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Disease mutations in the prion-like domains of hnRNPA1 and hnRNPA2/B1 introduce potent steric zippers that drive excess RNP granule assembly

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Approximately 1% of human proteins harbor a prion-like domain (PrLD) of similar low complexity sequence and amino acid composition to domains that drive prionogenesis of yeast proteins like Sup35. PrLDs are over-represented in human RNA-binding proteins and mediate phase transitions underpinning RNP granule assembly. This modality renders PrLDs prone to misfold into conformers that accrue in pathological inclusions that characterize various fatal neurodegenerative diseases. For example, TDP-43 and FUS form cytoplasmic inclusions in amyotrophic lateral sclerosis (ALS) and mutations in TDP-43 and FUS can cause ALS. Here, we review our recent discovery of discrete missense mutations that alter a conserved gatekeeper aspartate residue in the PrLDs of hnRNPA2/B1 and hnRNPA1 and cause multisystem proteinopathy and ALS. The missense mutations generate potent steric zippers in the PrLDs, which enhance a natural propensity to form self-templating fibrils, promote recruitment to stress granules and drive cytoplasmic inclusion formation. PrLDs occur in ~250 human proteins and could contribute directly to the etiology of various degenerative disorders.

Keywords: prion, prion-like domain, hnRNPA1, hnRNPA2/B1, amyotrophic lateral sclerosis, multisystem proteinopathy

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Prions are proteins that access a variety of infectious, self-templating amyloid forms.[1-5] Typically, prions confer phenotypic changes that can spread within an individual, between individuals and even between species.[1-5] In yeast, prions can promote survival by generating diverse and heritable phenotypic traits in response to specific environmental cues or stress.[6-11] Canonical yeast prion proteins are unified by the presence of a prion domain of low complexity sequence that is enriched in glycine as well as the uncharged polar amino acids: asparagine, glutamine, tyrosine and serine.[12-15] Yeast prion domains can switch between rapidly fluctuating unfolded conformations (non-prion forms) and a myriad of infectious amyloid folds termed prion strains.[1,16-18] Deletion of the prion domain precludes prion behavior and appending the prion domain to innocuous reporter proteins enables them to access prion states.[12,19] Remarkably, it is the amino acid composition of the yeast prion domain rather than its precise primary sequence that endows prion behavior.[14,15,20] This unusual property has informed the design of algorithms capable of accurately identifying new yeast prions.[10,13,14]

Strikingly, when applied to the human proteome, these prion domain prediction algorithms uncover "prion-like" domains (PrLDs) in ~1% of human proteins.[12,21] The function of predicted PrLDs in humans remains unclear. However, ~20% of PrLD-containing proteins are RNA-binding proteins and transcription factors are also enriched.[12,22] Conspicuous among human PrLD-containing proteins are TDP-43 and FUS,[5,12,23] which are predominantly nuclear RNA-binding proteins that frequently mislocalize and form cytoplasmic inclusions in degenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD)

and inclusion body myopathy (IBM).[23-26] Indeed, rare cases of ALS and FTD are caused by mutations in TDP-43 and FUS, and these mutations can occur within the predicted PrLD.[27] The PrLD drives assembly of TDP-43 and FUS into oligomeric structures and linear polymers.[28-31] Moreover, ALS-linked mutations in the PrLD of TDP-43 can accelerate these misfolding events.[29] However, a pathogenic role for PrLD-mediated misfolding has not been clearly established. Nevertheless, the presence of PrLDs in TDP-43 and FUS has led to conjecture that they represent just "the tip of the iceberg" and that PrLD-mediated misfolding of other proteins might underlie many age-related diseases.[12,23,32,33] A hint that the PrLD of FUS as well as the PrLDs of two related RNA-binding proteins, TAF15 and EWSR1, might contribute to disease also comes from deleterious genomic rearrangements that cause sarcoma and leukemia.[34] Here, a chromosomal translocation event appends a large portion of the PrLD of FUS, TAF15 or EWSR1 to the N-terminal end of a transcription factor.[35-37] Given the portable nature of yeast prion domains,[19] the translocated PrLD likely promotes misfolding and dysfunction of the transcription factor, which then causes cancer. Intriguingly, TAF15 and EWSR1, are mutated (outside the PrLD) and are found in cytoplasmic inclusions in some forms of sporadic ALS.[32,33] Moreover, wild-type (WT) TAF15 and EWSR1 form cytoplasmic inclusions in FTD-FUS.[25] However, familial forms of neurodegenerative disease caused by TAF15 or EWSR1 mutations remain to be identified.[38]

