

Featured Article

# Minimotifs dysfunction is pervasive in neurodegenerative disorders

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## Abstract

Minimotifs are modular contiguous peptide sequences in proteins that are important for posttranslational modifications, binding to other molecules, and trafficking to specific subcellular compartments. Some molecular functions of proteins in cellular pathways can be predicted from minimotif consensus sequences identified through experimentation. While a role for minimotifs in regulating signal transduction and gene regulation during disease pathogenesis (such as infectious diseases and cancer) is established, the therapeutic use of minimotif mimetic drugs is limited. In this review, we discuss a general theme identifying a pervasive role of minimotifs in the pathomechanism of neurodegenerative diseases. Beyond their longstanding history in the genetics of familial neurodegeneration, minimotifs are also major players in neurotoxic protein aggregation, aberrant protein trafficking, and epigenetic regulation. Generalizing the importance of minimotifs in neurodegenerative diseases offers a new perspective for the future study of neurodegenerative mechanisms and the investigation of new therapeutics.

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## Keywords:

Minimotif; Posttranslational modification; Trafficking; Binding; Aggregate; Epigenetics; Genetics; Histone code; GWAS

## 1. Introduction

Minimotifs, also called short linear motifs, are short contiguous peptide sequences or sequence patterns that encode molecular functions. These functions range from the binding of a protein to other proteins and molecules, posttranslational modification (PTM) of a protein, or trafficking of a protein to a subcellular compartment. Two main minimotif databases, Minimotif Miner and the Eukaryotic Linear Motif resource, now house more than one million minimotif instances [1–6]. Recent proteome-wide analysis

of minimotifs with 1000 genomes data determined that the vast majority of minimotifs are fixed in humans, suggesting their importance in cellular function [7,8]. At the fundamental level, each minimotif is defined as a sequence or sequence pattern in a source protein and an activity that connects the motif to a target protein. The target protein can be an enzyme catalyzing a PTM, another molecule such as a protein, or a trafficking receptor.

The conservation of most minimotifs and their role in evolution suggests that they might render a significant vulnerability to diseases [5,7–9]. In the first comprehensive review of minimotifs in human diseases in 2007, our group noticed that minimotifs were involved in disease, particularly infectious diseases, and others have expanded upon this observation [5,9,10]. There are at least 35 minimotifs mutated in more than 20 diseases, including both rare and common disorders [11]. These include the three general

Conflict of interest: The authors declare that they have no competing interests.

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<https://doi.org/10.1016/j.trci.2018.06.005>

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classes; binding, PTM, and trafficking minimotifs. Several annotations for diseases are listed and described on the Eukaryotic Linear Motif resources website. Approximately 0.1% of missense mutations in the COSMIC somatic cancer mutation database overlap with a minimotif sequence. There are 100s of minimotifs encoded by viruses that are used to hijack host cell processes, several essential for the viral life cycle.

Another line of evidence for the importance of minimotifs in disease is the emergence of Food and Drug Administration (FDA)-approved minimotif mimetic drug therapeutics [11]. For example, protein kinase inhibitor drugs such as Gleevec (imatinib mesylate), Iressa (gefitinib), Sprycel (dasatinib), and Stutnet (sunitinib) among others block phosphorylation of minimotifs and are useful for cancer treatment and immunosuppression [12]. Drugs such as Lotensin (benazepril), Novastan (argatroban), Januvia (sitagliptin), and many HIV protease inhibitors inhibit different proteases that cleave minimotifs and are also minimotif-directed therapeutics. Peptide hormones tend to have core clusters of amino acids that bind to receptors. For example, peptide therapeutics or chemical agonists such as Supprelin LA (histrelin) for the gonadotropin releasing hormone receptor, Byetta (exenatide) for the glucagon-like peptide 1 receptor, Somatuline Depot (lanreotide) for somatostatin receptors, and opiates such as Demerol (meperidine) for opioid receptors mimic the determinants that bind receptors. There are also antiviral drugs to several lipidation enzymes that modify minimotifs, and Aggrastat (tirofiban) is a drug that mimics the Arg-Gly-Asp peptide ligand for integrins.

In this review, by exploring the specific role of minimotifs in neurodegenerative disease (NDs), we consolidate significant evidence that supports our hypothesis that minimotifs have distinct and generalizable functional roles in ND etiology. Minimotifs are the key connections in the cellular molecular network, so it is not surprising that they are vulnerable to pathological dysfunction and that all NDs have multiple dysfunctions of minimotifs. By questioning whether the modification of minimotifs is causal or a consequence of other precipitating events, we recognize both are contributing factors to NDs. From the causal perspective, some of the familial genes in NDs have mutations that disrupt minimotif sources proteins or targets. Minimotifs may also be causal through PTM of the histone code and epigenetics. Other, less direct roles of minimotifs in pathogenesis are through protein trafficking, PTMs, neurotoxic protein aggregation, and protein aggregate clearance.

Because there are approximately 100 NDs known [13], in this review, we have focused on only the major NDs: Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and tauopathies, although where relevant, other NDs are mentioned.

## 2. Types of minimotifs in neurodegenerative diseases

All three types of minimotifs: binding, modifying, and trafficking, have important roles in neurotoxic aggregate

formation and clearance, epigenetics through the histone code, protein trafficking, and PTMs of ND-related proteins (Figs. 1 and 2). Some of the genes with familial inheritance in NDs harbor mutation in minimotifs or enzymes that modify minimotifs, as summarized in Tables 1 and 2 and discussed below.

### 2.1. PTM minimotifs in neurodegenerative diseases

Previous reviews of NDs summarize the important roles of PTMs, one general category of minimotifs. There are more than 500 types of covalent modifications, with most, if not all proteins in the human proteome having one or more PTMs [74]. These PTMs perturb local and global structure, thereby altering protein binding, trafficking, half-lives, activities, and signaling. Therefore, it is not surprising that several of these PTMs have substantial roles in the pathology of NDs.

#### 2.1.1. Glycosylation/glycation

Half of all proteins in most cell types undergo glycosylation and are N-glycosylated at the minimal consensus sequence Nx [S/T] and/or O-glycosylated on Ser or Thr with no specific sequence determinants [75–77]. Protein glycosylation stabilizes protein structural folds and facilitates protein trafficking, protein quality control, receptor activation, and endocytosis [77,78]. Human mutations causing substitutions at N-glycosylation sites can result in severe disease pathology; for example, a familial mutation encoding a T183A substitution in a NxT glycosylation minimotif in prion protein (PrP) causes Creutzfeldt-Jakob disease (CJD) [11,79–82].

Curiously, there are also several germline mutations immediately juxtaposed to an encoded N-glycosylation consensus sequence: a P504L substitution in WFS1 of Wolframs syndrome, E196K, V180I in PRP (P04156) for CJD, and E196K also for Gerstmann-Straussler disease [83–85]. However, whether these mutations alter glycosylation and influence pathogenesis is not yet clear.

