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TDP-43: A Novel Neurodegenerative Proteinopathy

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Summary

Over the past decade it has become clear that there is significant overlap in the clinical spectrum of frontotemporal lobar degeneration and amyotrophic lateral sclerosis. The identification of TDP-43 as the major disease protein in the pathology of both frontotemporal lobar degeneration with ubiquitin inclusions and amyotrophic lateral sclerosis provides the first molecular link for these diseases. Pathological TDP-43 is abnormally phosphorylated, ubiquitinated, and cleaved to generate carboxy-terminal fragments in affected brain regions. The normal nuclear expression of TDP-43 is also reduced leading to the hypothesis that sequestration of TDP-43 in pathological inclusions contributes to disease pathogenesis. Thus, TDP-43 is the newest member of the growing list of neurodegenerative proteinopathies, but unique in that it lacks features of brain amyloidosis.

Introduction

A wide variety of neurodegenerative diseases are characterized pathologically by the accumulation of intracellular or extracellular protein aggregates composed of amyloid fibrils [1]. For example, the pathology of Alzheimer's disease (AD) is defined by senile plaques and neurofibrillary tangles composed of β-amyloid and microtubule-associated protein tau, respectively, and Lewy bodies composed of α-synuclein are the disease-defining lesions of Parkinson's disease. Until recently, the neuropathology of both frontotemporal lobar degeneration with ubiquitin inclusions (FTLD-U) [2], the most common phenotype associated with the FTLD syndrome, and amyotrophic lateral sclerosis (ALS) [3] were defined by non-amyloidogenic ubiquitinated inclusions (UBI).

FTLD, the second most common form of presenile dementia, refers to a heterogeneous group of neurodegenerative disorders that have in common behavioral and/or language dysfunction [2]. Some affected individuals manifest a movement disorder such as parkinsonism or motor neuron disease (MND). While the designation FTLD reflects the prominent frontal and temporal lobe degeneration, multiple neuropathological abnormalities are identified in these patients [4]. Two broad pathological subdivisions of FTLD are recognized: brains with tau-positive inclusions (i.e., tauopathies) and brains with UBI that are not detected with antibodies to tau, α-synuclein, and β-amyloid (i.e., FTLD-U). Up to 40% of FTLD show a familial pattern of inheritance with three different genetic abnormalities associated with FTLD-U pathology

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including mutations in progranulin (*PGRN*) [5,6] and valosin-containing protein (*VCP*) [7-9] as well as linkage to a novel locus on chromosome 9p [10-12].

ALS, the most common adult-onset MND, is characterized by rapidly progressive weakness, muscular wasting, and spasticity resulting in death within a few years [13]. There is loss of both upper and lower motor neurons with UBI, typically filamentous skeins or compact round bodies, in the surviving motor cells. Familial forms of ALS (fALS) with Mendelian inheritance account for ~10% of cases and are associated with numerous genetic loci including mutations in five genes: Cu/Zn superoxide dismutase (*SOD1*), alsin, senataxin, vesicle-/synaptobrevin-associated membrane protein B, and dynactin. Mutations in *SOD1* gene are the most common accounting for ~20% of fALS.

Until recently, it was unclear whether the ubiquitin pathology in both FTLD-U and ALS was associated with the aggregation of a specific protein or through a generalized defect in protein ubiquitination and degradation. However, this past year, the transactive response (**T**AR)-**D**NA binding **P**rotein with a molecular weight of **43** KDa (TDP-43) was identified as the major disease protein in the UBI of FTLD-U and ALS [14]. The identification of TDP-43 pathology in both of these disorders provided a mechanistic link for the following: 1) a large proportion of ALS patients manifest a range of behavioral and cognitive changes that lie on the spectrum of FTLD [15]; 2) MND is commonly observed in FTLD-U patients [16]; 3) there is significant overlap in the ubiquitin pathology observed in ALS and FTLD-U [17]; and 4) identification of genetic loci and mutations in specific genes in families with co-segregation of both ALS and FTLD [18]. In this review, we highlight work over the past twelve months on TDP-43 and its role in the pathogenesis of FTLD-U and ALS.

