CellPress

Series: Current Trends in Aging and Age-Related Diseases

Review

Common Molecular Pathways in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are age-related neurodegenerative diseases in which predominantly motor neurons and cerebral cortex neurons, respectively, are affected. Several novel ALS and FTD disease genes have been recently discovered, pointing toward a few overarching pathways in ALS/FTD pathogenesis. Nevertheless, a precise picture of how various cellular processes cause neuronal death, or how different routes leading to ALS and FTD are functionally connected is just emerging. Moreover, how the most recent milestone findings in the ALS/FTD field might lead to improved diagnosis and treatment is actively being explored. We highlight some of the most exciting recent topics in the field, which could potentially facilitate the identification of further links between the pathogenic ALS/FTD pathways related to autophagy, vesicle trafficking, and RNA metabolism.

Amyotrophic Lateral Sclerosis and Frontotemporal Dementia – Components of a Phenotypic Neurodegenerative Disease Spectrum

Classic **amyotrophic lateral sclerosis (ALS**; see Glossary) and **frontotemporal dementia (FTD**) represent parts of a spectrum of classical neurodegenerative diseases with an incidence of approximately 2–3/100 000 and 3–4/100 000 per year, respectively [1,2]. ALS is a multisystem degenerative condition clinically characterized by the predominant loss of motor neurons and progressive weakness of voluntarily innervated muscles, including muscles of the respiratory apparatus. This leads to almost complete **paresis** after a few years, and death occurs usually from respiratory failure [3]. By contrast, FTD comprises a group of disorders with a principally different clinical phenotype, caused by degeneration of **cortical neurons** and **basal ganglia**. This results not only in cognitive and language deficits but also changes in personality and behavior [4]. FTD is therefore distinct from the 'classical' Alzheimer's disease (AD) type of dementia. It is frequently also termed frontotemporal lobar degeneration to specify that the disease phenotype goes beyond dementia and cognitive defects.

Despite the distinct neurological and psychiatric symptoms, ALS and FTD are tightly linked [4]. Case reports of a co-occurrence of ALS and FTD symptoms in the same patients date back to the 19th century, while the view of ALS in most textbooks after World War II was that of a pure motor neuron disease. The connection between both diseases was gradually rediscovered in the 1980s. In 2006, Neumann *et al.* [5] showed that ALS and FTD comprised cytoplasmic protein deposits consisting of the protein **transactive response DNA-binding**

Trends

The link between amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), described in the 19th century based on clinical observations, has been (re)discovered and confirmed by both neuropathological and genetic findings.

Recent results from human genetics have revealed common functional pathways in ALS/FTD pathogenesis. Protein products of most ALS genes act in pathways regulating autophagy and vesicle trafficking, RNA metabolism, or cytoskeleton dynamics. The functional role of impaired DNA damage repair remains to be shown.

Selective autophagy connects at least four different ALS/FTD genes in one putative functional pathway [*TBK1*, *SQSTM1*/p62, *OPTN*, and chromosome 9 open reading frame 72 (*C9ORF72*)].

RNA granules are regulated by liquid-phase transition involving RNA proteins with disordered, aggregation-prone protein domains. This principle provides a plausible explanation on how RNA-binding protein mutations and protein aggregation could be linked to RNA dysregulation, and subsequently, to neuronal degeneration.

New genetic mouse models based on recently discovered ALS genes are currently being evaluated.

protein 43 kDa (TDP-43). Finally, in 2008, mutations in the *TARDBP* gene [coding for TAR DNA-binding protein 43 (TDP-43)] were identified as causative for both ALS and FTD, sometimes even in the same family or in the same patient [6,7]. The identification of *TARDBP* as a shared ALS/FTD gene was followed by a wave of discoveries continuing until the present day, revealing that mutations in several other genes such as chromosome 9 open reading frame 72 (*C9ORF72*) [8,9], *VCP* [10], or *TBK1* [11] could cause both ALS and FTD. ALS and FTD have thus been increasingly regarded as part of a disease spectrum [4]. These illnesses have been further linked by an overlapping neuropathology, mainly characterized by TDP-43-positive cytoplasmic neuronal inclusions in most ALS, and a large proportion of FTD brains [5]. These discoveries have led to a completely different understanding of ALS and FTD in recent years, with research in these pathologies developing into one of the most active fields of neurological science. Nonetheless, the cellular basis of ALS and FTD remains unknown.

In this review, we summarize some of the current knowledge on the latest development of ALS and FTD and discuss the common downstream mechanisms of known ALS genes and the putative common denominators on how they are functionally linked. We also examine how the **premanifest phase** of ALS and FTD might be characterized, and how otherwise physiological age-related events might contribute to disease manifestation of a pre-existing disease predisposition. Will it be possible to generate more innovative and predictive ALS disease models (*in vitro* and *in vivo*) based on recent genetic and cell biological discoveries? (Box 1 and Outstanding Questions).

Human Genetics and Neuropathology of ALS – Guideposts to Molecular Events

Overall, a positive family history for ALS or FTD is recognized in approximately 5% of all ALS patients [1,12], but a higher contribution of genetic factors can be assumed, given that inheritance may be missed due to incomplete penetrance or because of an **oligogenic** mode

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Box 1. The Clinician's Corner

Neuropathology and human genetics have led to the (re)discovery that amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are tightly linked diseases.

Both diseases can be caused by the same mutation in different members of one same family, and comorbidity in a same patient is frequent. This has also led to a new definition of 'familial' ALS/FTD, already assumed when one family member has ALS and another one presents dementia or 'psychosis' (as FTD patients may be misdiagnosed with schizophrenia).

