Journal of Human Genetics (2018) 63:927–933
https://doi.org/10.1038/s10038-018-0463-6

BRIEF COMMUNICATION

Novel splice-site variant of *UCHL1* in an Indian family with autosomal recessive spastic paraplegia-79

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Received: 12 December 2017 / Revised: 20 March 2018 / Accepted: 10 April 2018 / Published online: 7 May 2018

Abstract

Spastic Paraplegia-79 (SPG79) is an autosomal recessive type of childhood onset complicated by hereditary spastic paraplegia. SPG79 is characterized by spasticity, paraplegia, optic atrophy, cerebellar signs, and other variable clinical features. Recessive, disease causing variants in *Ubiquitin C-terminal hydrolase-L1* (*UCHL1*) gene have been implicated as a cause for SPG79 in two families till now. In this study, we report on a third family of SPG79 with two similarly affected siblings, harboring a novel homozygous splice-site variant in the *UCHL1* gene (NM_004181.4: c.459+2T>C). The variant was identified by whole-exome sequencing and validated by Sanger sequencing in the family.

Introduction

Spastic paraplegia (SPG) is a highly heterogeneous group of inherited progressive neurodegenerative disorders, and so far more than 80 genes are known to cause SPGs. SPGs are broadly classified as pure and complicated SPG based on presence or absence of additional clinical manifestations (other than SPG) respectively. SPGs are inherited as autosomal dominant, autosomal recessive, and X-linked disorders. So far 44 genes are described to cause autosomal recessive SPG, majority of these being childhood onset and complicated type of SPG (OMIM).

Spastic Paraplegia 79 (SPG79) is an autosomal recessive SPG, characterized by early onset of SPG and optic atrophy. Additional variable features include peripheral neuropathy, cerebellar signs, seizures, borderline cognitive impairment, and abnormal neuroimaging findings [1]. Recently Rydning et al. [1] described a Norwegian family with three affected siblings (monozygotic twins and their sister) with childhood onset progressive visual loss and spasticity, harboring a compound heterozygous variant (NM_004181.4:c.533G>A; c.647C>A) in *UCHL1* gene. In 2013, Bilguvar et al. [2] reported a Turkish family with three siblings affected with this complex form of neurodegenerative disorder (childhood-onset neurodegeneration with optic atrophy, ataxia, and SPG) and a homozygous missense *UCHL1* variant (NM_004181.4:c.20A>C). We describe a third family of Indian origin with two affected siblings harboring a novel homozygous, likely pathogenic, splice-site variant, NM_004181.4:c.459+2T>C in *UCHL1*, which was identified by whole-exome sequencing and validated by Sanger sequencing in the family.

Patients and methods

Patients (P1 and P2)

Both siblings (P1 and P2) presented with history of motor developmental delay and gait imbalance. Progressive spasticity was associated with generalized decrease in the muscle bulk, chest deformity, and joint contractures. Gradually both of them became non-ambulatory by age 5–7 years and there was associated poor vision. Other clinical findings included—tremors, other cerebellar signs, facial dysmorphism, myopathic face, microcephaly, and

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s10038-018-0463-6) contains supplementary material, which is available to authorized users.