Also unclear is the observation of altered glycosylation in NDs, a likely downstream event or epiphenomena. On a more global scale, proteomic analyses of AD brains revealed altered glycosylation of 131 GlcNacylation sites in 81 proteins. Another global study comparing brains and sera from HD transgenic mice to controls reveals differences in the levels and pattern of glycans [86]. Aberrant glycosylation of an additional ten glycosylated proteins in several NDs (AD, PD, and HD) was summarized previously [87]. Furthermore, acetylcholinesterase is abnormally glycosylated in both CJD and AD [38,87]. Nonenzymatic glycation and aberrant glycosylation of tau are prevalent in AD and FTD [14,44,88,89].

#### 2.1.2. Phosphorylation

Protein phosphorylation of Ser, Thr, or Tyr residues is apparent during the development of disease. During the pathogenesis of NDs, aberrant phosphorylation results in

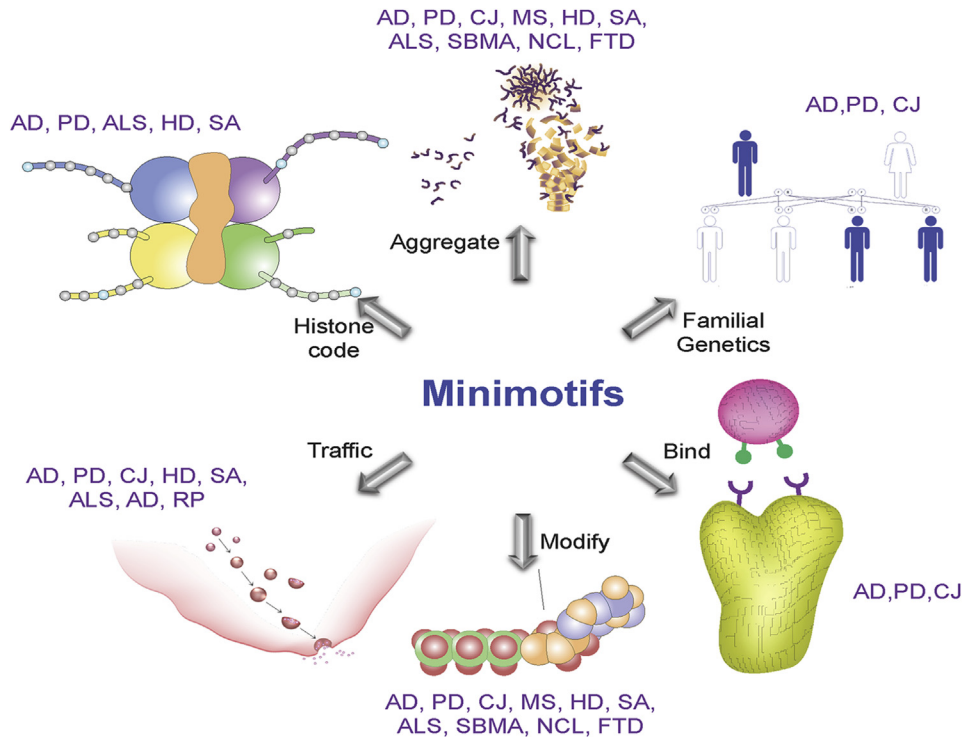


Fig. 1. General functions of minimotifs in NDs. Minimotifs perform multiple functions in NDs. Shown here are a selected functions or cell processes of minimotifs in NDs. Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CJ, Creutzfeldt-Jakob; FTD, frontotemporal dementia; HD, Huntington's disease; NCL, neuronal ceroid lipofuscinosis; PD, Parkinson's disease; RP, retinitis pigmentosa; SBMA, spinal and bulbar muscular atrophy; SA, spinocerebellar ataxia.

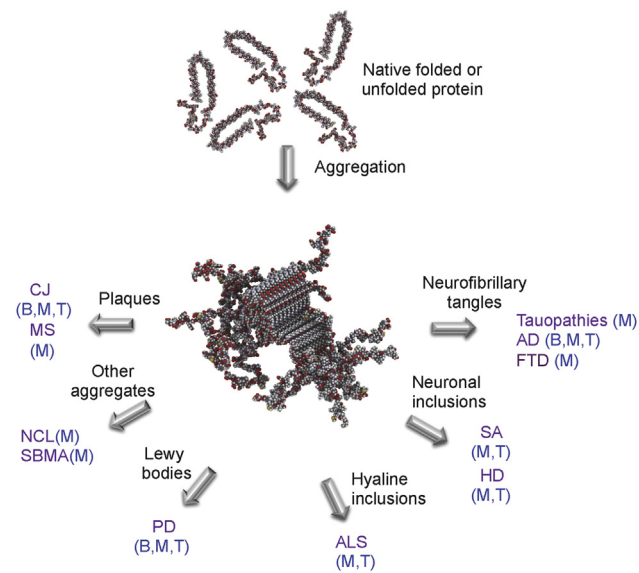


Fig. 2. Minimotifs in neurotoxic protein aggregation. The different types of aggregates in NDs are shown. The types of minimotifs for each aggregate are binding (B), trafficking (T), and posttranslational modification (M). Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CJ, Creutzfeldt-Jakob; FTD, frontotemporal dementia; HD, Huntington's disease; NCL, neuronal ceroid lipofuscinosis; PD, Parkinson's disease; RP, retinitis pigmentosa; SBMA, spinal and bulbar muscular atrophy; SA, spinocerebellar ataxia.

the misfolding and aggregation of neurotoxic proteins. For example, abnormally hyperphosphorylated tau is associated with aggregates in AD and other tauopathies [15]. In addition, more than 40 sites of phosphorylation in tau enhance or reduce its propensity to aggregate with S<sup>129</sup> being the most prominent [15,90]. The degree of tau phosphorylation differs in AD and FTD, which alters its propensity to aggregate and/or induce toxicity [45,46].

Although the causation and correlation between the phosphorylation and-aggregation of  $\alpha$ -synuclein ( $\alpha$ Syn), and toxicity of Lewy bodies remain unclear, the phosphorylation of  $\alpha$ Syn in Lewy bodies is well established [91,92]. Approximately 90% of  $\alpha$ Syn is phosphorylated in Lewy bodies, a hallmark of PD, and other synucleinopathies [91,52,93–97]. Of the 17 potential phosphorylation sites in  $\alpha$ Syn, S<sup>87</sup> is most tightly associated-with synucleinopathies [98]. Several potential reasons for the lack of consistency among different studies are different *in vitro* and *in vivo* comparisons, different efficiencies of kinases, and the balance between the phosphorylation and dephosphorylation of proteins in different model systems.

