Human HINT1 Mutant Proteins that Cause Axonal Motor Neuropathy Exhibit Anomalous Interactions with Partner Proteins

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Received: 17 June 2020 / Accepted: 16 December 2020
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Abstract

The 14 kDa histidine triad nucleotide-binding protein 1 (HINT1) is critical to maintain the normal function of motor neurons. Thus, a series of human HINT1 mutants cause autosomal recessive axonal neuropathy with neuromyotonia. HINT1 establishes a series of regulatory interactions with signaling proteins, some of which are enriched in motor neurons, such as the type 1 sigma receptor or intracellular domain (ICD) of transmembrane teneurin 1, both of which are also implicated in motor disturbances. In a previous study, we reported the capacity of HINT1 to remove the small ubiquitin-like modifier (SUMO) from a series of substrates and the influence of HINT1 mutants on this activity. We now report how human HINT1 mutations affect the interaction of HINT1 with the regulator of its SUMOylase activity, calcium-activated calmodulin, and its substrate SUMO. Moreover, HINT1 mutants exhibited anomalous interactions with G protein coupled receptors, such as the mu-opioid, and with glutamate N-methyl-D-aspartate receptors as well. Additionally, these HINT1 mutants showed impaired associations with transcriptional regulators such as the regulator of G protein signaling Z2 protein and the cleaved N-terminal ICD of teneurin 1. Thus, the altered enzymatic activity of human HINT1 mutants and their anomalous interactions with partner proteins may disrupt signaling pathways essential to the normal function of human motor neurons.

Keywords HINT1 • ARAN-NM • Type 1 sigma receptor • SUMO • NMDAR • ICD teneurin 1

Introduction

Histidine triad nucleotide-binding protein 1 (HINT1) is highly phylogenetically conserved [1] and belongs to the histidine triad (HIT) family. At the cellular level, this 14 kDa protein is ubiquitously expressed and in vitro systems forms homodimers and exhibits purine phosphoramidase activity [2] hydrolyzing lysyl-AMP that is generated by lysyl-tRNA synthetase (LysRS) [3, 4]. HINT1 is under regulation by Redox mechanisms [5, 6] exhibiting zinc- and calmodulin (CaM)-regulated protease activity to remove small ubiquitin-like modifier (SUMO) from target proteins [7]. In recent years, a rare form of hereditary peripheral neuropathy named autosomal recessive axonal neuropathy with neuromyotonia (ARAN-NM) has convincingly being related to mutations in the HINT1 gene. These patients present muscle weakness, wasting, and sensory loss, which starts in the distal parts of the limbs and slowly progresses in a length-dependent manner [8, 9]. HINT1 neuropathy is worldwide distributed and is particularly prevalent in populations in central and southeastern Europe. To date, the majority of diagnosed individuals are of European origin, and among the 15 HINT1 mutations reported, the most common are R37P, C84R, and H112N [10]. Notably, HINT1 mRNA levels in lymphoblasts from ARAN-NM-affected individuals were comparable to those in controls; however, only negligible expression of the mutated HINT1 proteins was detected [8]. In this sense, the targeted disruption of the HINT1 gene does not promote neuropathy-related phenotypes [11]. These findings suggest that the function of human mutants is altered to the extent that it compromises the viability of motor neurons. This would lead to the metabolism of these abnormal proteins by proteasome.

HINT1 is widely expressed in the central nervous system and in other tissues [12, 13]. At the cellular level, this protein is found in the plasma membrane, cytoplasm, and nucleus [1]. HINT1 behaves as a scaffold and/or chaperone in its...
regulatory interactions with a variety of signaling proteins. HINT1 binds simultaneously to the cytosolic C-terminal of the mu-opioid receptor (MOR), to protein kinase C (PKC), and to regulators of G protein signaling (RGS) proteins of the Rz family, such as RGSZ1 and RGSZ2, when they are coupled to neural nitric oxide synthase (nNOS) [14, 15]. With the aid of nitric oxide (NO) from nNOS and zinc ions from cysteine-rich domains of RGSZ2 proteins [5, 16], HINT1 binds the regulatory domain of Raf-1 and conventional PKCy and PKCα and inhibits their activity [16, 17]. HINT1 also binds to NR1 subunits of inotropic glutamate N-methyl-D-aspartate receptors (NMDARs) [16, 18] and, together with type 1 of sigma receptors (σ1Rs), coordinates the activity of G protein coupled receptors (GPCRs) with that of NMDARs [18].

