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Review Article

New Paradigm on Neurodegeneration and Possibly Autoimmunity from ALS/FTD and Its Genetic Hallmarks

Mike J. Chung[1], and Maria M. Plummer[2]

[1]NYIT College of Osteopathic Medicine, New York, USA

[2]Department of Clinical Specialties, NYIT College of Osteopathic Medicine, New York, USA

Abstract

Recognition of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) as a spectrum of neurodegenerative disease is rapidly evolving. Both disorders have been shown to overlap in their pathology, genetics, and clinical presentations. Recent investigations have identified genetic hallmarks that concretely bridge those highly heterogenic disorders as a spectrum. The discovery of hexanucleotide repeat expansions in chromosome 9 open reading frame 72 (C9orf72), TAR-DNA binding protein (TDP)-43 proteinopathy, and their implicated functions in neurodegeneration and autoimmunity suggests possible underlying pathogenesis of ALS/FTD and toxic neurodegeneration at large. The autoimmunity aspect of C9orf72 is particularly exciting as it may elucidate a novel paradigm that not only encompasses the origin of neurodegeneration, but also that of autoimmunity. Here, we briefly review the latest findings of functions of TDP-43 and C9rof72 and further discuss the possibility of an overarching theme in neurodegenerative diseases.

Keywords: Amyotrophic Lateral Sclerosis, Frontotemporal dementia, TDP-43, C9orf72, Autophagy, Autoimmunity

Introduction

Amyotrophic lateral sclerosis (ALS) is an insidious neurodegenerative disorder that primarily affects motor neurons, leading to progressive muscular atrophy, weakness, and paralysis, which often lead to respiratory failure and death within 3-5 years of symptom onset [1]. ALS is one of the most common motor neuron diseases with an incidence between 0.6 and 3.8 per 100,000 and prevalence between 4.1 and 8.4 per 100,000 [2]. An increasing number of patients diagnosed with ALS raises the need for better awareness and in-depth research of the disorder [2]. The heterogenic nature of ALS makes most cases of ALS unknown. However, about 5 to 10% of the cases are developed in familial forms, associated with genetic mutations that have a wide range of functions [3]. The most frequently associated pathogenic variants are identified in C9orf72, SOD1, FUS, and TARDBP [4]. The implicated genes are typically passed down with dominant inheritance, although genotype does not completely dictate phenotype [5]. Interestingly, TDP-43 proteinopathy plays a major pathological hallmark in ALS cases, as it is present in up to 97% of ALS patients [6, 7, 8].

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***Corresponding author(s):** Mike J. Chung, NYIT College of Osteopathic Medicine, New York, USA

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Frontotemporal dementia (FTD) is another fatal neurodegenerative syndrome leading to progressive degeneration of the frontal and temporal lobes of the brain. Since the first description by Arnold Pick as "the relationship between senile atrophy of the brain and aphasia" in 1892 [9], FTD has been well characterized with progressive aberrations in behavioral, linguistic, and executive functions. FTD is consistently considered one of the most prevalent dementia subtypes with a prevalence of 15 to 22 per 100,000 and incidence of 2.7 to 4.1 per 100,000 [10]. However, the true prevalence is most likely underestimated, as the disorder is frequently missed and misdiagnosed due to its overlap with late-onset psychiatric diseases [11]. Although most FTD cases are likely sporadic, a considerable number of cases show familial aggregation and up to 40% autosomal dominant inheritance patterns [10]. Recent research has identified five genetic mutations associated with the disorder: C9orf72, GRN, MAPT, VCP, and CHMP2B [10]. For this review on the ALS-FTD spectrum disorder, we will focus on C9orf72 only.