Both Parkin (*PRKN*) and PTEN-induced putative kinase 1 (*PINK1*) genes are mutated in PD with approximately half of the early onset familial cases caused by *PRKN* [99]. Autoinhibition of Parkin, an E3 ubiquitin ligase, is

Table 1  
Neurodegenerative disorder minimotif summary

NDs	Minimotifs			
	Aggregate/gene	Familial genes	Activities	References
Alzheimer's disease (AD)	$\beta$ -amyloid plaques, neurofibrillary tangles/ <i>APP</i> , <i>MAPT</i>	<i>APP</i> , <i>PSEN1</i> , <i>PSEN2</i> , <i>MAPT</i>	Proteolysis Sumoylation Glycosylation Lipidation Acetylation Methylation Oxidative PTM Ubiquitylation Citrullination Trafficking Binding	[14–28]
Amyotrophic lateral sclerosis (ALS)	Hyaline inclusions/ <i>SOD1</i>	<i>SOD1</i>	Ubiquitylation Sumoylation Phosphorylation Lipidation Oxidation S-nitrosylation S-glutathionylation Trafficking Lipidation	[19,29–36]
Batten (neuronal ceroid lipofuscinosis)	Aggregation/ <i>CSP<math>\alpha</math></i> aggregation/ <i>DNAJC5</i>			[37]
Creutzfeldt-Jakob disease (CJD)	Prion (PrP) plaques/ <i>PRNP</i>	<i>PRNP</i>	Glycosylation Trafficking Phosphorylation Acetylation Methylation Ubiquitylation Citrullination Binding	[11,24,38–43]
Frontotemporal dementia/ degeneration (FTD)	Neurofibrillary tangles/ <i>MAPT</i>	<i>MAPT</i>	Phosphorylation Sumoylation Ubiquitylation Glycosylation	[44–48]
Huntington's disease (HD)	Neuronal inclusions/ <i>HTT</i>	<i>HTT</i>	Sumoylation Glycosylation Ubiquitylation Proteolysis Trafficking	[19,49–51]
Parkinson's Disease (PD)	Lewy bodies/ <i>SNCA</i>	<i>SNCA</i> <i>LRRK2</i> <i>GBA</i> <i>VPS35</i> <i>EIF4G</i> <i>DNAJC13</i> <i>PRKN</i>	Proteolysis Sumoylation Glycosylation Lipidation Acetylation Methylation Oxidative PTM Ubiquitylation Phosphorylation Citrullination Trafficking	[16,19,28,52–62]
Spinal and bulbar muscular atrophy (SBMA)	Aggregation/ <i>AR</i>		Sumoylation Ubiquitylation Phosphorylation of non-aggregating tau	[19,63–66]
Spinocerebellar ataxia (SA)	Neuronal inclusions/ <i>SCA</i>	<i>SCA</i>	Sumoylation Phosphorylation Ubiquitylation Trafficking Proteolysis	[19,50,67–71]

(Continued)

Table 1  
Neurodegenerative disorder minimotif summary (Continued)

NDs	Minimotifs			
	Aggregate/gene	Familial genes	Activities	References
Tauopathies	Neurofibrillary tangles/ <i>MAPT</i>	<i>MAPT</i>	Phosphorylation Glycosylation Acetylation Methylation Ubiquitylation SUMOylation	[72,73]

Abbreviations: PTM, posttranslational modification; CSP $\alpha$ , cysteine-string protein  $\alpha$ .

released on its phosphorylation at S<sup>65</sup> by PINK1 [100,101]. Activated Parkin initiates a downstream ubiquitylation pathway that regulates the quality control of proteins in PD [102].

Phosphorylation is also important for several genes with a familial linkage to NDs. At least part of leucine-rich repeat kinase 2's (LRKK2) pathogenicity in PD is due to its kinase activity and its substrate recognition likely by minimotifs that bind its WD40 domain [103]. Ataxin1 has polyglutamine (polyQ) tracts important for the pathogenesis of spinocerebellar ataxia (SA). Phosphorylation of S<sup>776</sup> in Ataxin1 at an Akt phosphorylation minimotif increases the cytotoxicity of longer polyQ tracts [67,68]. Phosphorylation likely has a protective role in ALS as a T2D phosphomimetic mutation stabilizes superoxide dismutase (SOD) in the presence of other destabilizing pathogenic mutations [104]. Haplotypes with this variant and a destabilizing SOD variant is one possible explanation for variable penetrance of the pathogenic mutations.

### 2.1.3. Lipidation

Fatty acid attachment to proteins anchors them to membranes [105]. Some common lipidation PTMs are palmitoylation, N-myristoylation, farnesylation, geranylgeranylation, GPI addition, S-diacylglycerol addition, and prenylation [105]. We do not know of any direct linkage of lipidation with proteins harboring mutations in NDs, suggesting that

lipidation is a downstream change. However, several proteins with established roles in ND pathogenesis are covalently linked to fatty acids supporting some role. Palmitoyl acyltransferases (PATs), enzymes that catalyze protein palmitoylation recognize the  $\Psi\beta\alpha\alpha\text{QP}$  minimotif ( $\Psi$ , an aliphatic amino acid;  $\beta$ , a C- $\beta$  branched amino acid Val, Ile, or Thr) in Huntingtin (HTT) [49,106–111]. ZDHHC17 is a PAT specific for neuronal proteins, including HTT. The reduced palmitoylated HTT in HD implies a reduced activity of PAT for HTT. Palmitoylated HTT has shorter polyQ tracts supporting a protective role against HD. Similar to palmitoylation, myristoylation of HTT is also reduced in HD [112]. One possible explanation is that the expansion of the polyQ tract interferes with this PTM. Palmitoylation of mutant superoxide dismutase 1 (SOD1) and mutant CSP $\alpha$  may play a role in the etiology of familial ALS and neuronal ceroid lipofuscinosis, respectively [29,37,113,114].

In AD, a palmitoylated amyloid precursor protein (APP) is restricted to the Golgi, enhancing its proteolytic processing favoring amyloidogenesis. Several other substrates of PATs associated with AD, PD, and HD were previously reviewed [111].

Other examples of lipidation impacting NDs are a glycosphingolipids. Aglycosphingolipid binding minimotif [K/H/R]<sub>x(1-4)</sub>[Y/F]<sub>x(4-5)</sub>[K/H/R], with at least one Gly at x<sub>(1-4)</sub> is present in  $\alpha$ Syn,  $\beta$ -amyloid peptide, and PrP [115,116]. Of the many gangliosides (GM),  $\alpha$ Syn

Table 2  
Minimotif enzymes, activities, and substrates in familial NDs

ND(s)	Minimotifs		
	Enzymes	Activity	Substrates
AD	PS1, PS2	Proteolysis	APP
ALS/FTD			SOD1
FTD		Phosphorylation	Tau
FTD		Binding	SQSTM1
FTD	TBK1	Phosphorylation	
FTD	VCP, CHMP2B, TARDBP, SQSTM1, DCTN1		
HD	-		HTT
PD	Parkin	Ubiquitylation	$\alpha$ -SP22
PD	LRKK2, PINK1	Phosphorylation	
PD	UCH-L1	Proteolysis	Ub
SMA	UBE1, DYNC1H1	Ubiquitylation	

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; HD, Huntington's disease; PD, Parkinson's disease; SMA, spinal muscular atrophy.



specifically binds GM1 and GM3, inhibiting fibril formation in PD. Conversely,  $\beta$ -amyloid binding to GM1 initiates fibril formation in AD [117,118]. PrP localization to lipid rafts stabilizes its nonpathogenic state. [L/V]<sub>x(1-5)</sub>YX<sub>(1-5)</sub>[K/R] is a cholesterol interaction consensus sequence.  $\alpha$ Syn has a VLYVGSK sequence matchin this pattern and upon, binding cholesterol causes  $\alpha$ Syn aggregation in neurons [116,119]. A recent review summarizes accumulating evidence for a role for prenylation of small GTPases in A $\beta$  production and secretion in AD [120].