HINT1 interacts in the plasma membrane and in the nucleus with potential transcription factors such as RGSZ2 [19, 20], the Pontin/Reptin complex [21], and the cleaved N-terminal intracellular domain (ICD) of transmembrane protein teneurin 1, which induces the activity of the microphthalmia-associated transcription factor [22]. Previous results show that HINT1 acts as a transcriptional repressor; it is recruited by the DNA damage response [23, 24], triggers apoptosis [25], and exhibits tumor-suppressive activity [26–28]. RGSZ2 and ICD teneurin 1 incorporate SUMO [7, 29], which modifies protein association and transcriptional regulation [30]. HINT1 removes SUMO from these proteins, and this SUMOylase activity appears to be altered in all the ARAN-NM-related HINT1 mutants described so far [7].

In this study, we investigated the interactions of human HINT1 mutants with SUMO and calcium-activated CaM, the substrate, and the regulator of HINT1 SUMOylase function, respectively. We also analyzed HINT1 binding to the C-terminal cytosolic regions of the MOR, to the NR1 subunit of glutamate NMDARs, to the RGSZ2 protein, and to ICD teneurin 1 and the σ1R. A set of non-human HINT1 mutations provided more information about the association of HINT1 with these proteins. Our data indicate that the signaling proteins studied exhibit anomalous interactions with most human HINT1 mutants, which could contribute to their deregulation to finally causing ARAN-NM.

**Materials and Methods**

**Recombinant Protein Expression**

The coding region of human full-length HINT1 (NM_005340: residues 1–126) and its mutated sequences, full-length RGSZ2 (NM_019958.4: residues 1–210), the ICD region of teneurin 1 (NM_011855: residues 2–317), the C-terminal region of MOR1 (AB047546: residues 286–398), C0-C1-C2 region of the glutamate NMDAR NR1 subunit (NM_008169: residues 834–938), and full-length σ1R (AF004927: residues 1–223) were amplified by RT-PCR using total RNA isolated from the mouse brain as the template.

Specific primers containing an upstream Sgf I restriction site and a downstream Pme I restriction site were used, as described previously [16]. The PCR products were cloned downstream of the Glutathione S-transferase (GST) coding sequence (for NR1 and RGSZ2) or HaloTag coding sequence (for HINT1, MOR, σ1R, and ICD teneurin 1) (Flexi® Vector, Promega) and the Tobacco Etch Virus (TEV) protease site. All the sequences were confirmed by automated capillary sequencing, and they were identical to the GenBank™ sequences. The vector was introduced into the E. coli BL21 (KRX #L3002, Promega), and clones were selected on solid medium containing ampicillin. After 3 h of induction at room temperature (RT) in the presence of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 0.1% rhamnose, the cells were collected by centrifugation and maintained at −80 °C.

The GST fusion proteins were purified under native conditions on GSTrap FF columns (#17-5130-01, GE Healthcare), and further purification was achieved by high-resolution ion exchange (#780-0001 Enrich Q, BioRad). The HaloTag fusion proteins were purified under native conditions with HaloLink Resin (G1915, Promega), and they were cleaved in bulk with ProTEV protease (#V605A, Promega); further purification was achieved by high-resolution ion exchange chromatography (#780-0001 Enrich Q, BioRad). Sequences were confirmed by automated capillary sequencing.

**In Vitro Interactions Between Recombinant Proteins: Pull-Down of Recombinant Proteins**

The recombinant HINT1 protein (200 nM) or HINT1 mutants were incubated either with Sepharose 4B (#17-0120-01, GE Healthcare; negative control) or together with the immobilized proteins CaM-agarose 4B (#17-0529-01, GE Healthcare), SUMO1-agarose (#UL-740, Boston Biochem) or RGSZ2, ICD teneurin 1, σ1R, the C-terminus of MOR1, and the C0-C1-C2 region of NMDAR NR1 subunit, which were covalent attached to NHS-activated Sepharose 4 fast flow (4FF, #17-0906-01, GE Healthcare) according to the manufacturer’s instructions.