Prior to the recent findings, ALS was often considered to be confined to motor deterioration due to motor neurons death, sparing cognitive functions. Similarly, FTD was rarely linked to motor dysfunctions outside of its cognitive, behavioral, and semantic aspects. In recent years, however, it has been well established that ALS and FTD can develop simultaneously in an individual [12]. Up to half of the patients with ALS demonstrate some degrees of frontal lobe dysfunction with 15% of cases sufficing diagnosis of FTD [13]. On the other hand, about 40% of patients with FTD exhibit significant motor dysfunction with up to 15% warranting ALS diagnosis [14]. Increasing awareness of the diseases and their overlaps has shifted a perspective on how to diagnose the syndromes more effectively [15]. Further investigations reveal the two seemingly distinct heterogenic diseases share some common genetic mutations in their origins. As commonly seen in many neurodegenerative diseases, FTD expresses pathognomonic inclusion bodies, and approximately

90% of FTD variants exhibit either TDP-43 proteinopathy (50%) or tauopathy (40%) [16]. When linked to ALS, however, all cases of FTD demonstrate a clear histological phenotype with TDP-43 aggregated proteins [17, 18, 19]. Moreover, all familial forms of the cases appear to be associated with hexanucleotide repeat expansions in the C9orf72 [19, 20], the most common genetic cause of ALS and FTD [21, 22]. Further investigation on C9orf72 repeat expansions has solidified the idea that both disorders can be on one disease spectrum with overlapping clinical manifestations and TDP-43 pathology [23, 24].

ALS-FTD spectrum disorders, TDP-43 proteinopathy, and C9orf72 expansion have shown irrefutable association over the last decade. The next natural question that follows is, what do such striking genetic hallmarks of the diseases do? Unsurprisingly, their dysfunctions seem to complement each other in the development of neurodegenerative diseases. Furthermore, recent discoveries of their involvement in autoimmunity may further broaden our perspective on the complicated relationship neurodegenerative diseases possess with other types of diseases.

First, this review will focus on the functions of TDP-43 and C9orf72 in the context of autophagy and how their malfunction may lead to the development of ALS-FTD spectrum syndromes. Secondly, we explore the autoimmunity development found in C9orf72 mutations and briefly review the implications other ALS-FTD genes have on immune functions. Lastly, we discuss how these findings broaden our perspective on foundational pathogeneses of ALS-FTD disorders and degenerative or autoimmune disorders at large.

TAR DNA-binding protein 43 (TDP-43) and its role in neurodegeneration

TDP-43 is a highly conserved protein of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and is encoded by the TAR DNA Binding Protein (TARDBP) gene (1.p36.22) [3]. This RNA/DNA-binding protein is ubiquitously expressed for its vital role in various cellular functions such as mRNA transport regulation, microRNA maturation, RNA metabolism, and stress granule formation [25]. The protein executes its functions as it shuttles between the nucleus and the cytoplasm via active and passive transport [26]. It also appears to play a critical role in the development of central neuronal cells in the early stages of embryogenesis [27]. Given how important proper TDP-43 functions are to neuronal cells, it is not surprising that TDP-43 mutations and dysfunction lead to lethal neurological diseases such as FTD and ALS. Several mouse-model studies have proposed that dysregulation of RNA metabolism may serve as a pathogenic mechanism of TDP-43 proteinopathies [28, 29], leading to progressive weakness or motor neuron degeneration. Defected functions of TDP-43 in the nucleus as a regulator of RNA metabolism leads to degradation or reduction of RNA and altered splicing sequences [30]. Mutant TDP-43 aggregation appears to interfere with nucleocytoplasmic transport of proteins and RNA through sequestration and mislocalization of various transport factors [31]. Another interesting revelation in recent years is that TDP-43 toxicity seems to be exacerbated by TDP-43-associated mitochondrial abnormalities through amplification of oxidative stress [25, 32]. Of relevance, pathogenic TDP-43 overexpression found in motor neurons demonstrates mitochondrial length reduction, movement impairment, fission, and fragmentation [33]. Numerous dysfunctions associated with mutations in the core of the TARDBP gene eventually converge to the formation of amyloid-like fibrils, which are pathognomonic hallmarks of FTD/ALS, with ensuing neuronal toxicity [34]. Additionally, TDP-43 has a vital role in regulating autophagy, which is critical for cleaning a pathogenic form of its oligomers [35, 36]. Loss of TDP-43 leads to autophagy dysfunction by impairing the autophagy-lysosome pathway and mTORC1 signaling [37], necessary steps in the proper autophagic process. As expected, FTD and ALS with TDP-43 origins exhibit inclusion bodies positive for autophagy markers and associated disease progression [36].