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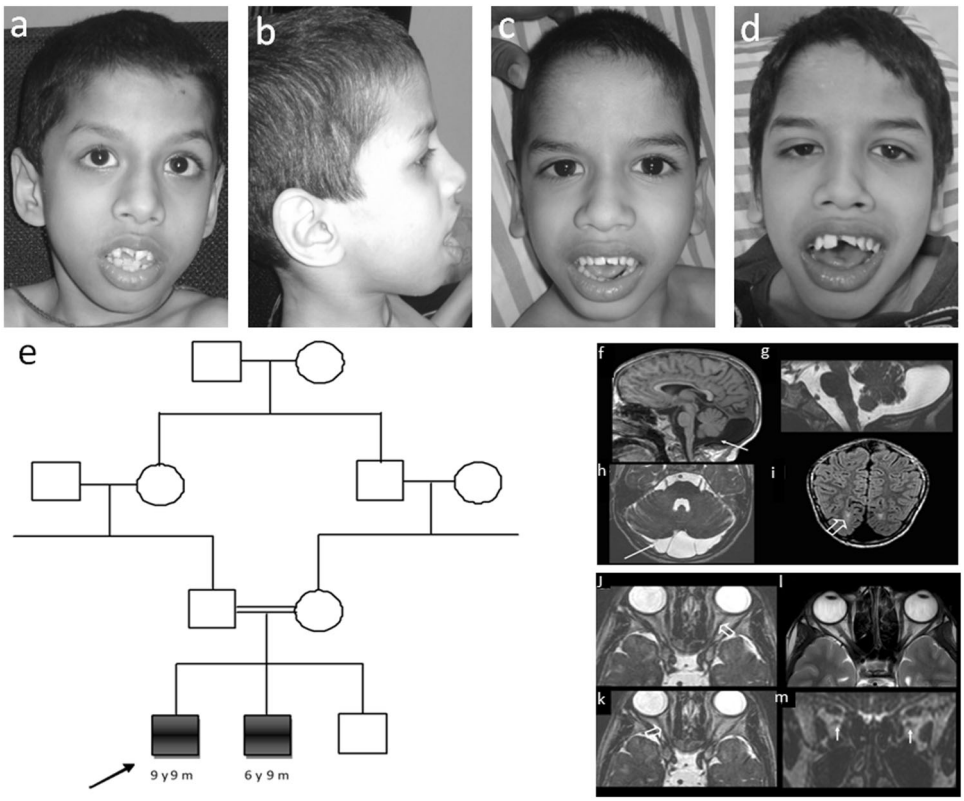

Fig. 1 a, b (Patient 1)—Down slant palpebral fissures, epicanthic folds, broad nasal base, thick full lips, open mouth, micrognathia, relatively large looking ears, and overall appearance of myopathic face. **c** (Patient 2 at age 6 years 9 months) and **d** (at age 13 years)—Down slanting palpebral fissures, epicanthic folds, broad nasal base, open mouth, thick full lips, micrognathia, relatively large looking ears, frontal upsweep, anterior unusual hair whorl, and with age overall myopathic facial appearance. **e** Pedigree of the family. **f**, **g** MRI findings for Patient 2—T1 and T2W MRI sagittal images sagittal and **h** axial image demonstrates large cysterna magna (arrow). Cerebellum and brain stem are well developed. **i** On FLAIR coronal MRI, there are areas of gliosis in both occipital white matter (open arrow). **j**, **k** T2W axial MR images of optic nerves of the patient show near symmetric, relatively small optic nerves (mean 24 mm) on both sides (open arrow). **l** Age-matched control shows a mean optic nerve measurement of 38 mm. **m** Coronal MR image of the patient also show near symmetric small sized, optic nerves (arrow)

fasciculations (both tongue and limb muscles). Cognitive functions were normal for age although IQ test at age 9 years for elder sibling (P1) and at age 6 years for younger sibling (P2) revealed scores of 59 and 56 respectively. Sensory system was normal in both siblings. Only elder sibling (P1) had recurrent seizures (age of onset 1.5 years), and was on anti-epileptic drugs. Detailed clinical history and examinations of the patients are given in Fig. 1 and Table 1 and also provided in Supplementary Note.

Whole-exome sequencing

Whole-exome sequencing was carried out on peripheral leukocyte DNA of the younger sibling (P2) to ascertain coding sequence variants. Exome sequencing and data analysis pipelines were described earlier [3]. The study protocol was approved by the Institutional Ethics Committees of respective institutes. PCR was performed using specific primers F-5′-GGCTAGGGGAAAAGGTACAGC-3′ and R-5′-TTGGCCTTGGGTGGATAACAT-3′, and Sanger sequencing was done using ABI 3130 Genetic analyzer (Life Technologies, Carlsbad, CA) following the manufacturer's protocol in control, parents, and patient samples to validate the variant. Pathogenicity of the variant was tested using online mutation prediction tools like MutationTaster2 [4], CADD [5], and Human Splicing Finder version 3 [6].

