at age 5 years, when he lost the ability to crawl and climb steps. Patient 2 had motor delay and spastic diplegia noted around age 2 years, after which she developed progressive motor deterioration, losing the ability to walk with a walker and requiring a wheelchair for mobility. Patient 3 had loss of ambulation after age 2 years.

Language, cognition, and epilepsy

ID was present in the three participants with the homozygous *ERLIN2* frameshift variants and absent in the two participants with the *ERLIN2* missense variants. Among those without ID, dysarthria and hypophonia were the noted speech difficulties. Patient 1 has sensorineural hearing loss, which has not been previously reported in association with *ERLIN2*-related disorders. One patient presented with generalized myoclonic absence seizures at the age of 2 years, which stopped after treatment with valproate.

Motor phenotype

The primary motor phenotype of the participants was axial hypotonia and appendicular spasticity. In four patients, spasticity affected lower extremities greater than upper extremities, and in the remaining individual, spasticity was limited to the lower extremities. More than half (3/5) of the cohort had dystonia. Patient 1 had intermittent dystonia affecting his arms. Patient 2 also had intermittent dystonic posturing affecting her arms and legs. Patient 1 and 2 did not undergo empiric carbidopa/levodopa trial. Hyperreflexia and predominantly lower extremity weakness were present in all five individuals. Patient 4 could crawl but not walk; the other four individuals had a spastic gait, with superimposed cerebellar features (tremor or dysmetria) in two individuals.

Neuroimaging features

Neuroimaging findings were variable. Thinning of the corpus callosum and other white matter changes were the primary abnormalities in two cases. Two individuals had a normal brain MRI. Patient 1 had arachnoid cyst that was fenestrated without any clinical improvement.

Table 1. Clinical features of the patients in our cohort.

	Patient 1	Patient 2	Patient 3	Patient 4 (sister of Patient 3)	Patient 5
	Family 1	Family 2	Family 3		Family 4
Sex/Age at last exam	Male/12 years	Female/10 years	Male/9 years	Female/3 years	Female/51 years
Genetics					
Inheritance/ Consanguinity	Recessive (parents are carriers)/Yes (parents are first cousins)	Mother heterozygous/ No	Recessive (parents are carriers)/Yes (parents are second cousins)	Recessive (parents are carriers)/No	de novo/No
Accession number	NM_007175.6	NM_007175.6	NM_007175.6	NM_007175.6	NM_007175.6
cDNA change	c.861_874dup14	c.407T> G	c.861_874dup14	c.861_874dup14	c.187C> A
Protein change	p.Asn292ArgfsX26	p.Val136Gly	p.Asn292ArgfsX26	p.Asn292ArgfsX26	p.Gln63Lys
Zygosity	Homozygous	Homozygous due to maternal uniparental isodisomy of chromosome 8	Homozygous	Homozygous	Heterozygous
Found in gnomAD	No	No	No	No	No
Variant detected by	Clinical exome sequencing	Clinical exome sequencing	Targeted gene panel	Targeted gene panel	Targeted gene panel
Previously reported?	No	Yes (previously reported with limited clinical information[1])	Yes (previously reported with no clinical information[2])	Yes (previously reported with no clinical information[2])	No
Growth parameters at last exam					
Head circumference	Normal	Normal	Normal	Normal	Normal
Weight	z-score −0.27	z-score −1.09	not available	not available	not available
Height	z-score −1.46	z-score −0.87	not available	not available	not available
Symptom Onset					
Age of onset	8 months	2 years	2 years	10 months	32 years
Initial symptoms	Motor delay, spastic diplegia	Motor delay, spastic diplegia	Motor delay	Motor delay	Spastic diplegia, wide base gait
Developmental regression (age of onset)	Yes (5 years)	Yes (unknown)	Yes (2 years)	Yes (1 year)	No
Nature of regression	Lost ability to crawl and climb steps	Lost ability to independent walking, worsening dysarthria	Lost ability to walk independently	Loss of standing position	No
Current motor abilities	Able to pull to stand, cruise, walk with posterior walker	Requires wheelchair	Able to stand, crawl, and walk with bilateral assistance	Unable to walk	Requires cane to walk
Language and cognition					
Current language abilities	200 + words, phrases	Age appropriate comprehension but difficulty speaking, hypophonia, difficulty opening mouth	Limited speech	Absent speech	Dysarthric speech
Intellectual disability	Yes	No	Yes (mild-moderate)	Yes	No
Behavioral dysregulation	No	No	No	No	No
Motor					
HSP phenotype	Complex	Pure	Complex	Complex	Pure
Axial hypotonia	Yes	No	Yes	Yes	No
Appendicular spasticity	Yes	Yes (spastic quadriplegia)	Yes	Yes	Yes
Motor impairment	Lower> upper limbs	Lower> upper limbs	Lower>> upper limbs	Lower>>upper limbs	Lower limbs

(*Continued*)

Table 1 Continued.