The interactions were studied in 300 μL of a buffer containing 50 mM Tris-HCl, pH 7.5, and 0.2% 3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonate (CHAPS) in the presence of 2.5 mM CaCl2 and mixed by rotation for 30 min at RT. After incubation, the pellets were recovered by centrifugation, washed three times in the presence of 2.5 mM CaCl2, solubilized in 2x Laemmli buffer, and analyzed by Western blotting.
Western Blotting

The detached HINT1 proteins recovered in the aforementioned procedure were resolved with SDS-PAGE in 4–12% Bis-Tris gels (#NP0341, Invitrogen, Fisher Scientific), with MES SDS as the running buffer (#NP0002, Invitrogen). The proteins were transferred onto 0.2 μm PVDF membranes (#162-0176, BioRad) and probed overnight at 6°C with anti-HINT1 antibody raised in rabbits (Immunostep) against the peptide sequence GYRMVVNEADGGG (93–106). The primary antibody was diluted in Tris-buffered saline (pH 7.7) (TBS) + 0.05% Tween 20 (TTBS) and detected using the appropriate horseradish peroxidase-conjugated secondary antibody, which was visualized by chemiluminescence (#170-5061, BioRad) and recorded on an ImageQuant™ LAS 500 (GE Healthcare). Because all the assays were performed with recombinant proteins, the immune-signals provided a single band of the expected size, which was used for the subsequent densitometry analysis. Accordingly, no other regions of the blots provided information and were routinely excluded from the analysis. The software automatically calculated the optimal exposure time for each blot to provide the strongest possible signal, from which the labelling could be accurately quantified. For each group of samples, protein immunosignals were measured using the area of the strongest signal (average optical density of the pixels within the object area/mm²; AlphaEase FC software). The gray values of the means were then normalized within the 8 bit/256 Gy levels [(256-computed value)/computed value].

Statistical Analyses

All graphs and statistical analyses were generated and made using the SigmaPlot/SigmaStat v.14 package (SPSS Science Software, Erkrath). Experiments were performed at least twice on separate experimental days. Western blot signals were expressed as the change relative to the controls (wild-type HINT1 interaction), which were assigned an arbitrary value of 1. Data using recombinant proteins were analyzed using one-way analysis of variance (ANOVA) followed by the Holm-Sidak multiple comparisons test. Statistical significance was defined as p < 0.05.

Results

Human HINT1 has 126 amino acids forming three helices, five β-sheets, and the rest linear sequences (DNASTAR NovaFold v15, Madison, USA). Currently, 15 HINT1 mutants have been reported to cause ARAN-NM, and protein analysis indicates that these mutants show limited localization in alpha helices (Fig. 1). The CaM-binding motif is located in the HINT1 N-terminal region (12–31 QPGGDTIFGKIRKEIPAKI) [33]. In a β-sheet close to the C-terminus, a series of hydrophobic amino acids (110–116: HIHLHVL) interweave to form the HIT and a typical SUMO-interacting motif (SIM) [34] (Fig. 1a).

HINT1 SUMOylation activity is triggered by calcium-activated CaM and/or NO [7], and in vitro, HINT1 binds calcium-activated CaM and SUMO1 [7]. All but the shortest HINT1 mutant Q62* were included in the study (Supplemental Fig. 9). Human HINT1 mutants R37P and G93D behaved as the wild-type in their interaction with SUMO1, the G89V greatly increased its association, while the other human mutants mostly reduced their interactions with this protein (Fig. 2a; Supplemental Fig. 2). Non-human HINT1 mutants targeting the HINT1 SIM domain V115D and L116Q [7] showed weak associations with SUMO1 (Supplemental Fig. 10), thus confirming the relevance of the SIM domain in HINT1 binding to SUMO proteins.

The human HINT1 mutants C38R and mostly C84R and G89V increased HINT1-CaM association. Non-human mutants, C84S and D87V, also increased this HINT1 interaction suggesting a role for HINT1 84–89 region in the interaction between these proteins. The other human HINT1 mutants reduced their interactions with CaM and the non-human T17A mutant located in the CaM-binding motif abolished this association [7] (Fig. 2b; Supplemental Fig. 10). While human HINT1 mutations H112N and H114R (HIT domain) reduced their interaction with CaM, non-human HINT1 mutants V115D and L116Q (SIM domain) greatly stabilized HINT1-CaM association (Supplemental Figs. 3 and 10).