Why are PrLDs so common in RNA-binding proteins? Typically, the PrLD is required for optimal RNA-binding protein functionality. For example, the PrLDs of hnRNPA2/B1 and hnRNPA1 are necessary for alternative splicing activity, stable RNA binding and for optimal RNA annealing activity.[39] PrLDs also mediate key protein-protein interactions. For example, the interaction between hnRNPA2/B1 and hnRNPA1 with TDP-43 is mediated through their PrLDs.[40,41] Emerging evidence suggests that phase transitions mediated by PrLDs found in specific RNA-binding proteins such as TDP-43, FUS and TIA-1 underlie the assembly of RNP granules, the crucibles of various aspects of RNA metabolism.[22,28,30,42] Thus, PrLDs and perhaps other low complexity domains are likely to play key roles in self-organizing the regulated spatial assembly of nonmembrane-bound compartments or collectives within the nucleus and cytoplasm that are devoted to discrete aspects of metabolism.[22,28,30,43-47] However, these essential activities also render PrLDs prone to accumulation in pathological inclusions in various fatal neurodegenerative diseases.[5,12,22,23]

Despite these advances in understanding the role of PrLDs in physiology and pathology, key questions in the field remain unanswered, including: (1) do other PrLD-containing proteins beside TDP-43 and FUS cause age-related proteinopathy? and (2) does the PrLD domain itself drive pathogenesis? Recently, we uncovered mutations in hnRNPA1 and hnRNPA2/B1 that impact the PrLD and cause familial forms of multisystem proteinopathy (MSP) and ALS.[21] Our studies suggest that RNA-binding proteins with PrLDs are likely to contribute broadly to degenerative proteinopathies and that the PrLD can play a critical role in pathogenesis.[12,21,22]

MSP, also known as IBM associated with Paget's disease of the bone (PDB), FTD and ALS (IBMPFD/ALS), is a devastating and progressive degenerative disorder afflicting muscle, brain, motor neurons and bone.[48,49] There is no cure and no effective therapy for this rare inherited syndrome, which presents with abundant TDP-43 pathology.[48-50] Elucidating the molecular basis of MSP is likely to afford clear insight into the pathogenesis of more common individual diseases. Indeed, MSP patients can experience isolated IBM, FTD, ALS or PDB, which can be indistinguishable from familial and sporadic forms of these disorders.[48,49,51,52] Some MSP cases are caused by mutations in the *VCP* gene,[53] which encodes a hexameric AAA+ ATPase VCP, a protein-remodeling factor that governs key steps in ubiquitin-dependent proteostasis and signaling by separating client proteins from multimeric protein complexes.[54] The initial realization that *VCP* mutations cause MSP spurred the discovery of pathogenic *VCP* mutations in the more common ALS,[52] FTD,[55] IBM,[56] and PDB.[57] Thus, rare MSP families provide an unbridled opportunity to identify fundamental molecular lesions that underpin more common age-related diseases. Hence, we sought to identify additional genetic mutations that cause MSP, which are likely to be broadly relevant in more common degenerative disorders.

We investigated two families afflicted with dominantly inherited MSP that were not caused by mutations in the *VCP* gene.[21] In family 1, exome sequencing and linkage analysis revealed that the sole novel nonsynonymous single nucleotide variant (SNV) that co-segregates with disease is in *hnRNPA2B1*.[21] hnRNPA2/B1 is a ubiquitous RNA-binding protein with a PrLD that is highly expressed in brain and muscle and exists as two alternatively spliced isoforms that differ by a 12 amino acid insertion close to the N-terminus.[58-60] The long isoform is referred to as hnRNPB1 and the short isoform as hnRNPA2. hnRNPA2 is the major isoform (> 90%) expressed from the *hnRNPA2B1* gene.[58,59] Both isoforms harbor a C-terminal PrLD: residues 197–353 in hnRNPB1 and residues 185–341 in hnRNPA2.[12,21] The MSP-linked mutation substitutes a highly conserved aspartate residue (D290 in hnRNPA2 and D302 in hnRNPB1) with valine in a region of the PrLD that is conserved in multiple human paralogues of the hnRNPA/B family.[21]