#### 2.1.4. Acetylation and methylation

The role of protein acetylation and methylation in NDs was previously reviewed [121]. These PTMs are common for histones and impact epigenetic inheritance, a topic addressed later in the epigenetics section. Protein acetylation and methylation increase the surface area of, and depolarize Lys and Arg residues, a PTM that generally drives new protein-protein interactions.

Protein acetylation is a reversible attachment of acetyl group to the  $\alpha$ -amino group of the N-terminus,  $\epsilon$ -amino group of the Lys residues, and many other amino acids [122]. Acetylation of Lys residues in tau inhibits its degradation and induces its aggregation in AD [16,123]. In particular, the K174Q substitution in tau mimics an acetylated Lys state, leading to more severe neuronal atrophy in mice. Conversely, acetylation of  $\alpha$ -tubulin, a PTM that increases its stability, is decreased in AD [121]. FTD mice models with the K174Q substitution have similar effects upon tau acetylation [44,47].  $\alpha$ Syn, HTT,  $\beta$ -amyloid, and PrP are all subject to acetylated with either a protective or a detrimental effect on ND pathology [122,124–128].

The  $\epsilon$ -amino moiety of Lys can be mono-, di-, or trimethylated, and Arg can be mono- or di-methylated. Methylated residues in tau are located in the microtubule binding repeat region, thus, may block its interactions with microtubules [16]. Tandem mass spectrometry analysis of a human AD brain identified seven monomethylated Lys residues (K<sup>44</sup>, K<sup>163</sup>, K<sup>174</sup>, K<sup>180</sup>, K<sup>254</sup>, K<sup>267</sup>, and K<sup>290</sup>) in tau and neurofibrillary tangles are immunopositive for methyl-lysine [16]. *In vitro* experiments demonstrate that methylation reduces deimination of Arg residues in myelin basic protein a modification that may sensitize T-cells for autoimmune attack of myelin in multiple sclerosis (MS) [129].

#### 2.1.5. Nitrosylation, glutathionylation, and other oxidative PTMs

There are several types of Cys redox PTMs and corresponding minimotifs in NDs [130]. During oxidative stress, two Cys residues can oxidize creating intermolecular disulfide bridges and protein aggregation. Some Cys thiol groups in SOD1 are oxidized to sulfenic, sulfinic, and sulfonic acids, decreasing the stability of the protein and rendering it prone to aggregation in ALS patients [29–31,131,132]. Similarly, S-glutathionylation of Cys residues in SOD1 induces its aggregation in ALS [32,33].

Aberrant protein S-nitrosylation in several NDs, including ALS, AD, and PD, has been previously reviewed [31,53]. A global analysis of mouse brain identified 31 nitro-tyrosine PTMs, more than half of which have been implicated in PD, AD, and other NDs [17,133,134]. The consensus motif for nitric oxide reaction with proteins is [K/R/H/D/E]C[D/E], where the Cys residues is covalently attached to nitric oxide [135,136]. Protein S-nitrosylation can induce protein misfolding and aggregation [135,137]. Aberrant S-nitrosylation of many proteins (parkin, PDI, DNM1L, CDK5, PRRX2, GAPDH, PTEN, AKT1, MAPK, IKBKP, and XIAP) may contribute to neurodegeneration through multiple pathways [53]. For example, aberrant S-nitrosylation of PDI decreases its enzymatic activity, thereby inhibiting its neuroprotective functions [53].

#### 2.1.6. Ubiquitylation

Many misfolded and damaged proteins are targeted for adenosine triphosphate (ATP)-dependent proteolysis by conjugation with ubiquitin, an 8.5 kDa protein. The role of ubiquitylation in NDs was previously summarized [138]. Proteins that aggregate in NDs tend to be ubiquitylated, likely for initiating targeted protein degradation. In the cerebral cortex of patients with AD, the cerebral spinal fluid of patients with CJD, and  $\alpha$ Syn in patients with PD, ubiquitylation of neurofibrillary tangles is increased [18,39,54,139]. Tau, the protein that oligomerizes in these tangles, is ubiquitylated at K<sup>254</sup> in AD, and the ubiquitylated tau may be blocked by Lys acetylation in FTD [44,123]. A PolyQ tract in Ataxin-1 induces its aggregation in the nucleus and leads to ubiquitylated SA type 1 (SCA-1), inducing cytotoxicity in SA [69]. Ubiquitylated polyQ tracts in HD and other triplet repeat NDs enhance intranuclear protein aggregation in neurons [50,140,141]. Additional PTMs in the polyQ tracts in 12 proteins involved in the pathology of 9 NDs are known [142]. SCF E3 ubiquitin ligase binds survival motor neuron protein through a phosphodegron signal, DSGxx[S/T], where the Ser is phosphorylated [143]. This suggests that oligomerization of the mutant survival motor neuron potentially sequesters the degradation signal and stabilizes survival motor neuron, a potential pathogenic pathway in spinal muscular atrophy [143,144].

#### 2.1.7. Sumoylation

Small Ubiquitin-like Modifiers (SUMOs) are ubiquitin-like proteins covalently attached to other proteins at  $\Psi$ KXE/D minimotifs. There are four SUMO genes in humans [145]. SUMO normally functions in protein stability, nuclear-cytosolic transport, and transcriptional regulation. Many key proteins in ND pathogenesis are sumoylated and implicated in disease mechanisms [19,146,147]. In AD, sumoylation of APP decreases A $\beta$  production, and sumoylation stabilizes tau by inhibiting its phosphorylation and ubiquitylation [72,147–149]. Similarly, in PD, sumoylation of  $\alpha$ Syn inhibits its aggregation [150,151].

The pathogenic fragment of HTT is sumoylated in HD, and SOD aggregation in ALS is also effected [152–155]. Pathogenic mutation of sumoylated residues in valosin-containing protein/p97 inhibits its translocation to the nucleus, the formation of stress granules, reduction of hexamer formation, and eventually, inhibition of its clearance. The amino acid substitutions for these mutants are prevalent in FTDs [48].