HINT1 directly associates with the cytosolic C0-C1-C2 region of the NR1 subunit of the glutamate NMDA receptor. In this scenario, human HINT1 mutants showed diminished interactions with this NR1 subunit, except of C38R and G89V mutants, which exhibited a strong and a very strong association, respectively, with this protein. Human and non-human HINT1 mutations in C38 and C84 produced similar effects, and these in the HIT and SIM domains practically abolished this interaction (Fig. 3a; Supplemental Figs. 4 and 10). HINT1 binds to an amino acid sequence at the beginning of the MOR C terminal cytosolic tail [35], which comprises the 354–357 (TSST) cluster. As observed for the association of HINT1 mutants with NR1 subunits, all but G89V diminished their binding to the MOR C-terminal sequence (Fig. 3b; Supplemental Fig. 5). Thus, NR1 and MOR Ct may share HINT1 binding surface, and in fact ex vivo assays suggested this possibility [18].

The RGSZ2 protein is a sumoylated regulator of G protein signaling and is found in the plasma membrane and in the nucleus [29]. HINT1 exhibits in vitro isopeptidase activity on sumoylated RGSZ2 [7] and forms stable complexes with RGSZ2 in the C-terminal region of the MOR [35]. Human HINT1 mutants showed a disparate pattern of interacting with RGSZ2, while some exhibited a strengthened association (C38R, G89V, etc.), other mutants interacted weakly with
RGSZ2 proteins. Human and non-human HINT1 mutants at C38 and C84 produced increases in this association. Human mutants at HIT domain, H112N and H114R, diminished HINT1-RGSZ2 interactions, and non-human V115D and L116Q (SIM domain) enhanced their interaction (Fig. 4; Supplemental Figs. 6 and 10).

Protein analysis revealed the almost absence of secondary structure in ICD teneurin 1 (Fig. 5). This protein interacts with HINT1, while calcium promotes and calcium-activated CaM diminishes this association [7]. Human HINT1 mutant proteins showed mostly weakened associations with ICD teneurin 1. As observed above for HINT1-CaM interaction, C84R and G89V increased their interaction with ICD teneurin 1 as also did non-human mutants, C84S and D87V. Thus HINT1 84–89 region may be shared by CaM and ICD teneurin1 in their binding to HINT1. Disruption of HIT domain also diminished this interaction (Fig. 5; Supplemental Figs. 7 and 10).

The association of HINT1 with σ1R is weak [18], and exception of C38R and G89V, most human HINT1 mutants exhibited even weaker interactions with the σ1R. Disruption of HIT and SIM domains caused similar changes to these
observed for ICD teneurin 1-HINT1 (Fig. 6; Supplemental Figs. 8 and 10).

Discussion

Because, the phylogenetically conserved HINT1 protein participates in a series of physiological processes at the plasma membrane, in the cytosol and in the nucleus, HINT1-regulated signaling pathways may be substantially altered by the mutations that cause ARAN-NM. Our present study shows that human HINT1 mutants exhibit anomalous interactions with a series of signaling proteins that interact in vivo with the wild-type form (see Introduction). These proteins include CaM, which regulates HINT1 SUMO protease activity, RGSZ2, which is implicated in GPCR signaling via G proteins, and ICD teneurin 1, which bind to HINT1 for transportation from the plasma membrane to the nucleus, GPCRs such as MOR and ionotropic glutamate NMDAR, both of which are regulated by HINT1 and σ1R [18, 35]. With a few exceptions, the human HINT1 mutant proteins showed reduced associations with the analyzed proteins compared with the HINT1 wild-type. Initially, crystallization studies revealed that HINT1 forms a homodimer with protomers interacting through their C-terminal sequences [31]. Subsequently, in vitro studies with the purified protein also confirmed its dimeric organization [32]. In the nucleus, HINT1 inhibits the transcription of target genes [36], forms a stable association with the Pontin/Reptin complex to inhibit the β-catenin transcriptional pathway [21, 37], and forms complexes with lysyl-tRNA synthetase (LysRS) and transcription factors such as MITF or USF2 [38, 39]. HINT1 displays nucleoside phosphoramidase/acyl-AMP hydrolase activity and SUMO protease activity [2, 7]. While the known ARAN-NM-related human HINT1 mutants lack or exhibit a deregulated isopeptidase activity [7], analysis of 5 human HINT1 mutants indicated alterations in their nucleoside phosphoramidase activity and/or in their capacity to form the dimer in vitro [32]. Thus, in vivo conversion of HINT1 from a homodimer to monomer, or a significant reduction of the enzyme’s catalytic efficiency [7, 32] probably contributes to the development of ARAN-NM. These studies suggest that in mature neuronal cells, HINT1 may exist as a monomer and homodimer, and even through its interaction with other proteins, it may result in heterodimers. The physiological regulation of such processes deserves further research.