Results

The quality information of exome-sequencing raw data and the variant filtering strategy are outlined in Table S1 and Table S2 respectively. After filtering steps, a total of three variants were shortlisted, among which a likely pathogenic variant was identified after detailed clinical correlation with the patient's phenotype (Table S3). The variant was a novel homozygous splice-site variation in intron 6 of the *UCHL1*

Table 1 Clinical and molecular findings in patients with SPG79 in the patients described in this study and in the patients described by Bilguvar et al. [2] and Rydning et al. [1]

	Bilguvar et al. [2013]			Rydning et al. [2017]			Present family	
	NG 1024-1	NG 1024-2	NG 1024-3	Patient III-4	Patient III-5	Patient III-6	P1	P2
Age[a] at examination	28	33	34	65	62	62	9.9	6.9
Age[a] at onset	5	7	5	10	10	10	2	2
First symptom	Visual loss	Visual loss	Gait imbalance	Visual loss	Visual loss	Visual loss	Motor developmental delay & seizures	Motor developmental delay & gait imbalance
Vision	−	−	−	Myopia	Light perception	Light perception	Poor	Poor
Optical atrophy	+	+	+	+	+	+	+	+
Nystagmus	+	+	+	+	+	+	+	+
Standing without assistance	−	−	−	−	−	−	−	−
Head titubation	+	+	+	+	−	−	−	+
Tremors	NA	NA	NA	+	+(Intention tremor in UL)	+(Intention tremor in UL)	+(not seen at 16 years of age)	+
Deep tendon reflexes (UL/LL)	++/+++	++/+++	++/+++	+/++	+/++	+/++	+	+
Up-going plantar reflexes	+	+	+	−	+	+	+	+
Generalized muscle atrophy	+	+	+	+	+	+	+	+
Spasticity	+	+	+	−	+	+	+	+
Microcephaly	NA	NA	NA	NA	NA	NA	+	+
Ophthalmoplegia	NA	NA	NA	−	Paresis upward gaze	−	−	−
Fasciculations	NA	NA	NA	−	+	+	+	+
Joint contractures	NA	NA	NA	−	+	+	+	+
Hand Myotonia	+	−	−	−	−	+	−	−
Cognitive impairment	NA	IQ74	IQ71	−	−	−	IQ56	IQ59
Cerebellar signs—dysmetria, dysarthria, ataxic gait	+	+	+	±	±	±	+	+
Sensory system (superficial, position & vibration sense)	Affected	Affected	Affected	Affected	Affected	Affected	Unaffected	Unaffected
Seizures	−	−	+	−	−	−	+	−

Table 1 (continued)

	Bilguvar et al. [2013]			Rydning et al. [2017]			Present family	
	NG 1024-1	NG 1024-2	NG 1024-3	Patient III-4	Patient III-5	Patient III-6	P1	P2
Facial dysmorphism	NA	NA	NA	NA	NA	NA	+	+
Chest deformity	NA	NA	NA	−	+	+	+	+
Pes cavus	NA	NA	NA	+	+	+	−	−
Visual evoked potentials (VEP)	NA	Increased latency time and reduced amplitude both eyes	Increased latency time and reduced amplitude both eyes	Nearly absent response	Nearly absent response	Nearly absent response	Absent wave forms	Absent wave forms
VEP electroretinogram	NA	Normal	Normal	Normal	Normal	Normal	NA	NA
Brian MRI (age[a])	Bilateral optic nerve and chiasm atrophy, Wallerian degeneration of the optic radiations, cerebellar and mild cerebral atrophy (NA)			Optic atrophy (63)	Optic atrophy and mild atrophy of superior vermis of cerebellum (63)	Optic atrophy (62)	Normal, not verified (8)	Large cisterna magna, bilateral areas of gliosis in occipital white matter, bilateral optic nerves atrophy (13)
Muscle biopsy	NA			Normal	NA	Chronic denervation	Not done	Not done
Mutations reported (zygosity)	c.20A>C (homozygous)			c.533G>A;c.647C>A (compound heterozygous)			c.459+2T>C (homozygous)	

NA not available; + present; − absent; *UL/LL* upper limb/lower limb

[a] All ages are in years; deep tendon reflexes = +normal, ++brisk, +++increasingly brisk