Chromosome 9 Open Reading Frame 72 (C9orf72) and autophagy in Neurodegeneration

Although C9orf72 has been consistently referred to as the most common genetic cause of ALS-FTD spectrum disorders, our understanding of its functions has been exponentially increasing only in recent years. To start, C9orf72 is likely involved in essential cellular processes as it is highly homologous among various species that are commonly used as model systems [38]. The gene is expressed the most in a subset of myeloid cells at the cellular level [39] while transcribed protein levels seem to peak in the brain and spinal cord [40]. Differences in transcription sites of C9orf72 identified in myeloid and central nervous system (CNS) tissues indicate diverse functions of C9orf72 depending on cell types [39]. One of the most identified functions of the gene in accordance with our interest is its autophagy regulation. C9orf72 product is structurally homologous to a Guanine Exchange Factor of Rab proteins (RabGEF) [41]. RabGEF is known to control membrane trafficking events such as autophagy and endocytosis. C9orf72 expression and its interaction with Rabs in neuronal cell lines indicate that C9orf72 likely has a function in membrane trafficking [42], and perhaps specifically in endocytic and phagocytic trafficking [43]. C9orf72 also appears to be involved in selective protein aggregation macroautophagy [44] with its interaction with a heat-shock chaperone, HSC70 [45]. Aggrephagy is a mechanism responsible for clearing aggregating proteins and macroautophagy is a type of autophagy that plays a role in neurodegeneration [46]. Whether C9orf72 hexanucleotide repeat expansion leads to a toxic gain of functions (GOF) [47] or loss of functions (LOF) [48] has been extensively discussed, but a recent study suggests that both GOF and LOF of C9orf72 products likely contribute to membrane trafficking defects [49]. With its presence as an autophagy regulator in neuronal cell lines as well [42], C9orf72 demonstrates its importance in maintaining CNS homeostasis, so much so that a reduced C9orf72 activity from repeat expansions is sufficient to disrupt vesicle trafficking and lysosomal biogenesis in motor neurons [45, 48]. The relationship between autophagic deficiencies and neurodegenerative aggregates is well established [50], and such disruption in cellular homeostasis likely relates to proteinopathies, such as TDP-43 and dipeptide-repeat proteins (DPR) aggregation [22]. Whether the inclusion bodies are active participants of neurodegeneration or inactive byproducts of the pathogenesis has remained elusive.

However, evidence is emerging to suggest that they contribute to the development to some degree [25, 32, 34].