### 2.1.8. Citrullination

Citrullination is an irreversible PTM type of minimotif [156]. Peptidylarginine deiminases deiminate Arg residues in proteins converting this amino acid to citrulline. Because arthritic patients have anti-citrullinated protein antibodies, citrullination may be related to some of the neuroinflammatory aspects of NDs. Abnormal protein citrullination is known for AD, PD, including Lewy bodies, and in prion diseases [157–159]. Autoimmune attack of citrullinated proteins may be prevalent in ND neuroinflammation [160,161]. Although MS is not considered a ND, citrullination of the major myelin component, MBP may play a role in the autoimmune attack of myelin in MS [162]. Deimination depolarizes MBP and is a modification that may trigger the demyelination of axons in MS [163].

### 2.1.9. Endoproteolysis

Proteolysis is central to the degradation of misfolded proteins by the ubiquitin-proteasome system and autophagy, both driven through minimotifs and central to NDs [164]. The inherent role of endoproteolysis in neurodegeneration was founded in the familial genetics of APP processing to  $\beta$ -amyloid and was recently reviewed [165]. In early onset AD,  $\beta$ -secretase and  $\gamma$ -secretase cleave APP-producing amyloidogenic  $A\beta_{40}$  or  $A\beta_{42}$  [87,166,167]. Familial mutations in the protease processing minimotif sites in APP cause overproduction of amyloidogenic peptide fragments.

Other NDs have proteolysis minimotifs as well. In patients with PD, familial mutations were identified in UCHL1, a protease that cleaves ubiquitin and colocalizes with Lewy bodies. Furin prohormone convertase cleavage site (KGIQKREA) cleavage of putative type-II single-spanning transmembrane precursor protein (BRI) yields a small C-terminal amyloidogenic peptide fragment [168]. These fragments are present in the amyloid fibrils of patients with Familial British dementia [169–171]. Several proteases cleave HTT at minimotifs producing peptide aggregation and neurotoxic peptides [172–176].

## 2.2. Trafficking and autophagy minimotifs in neurodegenerative diseases

Minimotifs are important in protein trafficking to and from organelles and are relevant to several NDs [1,177,178]. Defects in endocytic and synaptic vesicle trafficking, Golgi trafficking, retrograde transport, and lysosomal autophagy are apparent in PD and AD

[177,179,180]. Trafficking minimotifs are essential for most protein trafficking events, have been previously reviewed, and a few typical examples are presented [180–183]. LRKK2 is a kinase/GTPase with mutations in patients with familial PD [184]. LRRK2 has a WD40 interaction domain that binds to synaptic vesicles and several LRRK2 interactors function in vesicular trafficking [103,185]. Through phosphorylation of S75 in endophilin, LRKK2 plays a role in synaptic vesicle endocytosis in *Drosophila* [186]. LRKK2 is also involved in retrograde trafficking through a functional association with VPS35, another protein mutated in familial PD.

The role of trafficking of APP in AD pathogenesis has recently been reviewed [167]. A KFERQ minimotif at the end of APP binds SCG10 and is essential for trafficking APP to lysosomes for degradation [20,187]. The KDEL receptor binds ER resident proteins containing the KDEL minimotif and returns them to the endoplasmic reticulum [188]. The receptor is redistributed to lysosomes and stimulates autophagy, when aberrant SOD in ALS,  $\alpha$ Syn in PD, and HTT in HD are expressed [189].

The cytoplasmic domain of APP contains a YxNPxY sequence, an internalization minimotif that traffics APP to endosomes [21,190]. The same minimotif in APP binds to adaptor proteins, including LDL-Rs, FE65, X11, SNX17, and Dab2, which regulate APP trafficking and thereby regulates  $\beta$ -amyloid production [21].

Mutant ataxin-1 protein has a nuclear localization signal, which is required to mediate toxicity of variants with long polyQ tracts in SAs [67,191]. Mutations in the VxPx> minimotif in rhodopsin cause autosomal dominant retinitis pigmentosa, a progressive ND of photoreceptor neurons [192]. This C-terminal minimotif is essential for trafficking of rhodopsin from the trans-Golgi-network [193]. This minimotif also binds to adenosine diphosphate (ADP)-ribosylation factor 4, a rhodopsin transport carrier, regulating the otherwise bulk flow of proteins to photoreceptor rod outer segments.

Chaperone-mediated autophagy, in addition to ubiquitylation, is another mechanism that maintains protein quality control in NDs [194]. The substrates of autophagy contain a KFERQ-like minimotif that is exposed potentially only after a PTM-induced conformational change. These minimotifs interact with the chaperone complex and are translocated to lysosomes via lysosomal-associated membrane protein 2A, a receptor that triggers autophagy. A VKKDQ minimotif in  $\alpha$ Syn targets it for autophagy [55,195]. Mutant  $\alpha$ Syn encoding A30P and A53T substitutions may cause familial PD by interacting with lysosomal-associated membrane protein 2A. These mutant proteins fail to translocate into the lysosomal lumen, efficiently stalling the process of autophagy.

Autophagy may be a step in the pathology of PD and HD. Autophagy-targeting minimotifs (QVEVK, KDRVQ) in tau are important in a neuronal cell model of tauopathies [22]. On binding the chaperone complex, Cathepsin L, a lysosomal cysteine protease cleaves mutant tau proteins producing

peptide fragments that aggregate [22]. A more detailed overview of autophagy enumerates eight proteins containing 17 confirmed and putative autophagy-targeting minimotifs in proteins involved in PD etiology [196]. KDRVN and NEIKV minimotifs in HTT are recognized by the autophagy machinery, but mutant HTT with an extended C-terminal polyQ track delays targeting to the lysosomes. This may result in severe HTT aggregation in the neurons of patients with HD [197].

### 2.3. Binding minimotifs in neurodegenerative diseases

One of the most important activities of minimotifs is for proteins binding other molecules, which is central to many cellular pathways and processes. Interestingly, many types of binding motifs propagate neuronal atrophy in NDs. A linkage analysis of a Turkish kindred identified an autosomal recessive variant encoding a D458V mutation in the C-terminal PDZ binding motif of SANS protein in patients with the atypical Usher syndrome, a neurodegenerative hearing loss syndrome [198,199]. Through an SH3 binding minimotif, RTPPKSP, tau binds the SH3 domains of Fyn and Src nonreceptor tyrosine kinases, which also phosphorylate tau [23,200]. This minimotif is relevant because hyperphosphorylated tau is a hallmark of AD [201]. MED25 binds SH3 domains of Abelson family protein kinases. A mutation encoding the A335V mutation in the proline-rich SH3 binding motif of MED25 decreases binding specificity imparting interactions with a broader range of nonphysiological proteins with SH3 domains. This mutation causes Charcot-Marie-Tooth disease 2B2, a peripheral neuropathy ND [202]. A YENPTY minimotif in APP interacts with PTB domains of Mint, a protein that trafficks and processes APP [203–205]. Ataxin has a RxxSxP 14-3-3 binding minimotif that enhances the toxicity of its polyQ tracts in SA [68].

The GxxxGxxxG glycine zipper minimotif binds cholesterol and has an established role in oligomerization of proteins, including A $\beta$  and PrP [24,206,207]. Cholesterol binding to APP may contribute to amyloidogenesis and AD [24]. In the postsynaptic terminal,  $\beta$ -amyloid recruits PTEN, a lipid phosphatase in a PDZ domain-dependent manner inducing a malfunctioning state of amyloids [25]. A PTEN mutant producing a protein lacking its PDZ domain protects against amyloid toxicity.