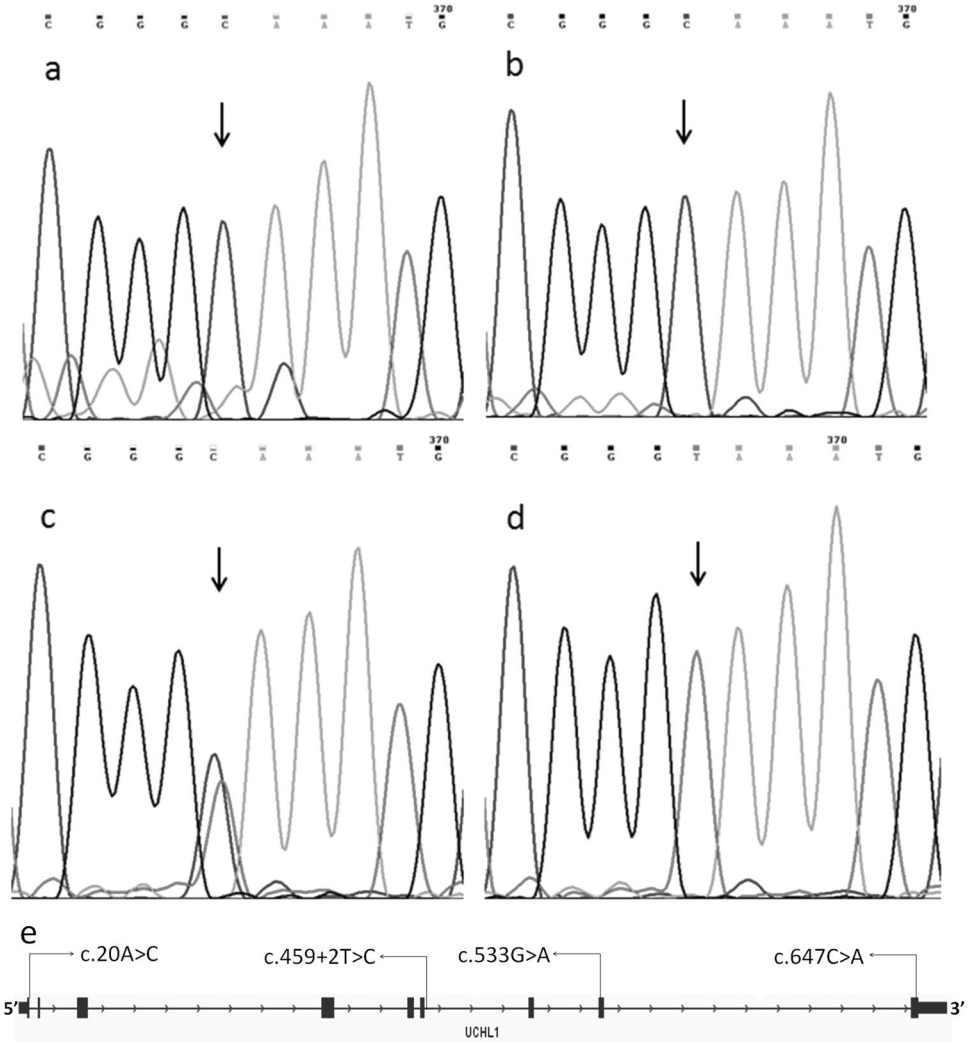

Fig. 2 Sanger validation of the *UCHL1* variant in family. The variation c.459 + 2T>C of *UCHL1* is found in homozygous state in probands (**a**, **b**) and is heterozygous in their mother (**c**). Normal control has the homozygous wild-type allele (**d**). **e** A schematic representation of *UCHL1* gene structure (exons 1–9) showing the earlier variants reported by Bilguvar et al. [2] (c.20A>C) and Rydning et al. [1] (c.533G>A;c.647C>A) and our variant (c.459 + 2T>C) in association with SPG79

gene (NM_004181.4:c.459 + 2T>C) which affects the invariant 5′ donor splice-site of exon 6. The variant was not present in 1000 Genomes project, Exome Variant Server, ExAC, gnomAD, and in our in-house exome database of 96 unrelated individuals from all over India. The in silico splice-site effect assessment of this variant by online tools scored low for the mutant splice-site and predicted possible splicing aberrations in the transcript due to this variant (Figs. S1 and S2). The variant was confirmed in the elder sibling (P1) and validated in the family using Sanger sequencing (Fig. 2). The variant has been submitted to ClinVar (ClinVar accession ID: SCV000746265.1)