In MSP family 2, exome sequencing and linkage analysis revealed five SNVs and one indel that co-segregated with disease[21,58,61] Among these, a mutation in *hnRNPA1* immediately leapt out because it was identical to the mutation in *hnRNPA2B1* identified in the MSP family 1.[21] *hnRNPA1* is highly expressed in tissues affected in MSP and exists as two isoforms produced by alternative splicing: hnRNPA1-B (amino acids 1–372) and hnRNPA1-A (missing amino acids 252–303). hnRNPA1-A (hereafter hnRNPA1) is the most abundant by ~20-fold. Both isoforms harbor a C-terminal PrLD: residues 186–372 in hnRNPA1-B and residues 186–320 in hnRNPA1.[12,21] The MSP-linked mutation substitutes a conserved aspartate (D262 in hnRNPA1 and D314 in hnRNPA1-B) residue with valine in a conserved portion of the PrLD.[21]

Stimulated by these observations we then searched for mutations in *hnRNPA1* or *hnRNPA2B1* in patients with ALS. Remarkably, in one dominantly inherited case of familial ALS in which known ALS genes were excluded, we identified a mutation affecting the identical, conserved aspartate residue in the PrLD of hnRNPA1. In this instance, the aspartate was substituted with asparagine (D262N in hnRNPA1 and D314N in hnRNPA1-B).[21] We also identified a missense mutation in hnRNPA1 (N267S in hnRNPA1 and N319S in hnRNPA1-B) in one classic, late-onset case of sporadic ALS in which mutations in known ALS genes were excluded.[21] Thus, mutations in the PrLD of hnRNPA1 are connected with both MSP and ALS.

Importantly, we uncovered several similarities in the pathology underlying muscle degeneration in sporadic IBM and in MSP caused by mutations in *hnRNPA2B1*, *hnRNPA1* or *VCP*.[21] In degenerating muscle of sporadic IBM and MSP patients, hnRNPA2/B1 was depleted from nuclei and found in sarcoplasmic inclusions, as was TDP-43.[21] Likewise, hnRNPA1 was depleted from the nucleus and mislocalized in sarcoplasmic inclusions in sporadic IBM and MSP caused by *VCP* or *hnRNPA1* mutations.[21] By contrast, in healthy muscle TDP-43, hnRNPA2/B1 and hnRNPA1 are localized predominantly to the nucleus.[21] In most cases, the sarcoplasmic inclusions formed by TDP-43, hnRNPA2/B1 and hnRNPA1 were physically separated and only occasionally overlapped.[21] Irrespective of etiology, hnRNPA2/B1, hnRNPA1 and TDP-43 pathology is observed in sporadic IBM and all forms of familial IBM.[21] It is noteworthy that it is not only the mutant hnRNPA2/B1 or mutant hnRNPA1 that forms cytoplasmic inclusions in disease. For example, WT TDP-43, WT hnRNPA2/B1 and WT hnRNPA1 all form cytoplasmic inclusions in VCP-related MSP.[21]

Collectively, these observations suggest that, in cases of sporadic IBM and in cases of MSP, a specific perturbation of a pathway involving VCP, hnRNPA2/B1 and hnRNPA1 induces the same phenotypic trajectory that elicits TDP-43 pathology and disease. In keeping with this possibility, hnRNPA2/B1 and hnRNPA1 interact with TDP-43 and function cooperatively to regulate various RNA processing events.[40,41] Moreover, degeneration in a Drosophila model of VCP-related MSP was suppressed by depletion of the fly homologs of TDP-43, hnRNPA2/B1 or hnRNPA1.[62] An intriguing possibility is that VCP regulates the disassembly or autophagic degradation of RNP granules populated by TDP-43, hnRNPA2/B1 and hnRNPA1.[63,80] In this case, mutations in VCP that reduce this putative clearance activity or mutations in either hnRNPA2/B1 or hnRNPA1 that promote incorporation into RNP granules, might cause disease via excessive and dysregulated RNP granule biogenesis that precedes pathological inclusion formation. Pathological accumulation might, therefore, reflect an imbalance in the normal assembly and disassembly of RNP granules. Indeed, upon stress, MSP-linked hnRNPA2/B1 or hnRNPA1 variants accumulated in a type of RNP granule, termed stress granules, more rapidly than their WT counterparts in cell culture.[21] Furthermore, MSP-linked hnRNPA2/B1 or hnRNPA1 variants, but not their WT counterparts, formed pathological cytoplasmic inclusions when expressed in mouse and Drosophila muscle.[21] Importantly, increased muscle degeneration caused by MSP-linked hnRNPA1 or hnRNPA2/B1 correlated with increased cytoplasmic inclusion formation.[21]