|	Patient 1	Patient 2	Patient 3	Patient 4 (sister of Patient 3)	Patient 5
	Family 1	Family 2	Family 3		Family 4
Dystonia	Yes (upper limbs)	Yes (hands and lower limbs)	Yes (hands, fluctuating)	No	No
Pyramidal tract signs	All limbs	All limbs	All limbs	All limbs	Lower> upper limbs
Tremor	No	No	Yes (occasional, hands)	No	No
Dysmetria	No	No	Yes (mild)	No	Yes
Gait pattern	Spastic gait (scissoring gait)	Spastic gait (scissoring gait, able to take few steps)	Spastic gait (scissoring gait with support)	Crawling	Spastic, ataxic gait
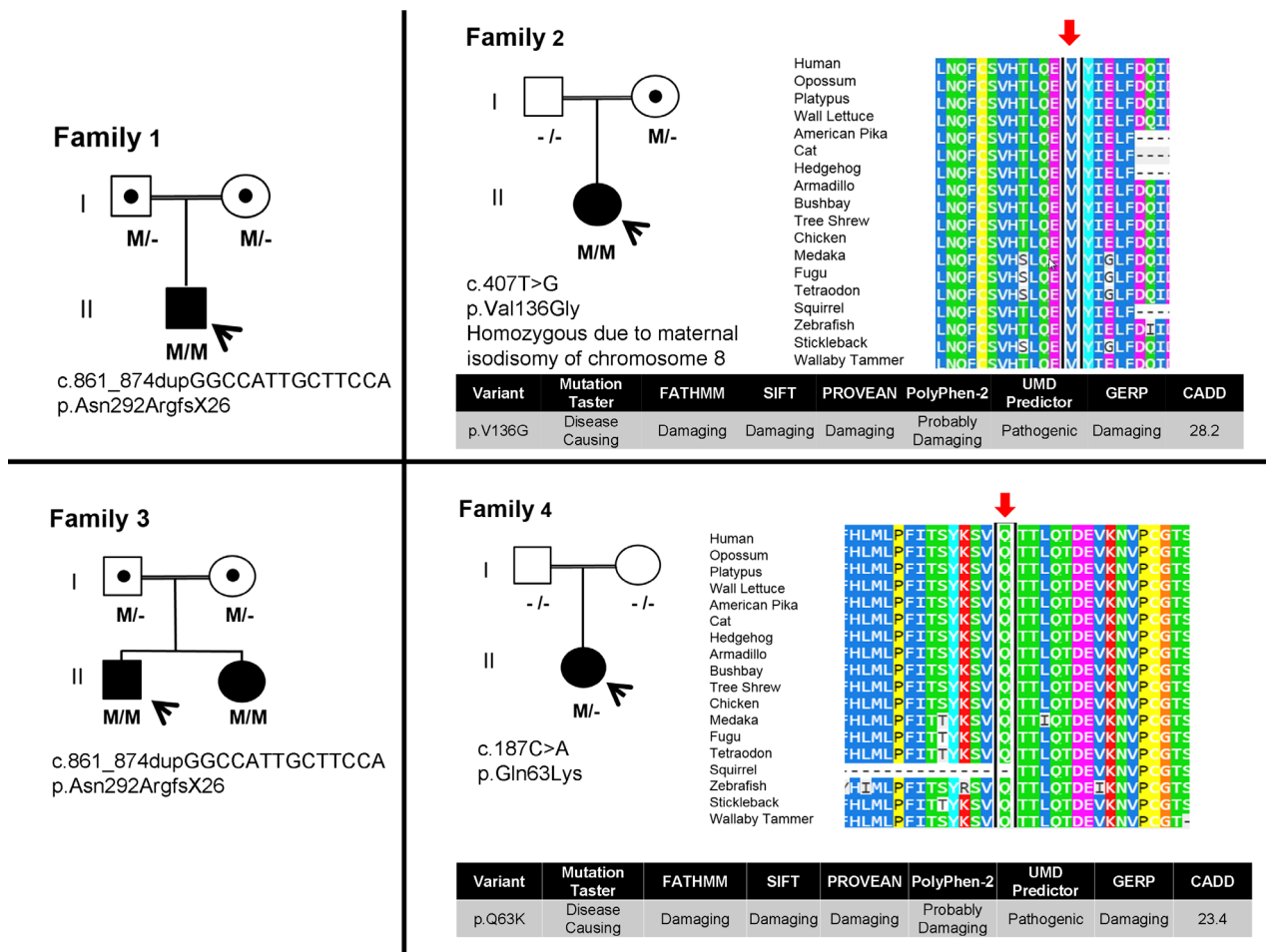
Figure 1. Pedigrees for all affected patients, multiple sequence alignment, and evidence of pathogenicity for the missense variants. In pedigrees, arrows denote probands; squares represent males and circles represent females; dotted circles/squares denote heterozygous carriers; M denotes the presence of an *ERLIN2* variant on one allele; hyphen denotes the presence of wild-type allele. We used the following pathogenicity prediction tools: MutationTaster (http://www.mutationtaster.org/), Functional Analysis through Hidden Markov Models (FATHMM);[22] Sorting Intolerant From Tolerant (SIFT);[23] Protein Variation Effect Analyzer (PROVEAN);[24] PolyPhen-2;[25] UMD-Predictor;[26] Genomic Evolutionary Rate Profiling (GERP);[27] and Combined Annotation Dependent Depletion (CADD).[28]

Discussion

The patients in our cohort constitute a wide spectrum of phenotypes consistent with previous reports of *ERLIN2*-related disorders (Table S2). There appear to be three clinical phenotypes associated with *ERLIN2* defects: pure HSP, complex HSP, and juvenile primary lateral sclerosis. The first two phenotypes are equally represented in the five participants from this study, who demonstrated progressive spasticity, weakness, and gait disturbance, with age of onset ranging from infancy to adulthood.