Discussion

We report on two siblings sharing a common phenotype of an early onset neurodegenerative disorder characterized by SPG, optic atrophy, seizures, and facial dysmorphism. Whole-exome sequencing identified a homozygous deleterious splice-site variant in *UCHL1* gene confirming the diagnosis of SPG79 (MIM#615491). This is the third report of a family with SPG79 and mutations in *UCHL1* gene.

The *UCHL1* encodes a thiol protease enzyme Ubiquitin carboxyl-terminal hydrolase-L1 (UCHL1) which is one of the most abundant proteins in the brain, comprising almost 1–2% of the total soluble protein [7]. Expression of *UCHL1* is seen in most neuronal cells, neuroendocrince cells, podocytes, and various other cells (due to its role in cancer pathology) [8]. The protein is mainly a deubiquitinating enzyme that has hydrolase activities in the ubiquitin–proteasome pathway. In vitro studies have also shown ubiquitin ligase activity in this pathway [9]. These studies suggest that UCHL1 enzyme may play an important role in maintaining the ubiquitin stability within the neurons [10].

Variants in *UCHL1* have been implicated in the late onset common neurodegenerative disorders like Parkinson's disease and Alzheimer's disease [11–14]. In 2013, Bilguvar

et al. reported a family with a early onset form of neurodegenerative disorder and a homozygous *UCHL1* missense variant [2]. They described three siblings born to consanguineous parents of Turkish origin with childhood-onset neurodegeneration, optic atrophy, ataxia, and SPG. All patients had the homozygous c.20A>C;p.Glu7Ala variant causing a loss of function of UCHL1 as observed in *UCHL1* knockout mice [15]. Recently, Rydning et al. [1] reported a second family with similar phenotype and a compound heterozygous variant in *UCHL1* gene (c.533G>A;p.Arg178Gln and c.647C>A;p.Ala216Asp). However, further studies showed contradictory functional consequences as the c.647C>A variant led to loss of function (insoluble protein), whereas the c.533G>A led to increased enzyme (hydrolytic) activity. The authors proposed that while the non-soluble c.647C>A variant results in reduction of functional UCHL1 protein and contributes to neurodegeneration, the increased enzymatic activity of the c.533G>A variant might offer a protective effect on cognitive function as their patients showed conspicuously well-preserved cognitive abilities than the patients described by Bilguvar et al. [2] despite similar neurological dysfunctions.

In the present study, we describe a third family of Indian origin with two siblings similarly affected with childhood-onset optic atrophy and spasticity harboring a homozygous splice-site variant c.459 + 2T>C in *UCHL1*. Additional features observed were recurrent childhood seizures in one of the probands (P1), facial dysmorphisms, and microcephaly in both the siblings. Cognitive functions appeared to be normal in both the siblings (during their home-visit interaction), similar to that observed by Rydning et al. group in their patients, although measured IQ scores were low (at young age). Apparent discrepancy of observed cognitive abilities (home-visit interaction and formal IQ testing) might be subjective and confounded by the presence of dysarthria, optic atrophy, and method used to measure IQ. The clinical features of the previously reported patients are comparable to that of the probands described in our study. However, progression seems to be more severe relative to previously reported two families, as both siblings were non-ambulatory at an early age (first decade). Both patients showed similar facial dysmorphic features, myopathic face and microcephaly, which have not been discussed in two previously reported families. Further studies are needed to understand facial dysmorphic features as a clinical feature of *UCHL1* gene mutations. Additional neuroimaging findings in our patient family (P2) and those unreported in previous two families, include large cisterna magna and bilateral areas of gliosis in occipital white matter (Fig. 1f–m). The hyperintense signals in occipital region do not appear to be due to hypomyelination. A comparison of clinical and molecular features of the probands with the previously described cases with mutations in *UCHL1* is given in Table 1. The splice-site variant identified by exome sequencing in intron 6 of the *UCHL1* gene, is likely to affect the invariant 5′ donor splice-site of exon 6.