Why do the disease-linked mutations in the PrLD of hnRNPA2/B1 and hnRNPA1 cause increased cytoplasmic inclusion formation in model systems and disease? The disease-linked mutations fall at the center of the PrLD and are predicted to enhance prion propensity, according to two prion domain prediction algorithms.[10,14,20,21] However, the most striking effect is revealed by ZipperDB, a structure-based threading algorithm, which scores 6-amino acid segments for their propensity to form two self-complementary β strands, termed "steric zippers," that form the spine of amyloid fibrils.[64] Hexapeptides with a Rosetta energy (RE) lower than –23 kcal/mol are predicted to form steric zippers, with lower energy predicting higher amyloidogenicity.[64] ZipperDB predicted that the D290V mutation in hnRNPA2 causes a mutant hexapeptide (residues 287–292: NYNVFG) to become highly amyloidogenic (RE = –25.5 kcal/mol), whereas the WT peptide (NYNDFG) is not (RE = –21.9 kcal/mol). Similarly, the D262V mutation in hnRNPA1 causes a mutant hexapeptide (residues 259–264: SYNVFG) to become highly amyloidogenic (RE = –26.4 kcal/mol), whereas the WT peptide (SYNDFG) is not (RE = –22.8 kcal/mol). Intriguingly, the ALS-linked D262N mutation in hnRNPA1 causes two hexapeptides to become more amyloidogenic. First, the hexapeptide comprising residues 258–263 (GSYNNF) becomes more amyloidogenic (RE = –25.3 kcal/mol) compared with the WT counterpart (GSYNDF), which interestingly also breaches the critical RE threshold (RE = –24.2 kcal/mol). Second, the hexapeptide comprising residues amino acids 259–264 (SYNNFG) also becomes more amyloidogenic (RE = –24.7 kcal/mol) compared with the WT version (SYNNFG, RE = –22.8 kcal/mol). Finally, the ALS-connected N267S mutation in hnRNPA1 causes a mutant hexapeptide (residues 264–269: GNYSNQ) to become amyloidogenic (RE = –23.6 kcal/mol), whereas the WT peptide (GNYNNQ) is not (RE = –22.3 kcal/mol). Thus, ZipperDB reveals that the disease-associated mutations introduce potent steric zipper motifs into the PrLD.[21]

Importantly, in both hnRNPA1 D262V and hnRNPA2 D290V a mutant hexapeptide is predicted by ZipperDB to have the highest fibrillization propensity in the entire PrLD.[21] For hnRNPA1 D262N, the mutant steric zipper motifs rank 2nd and 4th for fibrillization propensity in the PrLD.[21] For hnRNPA1 N267S, the mutant steric zipper motif is not as potent and ranks 11th in the PrLD.[21] The introduction of additional potent steric zipper motifs by the disease-causing mutations in a PrLD is likely to be significant for two reasons. First, introduction of similarly potent steric zipper motifs is sufficient to force fibril formation in model proteins, such as RNase A that would not ordinarily fibrillize.[65] Second, although many (if not all) proteins harbor steric zipper motifs, they are usually buried or contorted in protein structures, in a way that they are unable to make

the intermolecular contacts necessary for fibril formation.[64] This is not the case for hnRNPA1 or hnRNPA2, as their PrLDs are likely intrinsically disordered.[21] Thus, the potent steric zipper motifs of mutant hnRNPA1 and hnRNPA2 are available to make intermolecular contacts and drive fibril formation.

Taken together, multiple algorithms predict that the PrLDs of hnRNPA2/B1 and hnRNPA1 are intrinsically disordered, but poised to access higher order self-templating structures.[21] We experimentally assessed the ZipperDB prediction for the hnRNPA2 D290V and hnRNPA1 D262V variants. Remarkably, the synthetic mutant hexapeptides of hnRNPA2 (NYNVFG) and hnRNPA1 (SYNVFG) rapidly assembled into amyloid fibrils, whereas the WT peptides did not.[21] Thus, the MSP-causing D290V mutation in hnRNPA2 and D262V mutation in hnRNPA1 generate highly amyloidogenic hexapeptides.