### 2.4. Minimotif cooperativity

Minimotif cooperativity is perhaps one of the most understudied aspects of minimotif function and is likely an important factor for understanding molecular dysregulation in NDs. Many proteins, including those associated with NDs, have more than five minimotifs, and there are now numerous examples where one minimotif can induce or create a new minimotif, or compete with other minimotif targets for the same site. Several examples of minimotif cooperativity are mentioned in the other sections, and a few are highlighted here. In PTM minimotifs, certain residues either compete for a particular PTM or induce further modifications. For

example, phosphorylation often precedes modifications such as sumoylation and ubiquitylation [92]. In patients with AD, tau phosphorylation follows an initial glycosylation modification [88,208]. When MARK2 phosphorylates tau, it is no longer recognized by E3 ligase, preventing its degradation [209]. Methylation minimotifs in tau are positioned to engage in cross talk with phosphorylation [16].

## 3. Minimotifs in aggregates

Protein aggregation is common in the etiology of most NDs [210].  $\beta$ -Amyloid and tau are the major constituents of neuritic plaques and neurofibrillary tangles, respectively. Lewy bodies are intracellular aggregates of  $\alpha$ Syn in PD and other synucleinopathies. Proteins with extended polyQ repeats aggregate into nuclear and cytosolic inclusions in HD, SA, and dentatorubral-pallidoluysian atrophy. A hallmark of ALS is cytoplasmic inclusions of SOD1.

Although abnormal protein aggregation in neurodegeneration is well established, the generalized role of minimotifs in aggregation was not previously emphasized. There are several genes with familial inheritance in NDs that encode minimotif target enzymes or their substrates. These genes, minimotifs, and their activities are listed in Table 2.  $\beta$ -Amyloid, the central component of plaques, is ubiquitylated and phosphorylated, PTMs that regulate the stability of plaques [211–213]. Most tauopathies have the common molecular abnormality of hyperphosphorylation of tau, which aggregates into neurotoxic inclusions [214]. Furthermore, there are at least a dozen other PTMs that modulate tau aggregation [72,215]. Lewy bodies are ubiquitylated, sumoylated, phosphorylated, nitrosylated, and also have a unique PTM created by a dopamine radical adduct [150,216,217].

At least 48 PTMs of protein have been identified in NDs, and other neurotoxic proteins with long polyQ tracts have many PTMs [218,219]. Because minimotifs are located on the surfaces of all proteins and cover almost the entire surface of many proteins [220–223], it is not surprising that perturbing minimotifs results in abnormal neurotoxic aggregation. Given their prevalence in neurotoxic aggregate formation and stability, a better understanding of how minimotifs cooperate to induce and inhibit neurotoxic aggregates would provide a better understanding of ND etiology and open the door for new types of therapeutic intervention. As such, minimotif mimetics should be further considered as targets for reducing aggregation in NDs.

## 4. Genetics and epigenetics of minimotifs in NDs

Over the past decade, genome-wide association studies (GWASs) have advanced our understanding of the genetic basis of human disease [224]. Similar to other disorders, NDs have a familial component with rare variants of large effect and a more prevalent sporadic component with many common variants of small effect. Given that minimotifs are located at the interface between proteins and are



important constituents of cellular networks, it is no surprise that several of these motifs have been highlighted in GWASs and in the biology of key cell types such as neurons, astrocytes, and microglia. Some of the earliest advances in genetics were derived from genetic linkage and positional cloning in NDs and have led to newer studies relying on genome-wide quantitative trait analyses such as biomarkers or endophenotypes [225].

#### 4.1. Genetics

Through the combination of high-throughput genotyping platforms and next-generation sequencing technologies, disease-causing genetic variants and genetic factors necessary for protection against disease have been discovered at an unprecedented rate. While such technologies have transformed monogenic disease research, several challenges continue to hamper the discovery of actionable genomic variants in complex disorders. For example, variants of unknown significance and an abundance of neutral variants or variants with modest effect size continue to be produced at a staggering rate. As such, leveraging next-generation sequencing for discovery has been contingent on carefully constructed studies of large sample sizes and judicious selection of participants with accurate and detailed phenotyping. Here, we focus on AD and PD as exemplars of NDs, and we also discuss a pervasive role of genetic variants in minimotifs.

##### 4.1.1. Alzheimer's disease

AD is a common ND and a leading cause of dementia with progressive loss of memory, problem-solving skills, and communication. Due to recent improvements in life expectancy, AD is predicted to affect 1 in 85 people globally by 2050 [226]. As a heterogeneous disease, AD is caused by a combination of environmental and genetic factors and current estimates of AD heritability lie between 60-80% [227]. Late-onset AD accounts for more than 95% of all AD cases and is caused by a complex underlying genetic architecture. To date, over 40 GWASs and meta-analyses have identified a few hundred genes and single nucleotide polymorphisms; these include variants in apolipoprotein E (*APOE*) among other notable gene candidates [228]. In contrast to late-onset AD, early onset AD is caused by highly penetrant variants located primarily in three genes with minimotif functions, *APP* (located at 21q21.2), presenilin 1 (*PSEN1*, located at 14q24.3), and presenilin 2 (*PSEN2*, located at 1q42.13) [226]. Structural variants are associated with complex neurological traits; for example, duplication of *APP* causes early-onset AD with cerebral amyloid angiopathy [229,230]. To empower current association studies, new attempts at incorporating quantitative endophenotypes such as age at onset analysis and expression quantitative trait loci are being conducted [231].

Minimotif activities are prevalent in genes associated with AD. On examining disease genes with penetrant variants, we identified at least 11 activities that could be associ-

ated with disease: proteolysis, sumoylation, glycosylation, lipidation, acetylation, methylation, oxidative PTM, ubiquitylation, citrullination, trafficking, and binding (Tables 1 and 2). Intriguingly, more than half of all *APP* mutations are located at the secretase proteolytic cleavage sites or the transmembrane domain, a region on exon 16/17 ([www.molgen.ua.ac.be/ADMutations](http://www.molgen.ua.ac.be/ADMutations)). In addition, loss-of-function (LOF) animal and cellular models of AD suggest that such minimotif activities are indeed perturbed. As such, we predict that coding and structural variants that impact minimotif function will be key nodes in ND [232].

##### 4.1.2. Parkinson's disease

As the second most common ND after AD, PD results from the loss of dopaminergic neurons in the midbrain and the accumulation and aggregation of  $\alpha$ Syn in Lewy bodies [233,234]. While rare before the age of 60 years, up to 4% of the population at the age of 80 years has PD [235]. Similar to AD, the genetic architecture of PD is complex, and only 5-10% of all patients suffer from an apparent monogenic form of PD [235]. These rare and penetrant variants are located within 19 disease-causing genes such as  $\alpha$ Syn (*SNCA*, located at 4q22.1), *LRRK2* (located at 12q12), and acid  $\beta$ -glucosidase (*GBA*, located at 1q22) and segregate with the disease in an autosomal dominant or recessive pattern [236]. GWASs have recently identified more than 40 risk loci, including candidate genes encoding minimotifs that *LRRK2* phosphorylates [237].