In wild-type HINT1, isopeptidase activity is prevented by zinc ions binding to Cys84 in the catalytic domain and promoted by their removal by calcium-CaM or NO [40, 41].
N-terminal region of HINT1 binds CaM, and accordingly the non-human T17A mutation prevents the CaM-mediated activation of HINT1 SUMOylase activity but not NO-mediated activation, which releases zinc bound to Cys84 [7]. The availability of NO necessary to disrupt the zinc-Cys84 interaction is facilitated by the colocalization of HINT1 with nNOS in the MOR environment in which HINT1 is associated with RGSZ2–nNOS complexes [42]. HINT1 protease activity is altered in all human HINT1 mutants, which exhibit mostly weak interactions with CaM and the substrate SUMO. HINT1 phosphoramidase activity is also altered in the five human mutants evaluated [32]. Thus, both HINT1 enzymatic activities may share critical amino acid residues at the catalytic site [2, 7, 32]. Notwithstanding, the H112N HINT1 mutant triggers apoptosis independent of its enzymatic activity [25]. Thus, the proapoptotic and tumor-suppressive role of HINT1 may reside in its regulatory interaction with third partner proteins. We may consider that human HINT1 mutations are distributed into two arbitrary regions of the sequence, the first cluster from F33S to K57N and the second from C84R to W123*. Some of the mutations within the first region result in isopeptidase activity, albeit deregulated; however, none of the mutations in the second cluster exhibit this activity [7]. The second region contains the catalytic Cys84-Asp87-His114 core and the conserved HIT domain, which alternates with the hydrophobic amino acids of the SIM.

The HINT1 protein in the plasma membrane also behaves as a scaffold and binds simultaneously to cytosolic regions of GPCRs such as MOR [42], protein kinases such as PKCγ and PKCα, Raf-1 [6, 43], and proteins of the RGS family (RGSZ1 and RGSZ2) [15, 35]. HINT1 competes with calcium-activated CaM and σ1R for their binding to the cytosolic C0-C1-C2 region of NR1 subunits of neural glutamate NMDAR [16]. As a result, σ1Rs promote NMDAR activity, while HINT1 and CaM diminish this activity [44]. A tight functional relationship exists between HINT1 and σ1R in the neural membrane. The interplay between these proteins promotes physical coupling and uncoupling between MOR and the NR1 subunit of NMDAR [18]. Indeed, in mice lacking HINT1 or σ1R, the functional regulation between MOR and NMDAR is lost, morphine does not recruit NMDAR function, and the direct activation of NMDARs does not reduce morphine analgesia [16, 35].

Our study indicates that except for a pair of mutations, human HINT1 mutant proteins bind poorly to σ1Rs, MORs, and NMDAR NR1 subunits. Thus, G89V and C38R mostly increased HINT1 associations with these proteins; however, these mutants exhibit an impaired or deregulated SUMOylase activity [7]. HINT1 regulation of NMDARs may be relevant to the development of ARAN-NM. In this context, human HINT1 mutants deregulate NMDAR interactions with other signaling proteins contributing to neurological disorders, including neurodegenerative diseases [45], and probably alterations in motor coordination. In fact, the progression of amyotrophic lateral sclerosis (ALS) is
delayed by drugs such as riluzole, which diminishes the function of NMDARs [46].

The association of HINT1 proteins with ICD teneurin 1 and σ1Rs is relevant to the normal functioning of motor pathways. HINT1 couples with ICD teneurin 1, which acts as a transcription factor in the nucleus [22], and its dysfunction has been associated with Alzheimer’s disease [47]. Teneurins (four members in humans, Ten1–4) promote neurite outgrowth, cell adhesion, dendritic morphology, axonal guidance, and synapse formation [48]. The two teneurins present in Drosophila, Ten-m, and Ten-a regulate neuromuscular synapse organization and target selection [49]. Ten-a is presynaptic, while Ten-m is mostly postsynaptic; neuronal Ten-a and muscle Ten-m form a complex in vivo. Elevated Ten-m expression regulates target selection in specific moto neurons and muscles via homophilic matching and functions with additional molecules to mediate precise neuromuscular connectivity. Pre- or postsynaptic teneurin perturbations cause severe synapse loss and impair many facets of organization transynaptically and cell-autonomously. In addition, three missense mutations have been identified in the human TEN4 gene, which are associated with patient families displaying essential tremor movement disorder [50]. Ten4 is a regulator of oligodendrocyte differentiation and plays a critical role in the myelination of small-diameter axons in the central nervous system [51]. Moreover, Ten4−/− mice exhibit a tremor-like phenotype, and a missense mutation in TEN1 gene was predicted to be potentially pathogenic for cerebral palsy, a clinically heterogeneous group of disorders affecting movement and posture [52].