Identification of TDP-43 as a major disease protein in FTLD-U & ALS

Characterization of the biochemical composition of the UBI in FTLD-U and ALS was complicated by the relatively low abundance and uneven distribution of the pathology. Unlike the amyloidogenic inclusions composed of β-amyloid, tau, and α-synuclein, the UBI were not clearly fibrillar; they were not detected using amyloid binding dyes such as Congo red, thioflavin S or silver stains. This observation suggested that FTLD-U and ALS are unique proteinopathies characterized by protein misfolding in the absence of brain amyloidosis, a signature of many neurodegenerative diseases. Moreover, the description of subtypes of FTLD-U pathology (Fig. 1) [4,19,20] raised the possibility of multiple disease proteins or pathways. To address this issue, novel monoclonal antibodies (Mab) were generated to high molecular weight insoluble protein extracts prepared from FTLD-U brains of distinct subtypes [20]. These Mab immunolabeled the UBI in the FTLD-U subtype from which they were generated while a subset of the Mab also immunoblotted disease-specific insoluble proteins extracted from affected FTLD-U brain tissue. These Mab facilitated an extensive analysis of protein extracts from FTLD-U brains and led to the identification of TDP-43 as the major component of the UBI [14]. Despite the pathological heterogeneity among FTLD-U subtypes (Fig. 1), immunohistochemistry with commercially available antibodies demonstrated TDP-43 in the UBI of all FTLD-U subtypes as well as sporadic ALS. Furthermore, biochemical analysis of TDP-43 demonstrated a signature profile of TDP-43 in detergent-insoluble, protein extracts from affected FTLD-U and ALS tissue [14]. Thus, pathological TDP-43 was abnormally phosphorylated, ubiquitinated and N-terminally truncated. The identification of these biochemical modifications suggested a specific role for TDP-43 in the pathogenesis of FTLD-U and ALS, rather than simply representing a non-specific entrapped protein within UBI.

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Biology of TDP-43

TDP-43, a 414 amino acid nuclear protein encoded by the *TARDBP* gene on chromosome 1, was initially cloned from a genomic screen for cellular factors that bind to the TAR-DNA element of HIV where it acts as a transcriptional repressor [21]. It is highly conserved and ubiquitously expressed in all tissues including brain [22,23]. The expressed protein contains two RNA-recognition motifs as well as a glycine-rich C-terminal sequence. It was also independently identified as part of a complex involved in the splicing of the cystic fibrosis transmembrane conductance regulator [24] and apolipoprotein A2 genes [25]. The glycine-rich domain in TDP-43 is required for the exon skipping and splicing inhibitory activity [26, 27], an observation consistent with the finding that the C-terminal domain binds to several proteins of the heterogeneous nuclear ribonucleoprotein (hnRNP) family involved in the biogenesis of mRNA [27]. TDP-43 was also recently shown to bind to the proximal promoter of the mouse SP-10 gene (acrosomal vesicle protein 1) involved in spermatogenesis and to regulate its expression [28]. Finally, TDP-43 may also act as scaffold for nuclear bodies called ‘GEMS’ through interaction with survival motor neuron (SMN) protein [29]. Thus, the physiological function(s) of TDP-43 are diverse and incompletely characterized but likely involve the regulation of multiple biological processes through its binding to single stranded DNA, RNA, and/or proteins.

TDP-43 pathology in FTLD-U and ALS

As demonstrated in our initial report [14] and rapidly confirmed in several follow up studies, TDP-43 is a specific and sensitive marker to detect the UBI in both FTLD-U [30-34] and ALS [32,35,36], including neuronal cytoplasmic inclusions (NCI), dystrophic neurites (DN), and neuronal intranuclear inclusions (NII). Notably, while physiological TDP-43 is detectable in the nuclei of unaffected neurons and some glial cells, TDP-43 pathology is associated with a dramatic reduction of normal nuclear TDP-43 staining, raising the possibility that an essential function of TDP-43 is lost in FTLD-U and ALS. Immunohistochemistry for TDP-43 also facilitated the detection of white matter pathology with numerous oligodendroglial cytoplasmic inclusions in a subset of FTLD-U and ALS cases that was not previously appreciated [32,36, 37].