We also assessed the propensity of purified, full-length hnRNPA2 and hnRNPA1 to misfold in isolation. After a lag phase, WT hnRNPA2 and WT hnRNPA1 gradually assembled into fibrils capable of seeding their own assembly.[21] This spontaneous assembly process was greatly accelerated by the specific disease-linked mutations. hnRNPA2 D290V rapidly assembled into self-templating fibrils with a minimal lag phase, as did hnRNPA1 D262V and D262N.[21] Thus, the disease-linked mutations enhance a natural propensity to form self-templating fibrils.

The mutant hnRNPA1 (D262V or D262N) and hnRNPA2 (D290V) fibrils could cross-seed assembly of their WT counterpart.[21] This effect was specific, as neither hnRNPA2 D290V fibrils nor hnRNPA1 D262V fibrils seeded the assembly of TDP-43, another RNA-binding protein with a PrLD.[21] We suggest that cross-seeding might initiate assembly of WT hnRNP. However, once fibrils are assembled, the more efficacious self-seeding process is likely to predominate and play a larger role in disease.

Importantly, deletion of the mutant steric zipper residues 287–292 from hnRNPA2 and 259–264 from hnRNAPA1 yielded proteins that did not fibrillize spontaneously.[21] In addition hnRNPA2$^{\Delta 287–292}$ did not fibrillize when seeded by WT or hnRNPA2 D290V fibrils and hnRNPA1$^{\Delta 259–264}$ did not fibrillize when seeded by WT or hnRNPA1 D262V fibrils.[21] Thus, the hexapeptide 287–292 in hnRNPA2 is critical for spontaneous and seeded fibrillization of full-length WT hnRNPA2 and D290V.[21] Likewise, residues 259–264 are essential for spontaneous and seeded fibrillization of WT hnRNPA1, D262V and D262N.[21] The WT versions of these hexapeptides do not fibrillize, indicating that the hexapeptide deletion is likely to disrupt a distinct natural steric zipper motif that drives assembly of the full-length WT hnRNP. Indeed, the adjacent hexapeptides shifted by one residue toward the N-terminus in WT hnRNPA2 (amino acids 286–291: GNYNDF; RE = –23.4 kcal/mol) or in WT hnRNPA1 (amino acids 258–263: GSYNDF; RE = –24.2 kcal/mol), are also predicted to be steric zipper motifs, although not as strongly as the disease-associated mutant hexapeptides. These steric zippers likely drive assembly of WT hnRNPA2 and hnRNPA1. Indeed, deletion of amino acids 259–264 in hnRNPA1 and 287–292 in hnRNPA2 also eliminates these WT steric zipper motifs and fibrillization, which suggests that no other steric zipper motifs in the WT PrLD of hnRNPA2 or hnRNPA1 are capable of driving fibrillization.[21] Collectively, these findings suggest that the disease-associated mutations increase the fibril-forming propensity of this critical region of the PrLD of hnRNPA2 (amino acids 286–292) and hnRNPA1 (amino acids 258–264), which is already predicted to have an intrinsic tendency to fibrillize in the WT hnRNP. Consequently, polymerization is accelerated and dysregulated in the mutant hnRNPs, whereas polymerization is less rapid and can be more tightly regulated in the WT hnRNPs. Moreover, these studies identify hnRNPA2$^{\Delta 287–292}$ and hnRNPA1$^{\Delta 259–264}$ as hnRNP variants that are aggregation resistant even in the presence of self-templating fibrils. These aggregation-resistant hnRNPA1 and hnRNPA2 variants could be expressed as a potential therapy to rescue any loss of hnRNPA1 or hnRNPA2 function in disease.