One of the novel clinical features seen in association with *ERLIN2*-related disorders is sensorineural hearing loss (seen in patient 1). This patient did not have any pathogenic variants detected in mtDNA sequencing, and no other variants were reported on clinical exome sequencing to suggest an alternative cause for the hearing loss. Thus, hearing loss may be an expansion of the clinical spectrum of *ERLIN2*-related disorders, and individuals with a new diagnosis should consider undergoing audiological evaluation.

The variants discovered in our cohort expand the genetic landscape of *ERLIN2*-related disorders. Four out of the five patients had alterations in *ERLIN2* that are previously unreported in association with the disorders (patient 2 is part of a prior report, but detailed clinical information about her in that report is limited[8]; similar is the case for patients 3 and 4[7]). In addition, patient 2 has a novel missense variant that is homozygous due to maternal UPD of chromosome 8, with her mother being a heterozygous carrier.

Four of the five patients in our study had homozygous variants, whereas patient 5 carried a de novo variant, confirming the recent report of an autosomal-dominant form of *ERLIN2*-related disorder. The vast majority of previous studies of *ERLIN2*-related disorders have identified biallelic variants (Table S2).[4,6,9–12] One report, however, identified a recurrent heterozygous missense variant (c.386G> C; p.Ser129Thr) in multiple affected individuals from two large unrelated families.[5] Overall, these data lend support to the notion that there are autosomal-recessive and autosomal-dominant forms of *ERLIN2*-related disorders, similar to a small number of other genetic causes of HSP, such as defects in *ATL1* (SPG3A),[13] *KIF1A* (SPG30),[14] *KIF1C* (SPG58),[15] *REEP1* (SPG31),[16] and *SPG7* (SPG7).[17]

Certain genotype-phenotype correlations emerged within our cohort. The patient with the de novo heterozygous *ERLIN2* variant (patient 5) appeared less severely affected compared to the other participants with homozygous *ERLIN2* variants, as was shown by a later age of symptom onset, absence of developmental regression, and absence of ID. In fact, among prior reports of *ERLIN2*-related disorders (Table 2), ID was absent in 5/6 unrelated individuals who had a missense *ERLIN2* variant on at least one allele (three families with affected individuals);[5,11,12,18,19] conversely, ID was present in all individuals with a homozygous truncating variant.[4,9,20,21] This observation suggests that *ERLIN2*-related disorders can present dosage-dependent clinical features likely due to reduced protein function. It is worth noting that our data lend further support to the notion that zygosity is no longer a barrier to defining a molecular diagnosis in HSP, suggesting that even more HSP genes might be associated with different modes of inheritance. If this is truly the case, it would have implications for the estimated diagnostic yield and could imply differences in disease-associated phenotypes. The question of why parental carriers of a single variant in *ERLIN2* are healthy, though harboring a heterozygous predictably pathogenic variant, remains unclear. It is possible that the *ERLIN2* missense variants causing disease in heterozygous state have different effect on protein, such as dominant-negative or gain-of-function effects, whereas the variants that cause disease only when biallelic cause loss/reduction of protein function [5]. This would need to be investigated with functional studies. A final comment worth mentioning is the maternal UPD seen in our report. This unusual modality of transmission should alert clinicians to carefully evaluate results of next-generation gene sequencing when counseling families, especially on risks of recurrence.

In sum, we define new clinical presentations and gene variants in *ERLIN2*-related pathology; we report a common pathogenic variant in families of South Asian ancestry; and we affirm that autosomal-dominant transmission can be a mode of inheritance associated with *ERLIN2*-related disorders. Further research is needed to elucidate disease mechanisms of *ERLIN2*-related disorders.

Acknowledgments

Author contribution

Conception and design of the study: S.S., A.D., D.E-F., F.M.S. Acquisition and analysis of data: S.S., A.D., J.S.C, L.S., I.R., A.P., A.F., D.E-F., F.M.S. Drafting a significant portion of the manuscript or figures: S.S., A.D., D.E-F., F.M.S., J.S.C.

Conflict of Interest

JSC is a consultant to Invitae. SS received consulting fees from Guidepoint. The other authors declare no conflict of interest related to this study.

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Supporting Information

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

Table S1. Additional clinical features of the patients reported in our cohort.
Table S2. Clinical and genetic summary of previously reported patients with ERLIN2-related disorders.