On the other hand, like teneurin 1, the HINT1-associated protein RGSZ2 has also been involved in Alzheimer’s disease as well as in cognition, panic disorder, schizophrenia [53], and several human cancers, such as lung, prostate, breast, and liver [19, 20]. In vivo regulation of σ1R and glutamate NMDAR promotes HINT1 binding to sumoylated RGSZ2 proteins [7]. The covalent attachment of SUMO does not affect RGSZ2 binding to GPCR-activated GaGTP subunits but abolishes its GTPase accelerating activity. By contrast, non-covalent binding of SUMO with the SIM located in the RGS box impedes RGSZ2 from interacting with GaGTP subunits.
Binding of SUMO to the RGSZ2 SIM that lies outside the RGS box does not affect GαGTP binding or GTPase accelerating activity (GAP) [53], but it could lead to regulatory interactions with other sumoylated proteins. Thus, RGSZ2 activity propagating GPCR signaling via activated G proteins is under regulation by SUMO and the HINT1 protein. While almost every mutation in the human HINT1 protein weakened the interactions with associated proteins, the interactions with RGSZ2 were more evenly altered, with almost half increasing and half decreasing the strength of their binding. Because HINT1 couples with ICD teneurin 1, which acts as a transcription factor in the nucleus [22], disruptions in HINT1-teneurin 1 or HINT1-RGSZ2 interactions together with the impairment of HINT1 isopeptidase activity may affect the function of these proteins in the nucleus, regulating gene expression, which may underlie the pathophysiology of ARAN-NM.

The σ1R is a transmembrane protein mostly located in the endoplasmic reticulum (ER) [54] but also at the plasma membrane [55–57] and at the nuclear envelope [58, 59]. Notably, σ1R is highly expressed in motor neurons [60, 61], and autosomal recessive loss-of-function mutations in σ1R are primarily associated with distal hereditary motor neuropathy [59, 62, 63] and amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD) [64–66]. Indeed, the σ1R E102Q mutation that causes juvenile ALS [67] eludes ligand control, exhibits anomalous response to calcium, and rapidly aggregates in the ER and associated compartments in transfected cells, provoking alterations in proteasomal degradation and calcium homeostasis [68, 69]. Moreover, the lack of σ1R exacerbates ALS progression in G93A-SOD1 mice, and σ1R−/− mice showed motor neuron degeneration pathology [70]. HINT1 does not establish strong interactions with σ1Rs [18], but both...
proteins cooperate to bring certain GPCRs under the regulation of glutamate NMDARs [18]. Given the high expression of α1Rs in motor neurons and the functional relation between HINT1 and σ1R, mutations in any of these proteins may impair a series of physiological pathways, provoking the onset of motor neuron pathology.

As mentioned, the targeted deletion of the HINT1 gene does not promote significant motor disturbances or peripheral neuropathy in mice [11]. This observation indicates that HINT1 functions are efficiently substituted by other proteins. In ARAN-NM patients homozygous for these mutations, HINT1 proteins are rapidly metabolized by the proteasome [8], suggesting that mutated HINT1 proteins challenge cell viability. However, its continued destruction does not prevent cell damage from accumulating until motor disease finally becomes apparent. If this is the case, an early expression block of HINT1 mutants would likely reduce the phenotypic expression of ARAN-NM. Interestingly, recent reports suggest a potential therapeutic role for HINT1 exogenous regulators in the clinical management of acute and neuropathic pain [71, 72]. The possible utility of such pharmacological interventions to alleviate the progression of HINT1-related ARAN-NM merits consideration.

In summary, HINT1 is highly phylogenetically conserved, suggesting that it is a protein with high biological relevance. Human HINT1 mutations that produce ARAN-NM give rise to abnormal associations with signaling proteins that regulate or are regulated by wild-type HINT1 in neuronal cells. Thus, in the absence of these interactions, mutated HINT1 proteins could accumulate causing cell damage. Therefore, the pathogenesis of this disease may be related to the deterioration of a signaling pathway particularly relevant to motor neurons and in which HINT1 is involved.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-020-02265-x.

Acknowledgments Elsa Cortés-Montero is a recipient of a Fellowship from MECD [FPU 15/02356]. We would like to thank Gabriela de
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