One of the limitation of this study involves the inability to ascertain further functional assessment of the variant to see the actual effect of the variant in splicing aberrations, however, a distinct phenotype–genotype correlation and variation in the consensus splice-site region ensures a possible deleterious effect of the variant in the disease etiology. Another discrepancy is that we could not show complete familial co-segregation as father and normal sibling did not give their consent to participate in the study.

Finally, we report a novel *UCHL1* splice-site variant in multiple members of a family of Indian origin affected with SPG79. This finding substantiates that mutations in *UCHL1* gene are the cause of this rare phenotype, and should be considered in the diagnosis of patients with early-onset optic atrophy and subsequent progressive neurodegenerative symptoms.

Acknowledgements We thank the families who cooperated with the evaluation of the subjects and consented for participation in this study.

Funding This work was supported by Science and Engineering Research Board (SERB), Government of India (SERB file no. YSS/2015/001681).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Rydning SL, Backe PH, Sousa MML, Iqbal Z, Oye A-M, Sheng Y, et al. Novel UCHL1mutations reveal new insights into ubiquitin processing. Hum Mol Genet. 2017;26:1031–40.
2. Bilguvar K, Tyagi NK, Ozkara C, Tuysuz B, Bakircioglu M, Choi M, et al. Recessive loss of function of the neuronal ubiquitin hydrolase UCHL1 leads to early-onset progressive neurodegeneration. Proc Natl Acad Sci USA. 2013;110:3489–94.
3. Das Bhowmik A, Dalal A. Whole exome sequencing identifies a novel frameshift mutation in *GPC3* gene in a patient with overgrowth syndrome. Gene. 2015;572:303–6.
4. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. Nat Methods. 2014;11:361–2.
5. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46:310–5.
6. Desmet FO, Hamroun D, Lalande M, Collod-Béroud G, Claustres M, Béroud C. Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. 2009;37:e67.
7. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science. 1989;246:670–2.
8. Doran JF, Jackson P, Kynoch P, Thompson RJ. Isolation of PGP 9.5, a new human neurone-specific protein detected by high

resolution two-dimensional electrophoresis. J Neurochem. 1983;40:1542–7.
9. Liu Y, Fallon L, Lashuel HA, Liu Z, Lansbury PT Jr. The UCHL1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. Cell. 2002;111:209–18.
10. Osaka H, Wang Y-L, Takada K, Takizawa S, Setsuie R, Li H, et al. Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. Hum Mol Genet. 2003;12:1945–58.
11. Zhang M, Cai F, Zhang S, Zhang S, Song W. Overexpression of ubiquitin carboxyl terminal hydrolase L1 (UCHL1) delays Alzheimer's progression in vivo. Sci Rep. 2014;4:7298.
12. Ragland M, Hutter C, Zabetian C, Edwards K. Association between the ubiquitin carboxyl-terminal esterase L1 gene (UCHL1) S18Y variant and Parkinson's disease: a HuGE review and metaanalysis. Am J Epidemiol. 2009;170:1344–57.
13. Liu Y, Chen YY, Liu H, Yao CJ, Zhu XX, Chen DJ, et al. Association between ubiquitin carboxy-terminal hydrolase-L1 S18Y variant and risk of Parkinson's disease: the impact of ethnicity and onset age. Neurol Sci. 2015;36:179–88.
14. Butterfield DA, Gnjec A, Poon HF, Castegna A, Pierce WM, Klein JB, et al. Redox proteomics identification of oxidatively modified brain proteins in inherited Alzheimer's disease:an initial assessment. J Alzheimers Dis. 2006;10:391–7.
15. Chen F, Sugiura Y, Myers KG, Liu Y, Lin W. Ubiquitin carboxyl-terminal hydrolase L1 is required for maintaining the structure and function of the neuromuscular junction. Proc Natl Acad Sci USA. 2010;107:1636–41.