C9orf72, TDP-43, microglia, and autoinflammation

Autoimmunity is often characterized by chronic, progressive inflammation and tissue damage [51], features that are also prevalent in neurodegenerative diseases as neuroinflammation. The term describes the inflammatory process within the CNS as a result of interactions between CNS cells and resident glial cells (astrocytes, microglia) and peripheral immune cells (monocytes, neutrophils, lymphocytes) in response to various types of insults [1, 52]. Recent research suggests the connections between C9orf72 dysfunction, autoimmunity, and inflammation. Mice with LOF mutations in C9orf72 develop inflammatory and autoimmune phenotypes, such as splenomegaly, lymphadenopathy, and increased inflammatory cytokines induction, leading to a high mortality rate [53, 54]. For example, the systematic autoimmune reaction seems to manifest as increased levels of circulating cytokines, such as IFN-γ, IL-2, IL-4, and many more in ALS patients [55]. Additionally, loss of C9orf72 induces the accumulation of various self-reactive antibodies such as anti-double-stranded DNA (dsDNA), a common diagnostic marker of systemic lupus erythematosus (SLE) [56, 57], which are common markers of many autoimmune disorders. Furthermore, intermediate-length C9orf72 expansions are observed in systemic, non-neurodegenerative disorders such as SLE and rheumatoid arthritis (RA) [58]. The discovery of C9orf72 expansions in systemic autoimmune disorders may further elucidate the connection between ALS and autoimmunity which were established decades ago [59, 60, 61]. Of relevance, the proinflammatory state induced by loss of C9orf72 likely originates from the critical role C9orf72 plays in proper functions of macrophages and microglia [54, 62], resident macrophages in the CNS that play an important role in clearing toxic residues and activating immune responses. The finding is consistent with the fact that C9orf72 expresses the highest in myeloid cells [39] and that C9orf72 mutations result in autophagic deficiencies [49]. The importance of C9orf72 in proper immune function is further demonstrated by studies illustrating that haploinsufficiency of C9orf72 is enough to promote changes in myeloid cell functions and systemic immune response [53, 54, 57].

Interestingly, TDP-43 also exhibits some importance in the proper functions of innate immune cells, with TDP-43 proteinopathies showing significant association with autoimmunity [63]. When taken out of microglia, depletion of TDP-43 seems to enhance phagocytic functions of microglia, improving amyloid clearance but also increasing synapse loss [64]. Possibly profound impacts TARDBP may have on microglial function and other possible pathogenic pathways in neurodegeneration warrant further investigations. Additionally, the origin of neuroinflammation suggested in loss of C9orf72 and TDP-43 is not necessarily limited to autoimmunity, as hypersensitivity can also develop from an accumulation of excitotoxicity, oxidative stress, and stress granules. Such damages seem to originate from abnormal autophagic function, which is implicated in both C9orf72 and TDP-43 mutations.

Vicious cycle: hypersensitivity through excitotoxicity, ROS, SGs, implicated by impaired autophagy

Impaired autophagy and abnormal protein aggregation work as interesting convergence points for TDP-43 and C9orf72 mutations. They significantly compromise cellular homeostasis, further enabling neurodegenerative pathogeneses. For example, comprised autophagy due to low C9orf72 expression seems to hypersensitize motor neurons through the accumulation of membrane glutamate receptors on motor neurons and trigger unregulated excitotoxicity [48, 65]. Furthermore, low C9orf72 activity may synergistically exacerbate glutamate excitotoxicity to motor neurons by DPR toxicity [48, 66]. Deficient autophagy, along with other vital homeostatic roles that TDP-43 and C9orf72 partake in, is also implicated in the accumulation of toxic cytoplasmic organelles, called stress granules. As their name suggests, stress granules are cellular organelles that appear in cells under stress. Principles and properties of stress granules are thoroughly examined in other reviews [67, 68, 69], and although there are several types of stress granules, this review will focus on cytoplasmic stress granules in the context of neurodegeneration and refer to them as SGs. Stress granules contain aggregations of various types of proteins and RNAs depending on stress conditions that impede translation, such as heat stress and oxidative stress, and the compositions of SGs vary accordingly [70]. SGs play a crucial role in maintaining appropriate RNA metabolism and cell signaling by preventing the accumulation of misfolded proteins, and their defects are implicated in many diseases, including neurodegenerative disorders [69, 71]. In relevance to this review, abnormal SGs have been associated with the pathogenesis of FTD/ALS with both TDP-43 and C9orf72 origins [28, 72], and TDP-43 localizes to SGs [73, 74]. Translational study indicates early pathological SG formation as a key mechanism of TDP-43 proteinopathy in FTD/ALS [75]. Conversely, the inability to efficiently sequester and clean abnormal aggregation of RNA binding proteins may lead to the formation of toxic SGs [69, 71]. Chronic buildup in toxic residues as previously mentioned remains in the CNS to persistently leave insults to resident cells, potentially hypersensitizing microglia in the process. Microglia are extremely sensitive to environmental changes and respond to both neurological and systematic stress signals [1]. In response to continuous activation, microglia can produce potentially deleterious neurotoxins, namely reactive oxygen species (ROS), which may further amplify the toxicity through SGs induction [76] or protein misfolding [77].