FTLD-U pathology is heterogeneous with respect to morphology, laminar distribution of pathological inclusions and relative proportion of intranuclear and cytoplasmic inclusions leading to the description of four distinct subtypes (Fig. 1) [8,14,19,20,30,38]. The relevance of the distinct patterns of pathology with respect to disease pathogenesis remains unclear. While some cases of FTLD-U do not fit neatly into a specific category, a correlation of distinct histologic subtypes was observed with familial forms of FTLD-U thereby supporting the significance of this classification [30]. For example, mutations in *PGRN*, a secreted growth factor associated associated with cell cycle progression and cell motility, were associated with type 3 pathology. Nearly all of the mutations in *PGRN* are predicted to cause premature termination of the coding sequence by nonsense mediated decay of mutant mRNAs leading to haploinsufficiency. By contrast, mutations in the gene *VCP* are characterized by type 4 pathology. VCP, a member of the AAA-ATPase gene family, associates with a number of protein adaptors to perform a plethora of cellular processes including ubiquitin-dependent protein degradation, stress responses, programmed cell death, nuclear envelope reconstruction, and Golgi and endoplasmic reticulum assembly. The mechanism whereby *VCP* gene mutations cause neurodegeneration remains unclear although disruption of ubiquitin-dependent protein degradation pathways has been implicated. Lastly, the recently identified locus on chromosome 9p is associated with type 2 FTLD-U pathology. As yet, no genetic alterations have been associated with FTLD-U type 1.

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The role of TDP-43 in sporadic ALS versus fALS has also been evaluated. TDP-43 was detected in the round and skein-like NCI as well as glial inclusions in affected brain regions from ALS patients with and without dementia [14,32,35,36]. Remarkably, while pathological TDP-43 was a consistent feature in non-*SOD1*-fALS, TDP-43 was not detected in the UBI of any patients with *SOD1* mutations [35,36]. Consistent with these findings is the reported absence of TDP-43 immunoreactivity in inclusions in mutant *SOD1* (G93A) transgenic mice [39]. In contrast, in Guam ALS and parkinsonism-dementia complex (PDC), a disease of unknown etiology affecting the Chamorro populations and characterized by extensive tau pathology, TDP-43 inclusions were detected in the spinal cord of both ALS and PDC cases but not in controls [40]. These TDP-43 inclusions were distinct from the tau pathology in the spinal cord. Interestingly, TDP-43 pathology was also detected in cortical and limbic regions from Guam-PDC cases, inclusions that were not detected with antibodies to the tau protein [40,41]. Thus, these results support the hypothesis that ALS and FTLD-U represent a clinical spectrum of neurodegenerative disease characterized by TDP-43 pathology (Fig. 1). However, the absence of TDP-43 in *SOD1*-fALS implies that motor neuron degeneration in these cases results from a different disease pathway that also affects motor neurons. However, this hypothesis is highly controversial [42].

The specificity of TDP-43 as a marker for FTLD-U lesions now permits the investigation of FTLD-U pathology in the setting of concurrent ubiquitin-positive pathology in other neurodegenerative diseases (Box 1) [14,30-32,43-45]. Surprisingly, additional TDP-43 pathology similar to that found in FTLD-U was reported in several other neurodegenerative diseases. This observation raised the possibility that amyloid deposition in the brain (i.e., neurofibrillary tangles and Lewy bodies) predisposes TDP-43 to misfold and aggregate to form non-fibrillar inclusions. However, the clinical significance of concomitant TDP-43 pathology in these other diseases is unknown.

Pathobiology of TDP-43

The identification of TDP-43 in the UBI of FTLD-U and ALS implicates a role for TDP-43 in disease pathogenesis. However to date, the proverbial ‘smoking gun’, i.e., genetic variation in the *TARDBP* leading to increased risk for disease, is lacking [46]. Although its functions are reported as a transcriptional repressor and splicing regulator [21-23], the mechanism whereby TDP-43 contributes to neuron degeneration is unknown (Fig. 2). Nonetheless, based on this functional data, a number of hypotheses have been generated. The sequestration of TDP-43 in inclusions could cause a loss of function defect and thereby result in transcriptional deregulation and aberrant splicing of pre-mRNA. For example, TDP-43 was recently demonstrated to stabilize low molecular weight neurofilament mRNA via a direct interaction with the 3′UTR [47]. Loss of TDP-43 activity could destabilize low molecular weight neurofilament mRNA thereby altering the stoichiometry of neurofilament subunits and leading to the formation of neurofilament aggregates as observed in ALS. The sequestration of TDP-43 could also alter the cellular distribution of SMN and hnRNP; however, changes in the expression and posttranslational modification of hnRNP were not observed in FTLD-U and ALS, and hnRNP were not detected in the UBI [48]. Alternatively, the C-terminal domain of TDP-43 that aggregates in the inclusions and is implicated in its splicing regulatory function [23,27], may have aberrant biological activities (i.e., toxic ‘gain of function’). It has also been hypothesized that PGRN might be a protein binding partner of TDP-43, involved in its trafficking to and from the nucleus [49]. Thus, dysfunction or dysregulation of PGRN could contribute to the abnormal compartmentalization of TDP-43. Finally, the abnormal phosphorylation of TDP-43 may disrupt important signaling pathways or directly affect the trafficking of TDP-43 itself, thereby leading to neuronal dysfunction. The development of cell culture and murine model systems will be critical to testing these hypotheses and elucidating the role of TDP-43 in the pathogenesis of FTLD-U. Furthermore, the development of genetic