In a similar minimotif analysis of PD, we note that 11 activities are potentially impacted. These include the following: proteolysis, sumoylation, glycosylation, lipidation, acetylation, methylation, oxidative PTM, ubiquitylation, phosphorylation, citrullination, and trafficking (Tables 1 and 2). To verify the relevance of these predictions, we examined how familial mutations in *LRRK2* might affect minimotif activities (Table 2). Focusing on phosphorylation, we noted that disease-associated mutations or substitutions, Y1699C and I2020T (nonphosphorylated residues), disrupted the phosphorylation of constitutively phosphorylated residues at the amino terminal, resulting in attenuated *LRRK2* function [238]. These findings, and additional LOF and gain-of-function (GOF) models, suggest that detailed analyses of minimotif function can identify feedback control mechanisms and novel networks that drive PD biological processes [239].

##### 4.1.3. Microglia and NDs

Microglia are the highly specialized macrophages of the central nervous system, accounting for 10–15% of all cells in the adult brain [240]. Significant advances have been made in understanding the roles of microglia in the development of the brain, such as in neurogenesis, synaptic pruning, surveillance, and homeostasis [241]. In contrast, defining the role of microglia in central nervous system disorders has proven to be more difficult. Given that microglial activation and neuroinflammatory processes play a critical role in the pathogenesis of NDs, biological components linked to

microglial biology have been associated with AD etiology through GWAS; these include the following: *TREM2* (triggering receptor expressed on myeloid cells 2), *CD33*, *CR1* (complement receptor 1), *ABCA7* (ATP-binding cassette, sub-family A, member 7), *SHIP1* (also known as INPP5D, inositol polyphosphate-5-phosphatase D), *APOE*, *CLU* (clusterin), *CD2AP* (CD2-associated protein), and *EPHA1* (EPH receptor A1) [242–246].

Complementing the human genetic studies, knockout mice models of genes from GWAS have allowed for cause-effect *in vivo* investigations into disease pathogenesis. As expected, a fraction of these genes is implicated in the regulation  $A\beta$  accumulation. For example, ApoE- and *CLU*-deficient APP transgenic mice exhibit earlier and more extensive  $A\beta$  deposition compared with control mice [247]. In addition, *CD33* inactivation in *App/Psen1* mice reduced  $A\beta$  accumulation and plaque burden, whereas *Abca7* deficiency in *App/Psen1* and *TgCRND8* mice accelerated  $A\beta$  generation [248–250]. Importantly, variants of *TREM2*, an immunoglobulin-like cell-surface receptor specifically expressed in brain microglia, confer a 2- to 4-fold increased risk for AD [245]. However, exactly how *Trem2* variants confer AD risk is still under investigation. Thus far, the most consistent and striking observation is a strong decrease in microgliosis surrounding  $A\beta$  plaques of AD in *Trem2* haploinsufficient and *Trem2* deficient mice; a similar impairment in microgliosis has also been reported in mouse models of prion disease, stroke, and MS, suggesting a critical role for *TREM2* in supporting microgliosis in response to pathology in the central nervous system.

A LOF mutation in the lipid sensing function, a minimotif activity of *Trem2* deficient mice may be the underlying mechanism for the loss of microglia proliferation and microglial response to  $A\beta$  plaques or reduced infiltration of peripheral macrophages [251,252]. In addition, *Trem2* deficiency leads to exacerbated aggregation of tau in a mouse model of tauopathy [253]. These findings demonstrate that *TREM2* has complex multiple roles in regulating  $A\beta$  and tau pathologies that may reflect its distinct functions at different stages in AD pathology [254]. These findings also highlight how minimotif activities are present in several proteins relevant to microglial biology.

Minimotifs are a source of functional genetic variation and are important targets in natural selection and evolution [7]. Given their prevalence in NDs, we must consider that common NDs may include a cumulative composition of many minimotif variants that cause network dysfunction. This could provide an explanation for missing heritability in NDs. One exciting possibility is that minimotifs may contribute to the genetic risk of neurodegeneration; however, this will require additional study.

#### 4.2. Epigenetics

Epigenetics refers to inheritance that is not mediated through DNA sequence but through covalent modifications

or noncovalent DNA interactions. Recent reviews highlight the emerging role of epigenetics in normal brain function and neurodegeneration [255,256]. Major mechanisms of epigenetics are DNA methylation and PTM of histones/nucleosomes, which is often referred to as the histone code [257]. Minimotifs are the foundation of the histone code. Enzymes that covalently modify minimotifs, including those in histones, are central to AD, PD, ALS, HD, and other polyQ track expansion disorders summarized in recent reviews [258–263].

The role of minimotifs in ND epigenetics is of general interest for ND beyond disease etiology. One emerging area of interest is drugging the enzymes that modify protein components of nucleosomes. Considering the broad role of epigenetics in NDs, there are several drugs such as histone deacetylase inhibitors that are being tested in preclinical investigation and are in different phases of clinical trials [256].

Epigenetics may also explain at least part of the ubiquitous “missing heritability” for common diseases such as the major NDs. Testing the role of epigenetics in NDs may prove difficult without considering the effects of minimotifs. This is because our group has previously reported histone code variability in the population [7]. Approximately 4% of the minimotifs in histone tails have common alleles in humans that are LOF for the PTM. This suggests that the histone code may be wired differently among many people, inferring differing epigenetic responses to the environment [7]. This may be critical to account for in any study that attempts to uncover the epigenetic basis of NDs.

#### 5. Minimotifs as therapeutic targets for NDs

The Therapeutics Peptide Database has no FDA-approved drugs for treating NDs [264]. Despite this lack of success, the investigation of minimotif-directed therapeutics has been, and remains of high interest and potential. Therapeutic interventions based on minimotifs have been discussed in detail for AD, PD, HD, and CJD [265–272]. Since APP was one of the first genes connected with familial NDs and the aggregation of  $A\beta$  is a predominate feature of late-onset AD, there has been much interest in inhibiting  $A\beta$  production. Inhibitors of  $\alpha$ ,  $\beta$ , and  $\gamma$ -secretase that inhibit APP processing at minimotifs,  $A\beta$  production, and aggregation is still under clinical study as was recently reviewed [273].