We suggest that the highly conserved aspartate residue, which is mutated in disease and is conserved among hnRNPA1, hnRNPA2 and hnRNPA3, functions as a gatekeeper residue that inhibits the intrinsic fibrillization propensity of the hnRNPA1 and hnRNPA2 PrLD. Gatekeeper residues are found in many proteins and are typically proline or aspartate. They function as β-breaker residues that effectively mask aggregation-prone sequences and promote native protein folding.[66,67] In functional amyloids such as those formed by the *E. coli* curli protein CsgA, gatekeeper residues ensure that amyloid formation is tightly regulated and only occurs at the right place and the right time.[67] Indeed, mutation of gatekeeper Asp residues in CsgA induces unchecked, rapid amyloidogenesis and severe toxicity.[67] We propose that the conserved aspartate in hnRNPA1 and hnRNPA2/B1 also enables tight regulation of fibril assembly such that it can be readily harnessed and even reversed for functional purposes, such as RNP granule biogenesis. Replacing this critical aspartate gatekeeper with valine or asparagine, as in the disease-linked variants, accelerates and dysregulates fibrillization and likely causes disease.[21,22]

Our work also raises several important questions for both the natural physiology of RNA-binding proteins with PrLDs as well as their precise role in pathogenesis. For example, what is the relationship between hnRNP behavior in the purified system (i.e., fibrillization) and its role in the cell (e.g., RNP granule assembly)? It appears likely that PrLD-driven polymerization of hnRNPs plays a key role in phase transitions required for RNP granule assembly,[28,30] but the RNA-recognition motifs (RRMs) are also likely to contribute. Indeed, RNA-binding proteins with multiple RRMs can phase transition to hydrogel structures in the presence of RNA ligands, even though they lack a PrLD.[47] Moreover, the RRMs of TDP-43 and FUS contribute to the localization of both proteins to stress granules.[68,69] We suggest that RNP granule biogenesis and, perhaps, contingent pathological misfolding events are likely to involve a complex interplay between multiple domains including the PrLD. Moreover, whether the PrLD accesses the

same self-templating strain conformation in functional RNP granules and pathological inclusions remains to be determined.[22]

A key related question is whether the binding of hnRNPA1 and hnRNPA2 to RNA is critical for MSP or ALS pathogenesis? In the case of TDP-43 and FUS, aggregation of the PrLD is not sufficient to confer toxicity. Rather, TDP-43 and FUS must aggregate *and* engage RNA to cause toxicity in multiple systems.[31,69-72] It will be important to determine whether the same is true for hnRNPA1 and hnRNPA2/B1. It is also not yet clear whether MSP is caused by a toxic gain-of-function of potentially misfolded hnRNPA1, hnRNPA2/B1 or TDP-43, by a toxic loss-of-function of these proteins or by a synergistic or additive combination of these non-mutually exclusive possibilities. It will be important to elucidate the critical perturbations in RNA metabolism that are caused by the misfolding of hnRNPA1, hnRNPA2/B1 and TDP-43 that lead to MSP. Synergistic effects of depletion of multiple hnRNPs appear likely due to the extensive cross-talk and co-operativity between hnRNPs that control alternative splicing decisions.[73,74] It is plausible that the critical perturbations may be different in the brain, muscle and bone in a manner that reflects selective vulnerability of these disparate tissues.

Diseases associated with pathological inclusions of PrLD-containing proteins frequently exhibit a "spreading" pathology, in which degeneration with intracellular inclusions initiates in one or several epicenters and subsequently spreads to neighboring tissue.[5,12,75] Our findings indicate that cell-to-cell transmission of self-templating conformers formed by hnRNPA1, hnRNPA2/B1 or TDP-43 could contribute to the spreading pathology that is characteristic of these diseases. It will be critical to determine whether self-templating fibrils formed by pure hnRNPA1, hnRNPA2/B1 or TDP-43 are capable of initiating and propagating disease when introduced into WT animal model systems. In this regard, it is interesting to note that the prion domain of the yeast translation termination factor, Sup35, can access transmissible conformers capable of spreading from cell to cell in mammalian cell culture as well as from tissue to tissue in *C. elegans*.[76,77]

Finally, human proteins bearing PrLDs appear to provide a treasure trove of opportunity to identify genes that cause human disease.[12,21,22,32,33] Several other RNA-binding proteins with PrLDs are emerging in disease.[22] For example, mutations in TIA1 cause Welander distal myopathy[78] and hnRNPA3 is found in inclusions in ALS cases caused by *C9ORF72* mutations.[79] Indeed, a substantial proportion of age-related degenerative proteinopathies for which the etiology is presently obscure may be due to unregulated polymerization of PrLD-containing proteins. We suggest that PrLD-bearing proteins that have not yet emerged in degenerative disease should be investigated as potential causative agents using a combination of gene sequencing and histopathological examination of protein localization.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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