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models will be essential to our understanding of the link between TDP-43 and mutations in multiple different genes including *PGRN* and *VCP*.

Conclusions

Despite the significant clinical, genetic, and neuropathologic heterogeneity within FTLD and ALS, TDP-43 is a common pathological substrate linking FTLD-U and ALS caused by different genetic alterations. This observation supports the hypothesis that FTLD and ALS represent two extremes of a clinicopathological spectrum of TDP-43 proteinopathies. An understanding of the role of TDP-43 in the pathogenesis of FTLD-U and ALS will have to integrate the biology of multiple distinct genetic elements. However, the absence of pathological TDP-43 in fALS with *SOD1* mutations implies that MND in these cases is not the familial counterpart of sporadic ALS.

While these are still early days in the understanding of the pathobiology of TDP-43, it is evident that a new classification of neurodegenerative disorders has emerged (Fig. 1). However, the TDP-43 proteinopathies are distinct from other protein misfolding neurodegenerative diseases because of the lack of amyloid fibrils and will likely lead to unique challenges. Nonetheless, the identification of TDP-43 in the pathological inclusions of FTLD-U and ALS will have significant implications for the diagnosis and treatment of FTLD and ALS. For example, the development of assays to monitor levels of normal and pathological TDP-43 in cerebrospinal fluid could be used as a diagnostic tool to distinguish TDP-43 proteinopathies from other clinically similar neurodegenerative disorders. Further, the development of imaging ligands that enable the detection of TDP-43 neuropathology in living patients will provide a tool not only for diagnosis but also for following the response of patients with a neurodegenerative TDP-43 proteinopathy to disease-modifying therapies. Finally, the recognition that TDP-43 pathology underlies and links FTLD-U and ALS will be a significant driver of efforts to develop mechanistically-based therapies for these disorders.

Box 1

Spectrum of TDP-43 pathology in neurodegenerative disease

The initial report identifying TDP-43 in the UBI of FTLD-U and ALS suggested that TDP-43 is a specific marker for these diseases [14]. However, a follow-up study identified TDP-43 not only in the UBI of FTLD-U and ALS but also in tau inclusions including the majority of Pick bodies in Pick's disease, as well as a subset of the neurofibrillary tangles in AD and tangle-predominant senile dementia and threads and coiled bodies in corticobasal degeneration [32]. TDP-43 was not detected in the tau pathology of progressive supranuclear palsy or the α-synuclein pathology in Lewy body disease and multiple system atrophy. While subsequent studies did not detect TDP-43 in the tau inclusions of both familial and sporadic tauopathies [30,31], these findings prompted further investigation into the specificity of TDP-43 pathology.

Amador-Ortiz and colleagues detected TDP-43 pathology in 71% of hippocampal sclerosis (n = 65) cases [45]. While this result is not surprising in light of the high prevalence of hippocampal sclerosis in FTLD-U, they also detected TDP-43 pathology in 23% of AD cases (n = 167). However, double-labeling for TDP-43 and phospho-tau demonstrated that the TDP-43-immunoreactive pathology was largely distinct from the neurofibrillary tangles. In a related study on Lewy body disease, co-morbid TDP-43 pathology was identified in 29% of cases with Dementia with Lewy body and AD pathology, 19% of Parkinson's disease dementia, and 7% of Parkinson's disease [44]. TDP-43 pathology was also a consistent feature in affected Chamorrans with Guam-PDC, but not in controls [40, 41]. Whether TDP-43 pathology represents concomitant FTLD-U pathology in these cases or is analogous to co-localization of α-synuclein pathology in AD remains to be determined.

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Nonetheless, these studies expand the concept of TDP-43 proteinopathies by implication of TDP-43 in a variety of neurodegenerative diseases characterized by the aggregation of fibrillar amyloid deposits.