The unexpected connection between C9orf72, TDP-43, autophagy, neurodegeneration, and perhaps autoimmunity?

Despite their heterogenicity, FTD and ALS demonstrate clear convergence in genetic hallmarks and pathogeneses through the overwhelming prominence of TDP-43 and C9orf72 mutations in the ALS-FTD spectrum. Moreover, the presence of TDP-43 and C9orf72 defects in other neurodegenerative diseases with varying clinical features indicate their importance in neurodegeneration [6, 32, 38]. Their eminence may be explained by the vital roles the genes take in proper cellular homeostasis and signaling.

Autophagy defect from loss of both hallmarks indicates one of underlying, ubiquitous pathogeneses in ALS-FTD, and possibly in various types of distinct neurodegenerative disorders as well. The idea is further supported by studies that show TDP-43 is also implicated in the development of Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) [6, 32], and C9orf72 mutations are involved in the development of HD phenotypes and Friedreich's ataxia (FRDA) [38]. In this review, we notice that pathological steps of ALS/FTD amplify one another in a cyclical pattern to disrupt cellular homeostasis and drive toxicity within the CNS. While identifying a beginning step of neurodegeneration in FTD/ALS remains challenging, we recognize autophagy dysfunction as the basis of numerous pathological events and one of the initial driving forces in the ALS-FTD spectrum.

Additionally, the recent discovery that C9orf72 and TDP-43 can be involved in the development of neurodegeneration and autoimmunity is nothing short of astonishing. Implications that C9orf72, TARDBP, and other ALS/FTD genes at large have on immune function [1, 57] bring up interesting questions. Are patients with ALS/FTD more vulnerable to autoimmune syndromes such as SLE, or vice versa? Can autoimmunity and neurodegeneration share a pathological origin? Current research reinforces the importance of altered immunity in the pathogenesis of ALS/FTD. Combined with the fact that the same genetic hallmarks are often implicated in various types of neurodegenerative disorders, the finding also suggests such aberration may be prevalent in other types of neurogenerative disorders. Moreover, the discovery supports the idea of underlying pathogenesis that bridges neurodegenerative disorders to autoimmune disorders. If autoimmune disorders and neurodegenerative diseases are related, how far can the underlying pathology permeate? Questions emerging from the discovery of autoimmunity from loss of C9orf72, along with other pathogenic genetic defects in ALS/FTD, need to be investigated further, as future studies may serve as the gateway to understanding the origins of autoimmunity and possibly to those of other types of pathologies.

Conclusion

Extensive investigations in recent years have illuminated ALS and FTD as neurodegenerative disorders on a spectrum with TDP-43 and C9orf72 as driving genetic hallmarks. As our understanding of their functions is exponentially expanding, we are discovering that TDP-43 and C9orf72 mutations converge in many aspects of FTD/ALS pathogenesis, where their implicated dysfunctions often overlap or complement one another. One notable point to make is that both TDP-43 and C9orf72 mutations are implicated in autophagy dysfunction. The defect seems to be the main factor in driving a vicious cycle of perpetual toxicity and proteinopathy, in which resident cells of the CNS degenerate due to compromised cellular homeostasis. Additionally, pathologic mutations in TDP-43 and C9orf72 have been shown to disrupt immune functions and induce autoimmunity, further reinforcing the concept of the pathologic cycle. The discovery of C9orf72 in autoimmune disorders such as SLE and RA also opens an interesting possibility that degenerative diseases and autoimmunity may originate from the same foundational, overarching pathology.

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