Several other minimotif-directed therapeutics are being investigated for reducing aggregation of neurotoxic proteins. One central therapeutic approach, although not directed toward minimotifs, is to lower levels of the neurotoxic protein by antisense oligonucleotide therapy [274,275]. The ubiquitin-proteasome system is strongly associated with NDs and is a target for new therapies with compounds that stimulate proteasome-mediated aggregate degradation or therapeutics that target specific proteins for proteome degradation [138,276]. Peptides such as SNWKWPGIFD, a

polyQ-binding peptide inhibit HTT aggregation in HD; GAVVT, a peptide in  $\alpha$ -Syn that inhibits self aggregation in PD; and a NPxY> PTB domain-interacting minimotif inhibiting APP endocytosis in AD are all being investigated as therapeutics [277-279]. Glycosaminoglycans mimetics are decoy inhibitors of covalent glycosaminoglycan attachment to A $\beta$ , blocking its aggregation [87]. Polyanions such as pentosane polysulfate interfere with aggregation of glycosylated PrP and have shown promise in a preclinical model. Several kinase inhibitors block GSK3 $\beta$ , CDK5, and other tau kinases to reduce tau phosphorylation, and hence their inhibition of aggregation is being investigated for AD and tauopathies [15].

Given the role of neuroinflammation and autophagy in A $\beta$  removal, drugs that enhance amyloid degradation are of growing interest. Many analogs and approaches to target autophagy machinery were previously reviewed [164,197]. Rapamycin is a FDA-approved drug that inhibits the kinase activity of mTOR and improves memory function in model organisms. Preclinical studies of mice show that rapamycin reduces A $\beta$  and tau-associated pathologies by increasing autophagy [87,280,281]. Although not related to minimotifs, lithium also induces autophagy and clearance of HTT, synuclein, and PrP [282].

Three histone deacetylase inhibitors were approved by the FDA for treating various types of cancer [283]. Since NDs have an epigenetic component (addressed earlier), histone deacetylase inhibitors and other drugs targeting epigenetic modifications may be useful for treating NDs. This first test of hypothesis is currently under investigation with a clinical trial testing treatment of AD with ORY-2001. ORY-2001 inhibits histone methylation and reduces cognitive impairment and neuroinflammation in a rodent model.

Although minimotif-targeted drugs are not a major class of therapeutics, as we learn more about minimotifs, we hypothesize that their broad influence on disease will likely become more evident and become useful for treatment of NDs.

## 6. Conclusion

Minimotifs are short peptide sequences that encode a molecular function. Their role in cancer and infectious diseases has been previously established [9-11]. In this review, we summarize the evidence supporting minimotifs as key regulators of ND pathogenesis and describe numerous examples of causative cases in familial NDs.

Collectively, numerous studies support centrality of minimotifs to NDs, much of which was previously reviewed [15,19,152,157,163,216,276,281,282,284-286]. Our observation of the generalized minimotif pervasiveness in NDs raises several interesting questions. Particularly, why are minimotifs so prevalent in NDs, and can this observation teach us anything more general about ND etiology?

We consider that answers to these questions may arise from the role of minimotifs in protein and larger networks. NDs have an additional layer of complexity when compared

with other human diseases. Many diseases are founded in physiological dysfunction of the cellular network that manifests as the emergent network properties of symptoms and even death. This network dysfunction is rooted in dysfunction of the molecular network with cells where minimotifs have an important role. However, networks are more complex in NDs because the symptoms arise from emergent properties of dementia, loss of motor control, and other mental health symptoms. These symptoms are due to breakdown of an additional network, the neuronal network. Cognitive states are considered emergent properties of a neuronal network [287].

Why are minimotifs a point of vulnerability? Thus far, about a million minimotifs have been experimentally verified with large growth anticipated [4]. Minimotifs are the key determinants that enable proteins work together in a network as a basis for emergent properties in the cell such as neurotransmission and neuroinflammation. Most complex networks, including biological networks and the minimotif/protein network, have topologies of cliquish small world networks and have the property of robustness.

Robustness protects the system against immediate shock through adaptive mechanism engineered over time through selection and evolution. Those mutations that target key vulnerabilities in the network cause failure and prenatal lethality.

While the protein/minimotif, cellular, and neuronal networks are evolutionarily tuned for robustness, like any network, they can fail, the failures of interest herein being NDs and death. The study of ND disease etiology has informed us of two general categories of disease failure, familial, and sporadic. Familial ND network failure may arise from hub protein dysfunction [288,289]. Hub proteins are generally highly enriched with minimotifs, and our review consolidates many types of minimotifs involved in familial NDs. Hub nodes can collapse an entire network because of functional overload and poor ability to redistribute information flow [290].

In sporadic NDs, GWAS studies have helped resolve a genetic architecture with the culmination of many genetic variants of small effect size. In this scenario, we suggest that this network dysfunction arises from a dynamic redistribution of flow through the network [289]. The genetic and polygenic risk scores are measures of reaching a mutational burden threshold and is consistent with this model. While many of the variants are in noncoding regions, disruption of key minimotifs likely contributes to the network dysfunction. Furthermore, the birth of billions of people provides a screen to identify how multiple variants can contribute to breaking network robustness manifested as NDs. This could also explain the higher prevalence of sporadic disease in general.

The role of minimotifs in network dysfunction may also provide an explanation for the slow progression of NDs, often over decades. ND can be considered a cascading failure of networks where several models have been proposed. For late-onset AD, the uncontrolled neuroinflammation

hypothesis proposes a feedback cycle that leads to shutdown of microglial phagocytosis of amyloid plaques [291–293]. This is an example of a cascading failure of a network where the disease manifest once a key threshold is breached with overload of a subnet [294]. Minimotifs are involved in immune responses and autophagy, and are likely part of the network dysfunction [295].

In conclusion, our summarization of NDs shows a pervasive role for many different types of minimotifs in familial mutations, aggregation, the histone code, epigenetics, and biomarkers. Minimotifs are likely a key part of network dysfunction in NDs.

### Acknowledgments

The National Institutes of Health grants R15GM107983, R01GM079689, R01LM010101, R21AI116411, P20GM121325 awarded and the NV Governor's Office of Economic Development Knowledge Fund awarded to M.R.S; U54GM104944 to J.C.C., R01MH101054 to X.N.C., and R01MH109706 to E.C.O. supported this work. The National Institutes of General Medical Sciences (NIGMS) supports research infrastructure through Institutional Development Awards (IDeAs) and Center of Biomedical Research Excellence (COBRE) awards. This work stems from a collaboration of the Nevada Institute of Personalized Medicine (NIPM) and the Center for Neurodegeneration and Translational Neuroscience (CNTN; P20GM109025). The collaboration is based on COBRE awards to the Cleveland Clinic Lou Ruvo Center for Brain Health and the University of Nevada Las Vegas (UNLV).

### RESEARCH IN CONTEXT

1. Systematic review: During the course of a literature on the role of minimotifs in neurodegenerative diseases we realized that minimotifs had a central role both in the genetic basis and etiology of general neurodegeneration.
2. Interpretation: Our findings indicate that minimotifs play a central role in NDs with familial mutations altering minimotifs and in the general disease etiology by altering protein trafficking, protein aggregation, PTMs, and epigenetics.
3. Future directions: Our review indicates the role of minimotifs in the pathology of NDs, which can be used to further investigate the underlying mechanisms leading to NDs and for research investigating new therapeutics.

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