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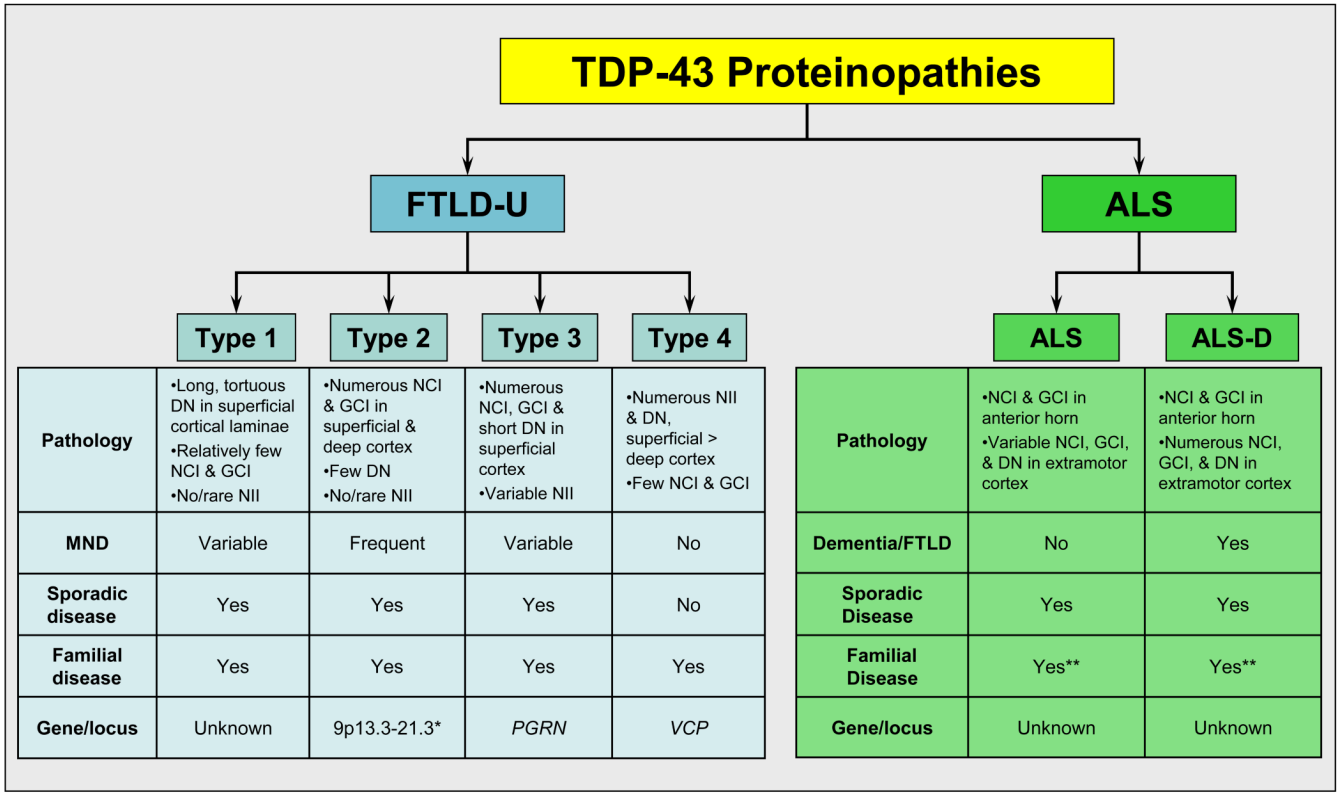

Figure 1. Proposed classification scheme for TDP-43 proteinopathies

Despite the significant clinical, genetic, and neuropathologic heterogeneity within FTLD and ALS, TDP-43 is a common pathological substrate linking FTLD-U and ALS caused by different genetic alterations. This observation supports the hypothesis that FTLD and ALS represent two extremes of a clinicopathological spectrum of one disease, TDP-43 proteinopathies. FTLD-U is subclassified based on distinct morphological, genetic, and clinical parameters while dementia is reported in a significant subset of ALS patients. ALS, amyotrophic lateral sclerosis; DN, dystrophic neurites; fALS, familial amyotrophic lateral sclerosis; FTLD, frontotemporal lobar degeneration; FTLD-U, frontotemporal lobar degeneration with ubiquitin inclusions; GCI, glial cytoplasmic inclusions; NCI, neuronal cytoplasmic inclusions; NII, neuronal intranuclear inclusions; MND, motor neuron disease; *PGRN*, progranulin; *SOD1*, Cu/Zn superoxide dismutase; *VCP*, valosin-containing protein.

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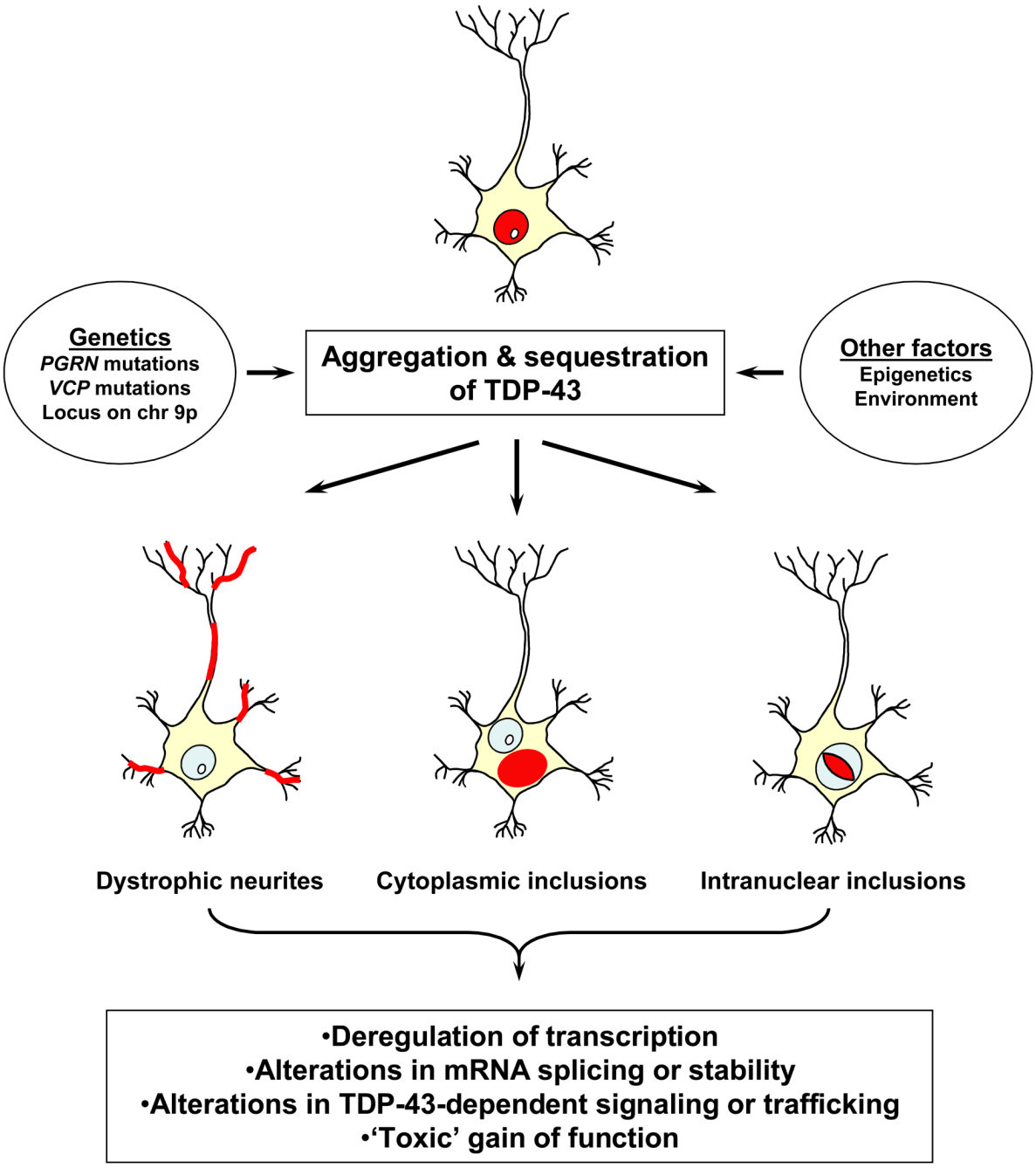

Figure 2. Model of TDP-43 disease pathogenesis

The aggregation of TDP-43 (depicted in red) in neurons as well as glia leads to its sequestration in cytoplasmic and intranuclear inclusions as well as dystrophic neurites. The sequestration of TDP-43 may be toxic due to loss of normal function. Alternatively, the aggregation of TDP-43, in particular the C-terminal fragment(s) may lead to a toxic gain of function.