Known ALS and FTD genes explain more than half of familial ALS/FTD cases in Caucasian populations, which can improve genetic counseling, also because it is increasingly recognized that typical phenotypes and disease courses can be assigned to specific genes (e.g., often times representing more aggressive disease courses in chromosome 9 open reading frame 72 (*C9ORF72*) mutation carriers, or in advanced age-onset *TBK1* loss-of-function mutation carriers). According to their frequency, familial patients should usually be screened first for mutations in *C9ORF72* and *SOD1* (superoxide dismutase 1), and then *TBK1*, *TARDBP*/TDP-43 (TAR DNA-binding protein 43 gene/transactive response DNA-binding protein 43 kDa), and *FUS*, until whole-genome sequencing finds its way into clinical routines.

Recent discoveries in the ALS/FTD field have outlined a few overarching cell biological topics that seem to play a central role in disease causation, specifically protein quality control, RNA regulation, and cytoskeletal dynamics.

Novel pathogenic insights will hopefully lead to innovative, ALS/FTD-relevant experimental *in vitro* and *in vivo* paradigms to be used for therapeutic compound screening and evaluation of novel treatment approaches.

As a consequence of ALS genetic research, a first genotype-dependent therapy, which is based on intrathecally delivered improved antisense oligonucleotides, is currently being tested in Phase I clinical trials.

of inheritance. The advent of next-generation sequencing has led to a wave of discovery of novel ALS-related genes. Altogether, mutations in more than 20 genes have been suggested to cause ALS/FTD in a mostly autosomal-dominant manner, although the level of evidence for attributed pathogenicity differs between these genes [12]. However, several of these disease genes have been repeatedly confirmed by different approaches (e.g., genome-wide or exome-wide association analysis combined with segregation analysis).

The ALS/FTD genes discovered in recent years seem to be diverse at first glance. However, their physiological functions and properties can be grouped according to their involvement in (i) protein quality control, (ii) cytoskeletal dynamics, (iii) RNA homeostasis, and (iv) DNA damage response (Box 2, Table 1, and Figure 1, Key Figure).

Despite the common functional processes that seem to apply to most ALS genes, a key question to understand in ALS is why these diverse genetic pathways lead to the same clinical syndrome. Moreover, it must be kept in mind that even though most ALS genes can be linked to few functional main pathways, their (known) physiological functions may also be conceptually misleading, as ALS-relevant functions may be unknown, and furthermore, toxic gain-of-function principles may be in place. As an example, the mutation-induced gain of a novel toxic property – rather than loss of the free radical scavenging function of the ALS-associated protein superoxide dismutase 1 (SOD1; discovered in 1993) – is most likely critical for disease causation [13]. This is supported by the fact that several ALS-associated mutations do not necessarily result in impaired SOD1 enzymatic function, and expression of mutant human SOD1 in mice – though not deletion of endogenous wild-type mouse SOD1 – causes motor neuron degeneration.

Box 2. Amyotrophic Lateral Sclerosis (ALS) Disease Genes Converge in Overarching Functional Processes

Protein Quality Control

Mutations in genes functioning in protein quality control pathways comprise a functionally connected group of ALS disease genes. Altered function or expression of *TDP-43* (transactive response DNA-binding protein 43 kDa) [61], *UBQLN2* (ubiquilin-2) [83], *OPTN* (optineurin) [63], *SQSTM1, VCP* [10,64], or chromosome 9 open reading frame 72 (*C9ORF72*) [8,9] genes leads to ALS, implicating that proteasomal protein degradation and proper autophagic activity are critical to maintaining healthy motor neurons during the lifetime of a human being.

Cytoskeletal Dynamics

Experimental evidence has implicated altered cytoskeleton dynamics and disturbed axonal transport processes in ALS, confirmed by data indicating that mutated *DCTN1* [84], *PFN1* [85], *NEFH* [86,87] or *TUBA4A* [88] genes, which regulated either actin or tubulin cytoskeleton, could be identified and implicated in disease in ALS patients.

RNA Homeostasis

Disturbance of RNA homoeostasis has recently emerged as another central pathogenic denominator in the ALS/frontotemporal dementia (FTD) disease continuum, as mutations in several genes of RNA-binding/processing proteins, for example, *TARDBP* (TAR DNA-binding protein 43 gene) [5,6], *FUS* [14,15], *MATR3* (matrin 3) [16], or *HNRNPA1* [17], have been shown to result in ALS.

DNA Damage Response

Additionally, it is noteworthy that a few ALS-associated genes have been implicated in the DNA damage response in mammalian cells *in vitro*. Specifically, *FUS*, *NEK1* [61,89], *C21ORF2* [90], or *SPG11* [91] have been implicated. Long-term accumulation of genomic DNA mutations might indeed contribute to age-dependent induction of ALS/FTD disease.

Glossary

Amyotrophic lateral sclerosis (ALS): a fatal neurodegenerative disease affecting predominantly motor neurons in the motor cortex and spinal cord, leading to progressive muscle weakness, including respiratory muscles. Death ensues usually a few years after diagnosis.
Basal ganglia: group of subcortical nuclei in the brain of vertebrates connected to various other brain regions, for example, the cerebral cortex, thalamus, or brain stem. The basal ganglia are involved in the control of voluntary movement, procedural learning, emotion, and other functions.
Cortical neuron: neuron that is located in the cerebral cortex.
Creutzfeldt–Jakob disease: most frequent example of a transmissible spongiform encephalopathy, a group of rare, degenerative, and fatal brain disorders caused by prions.
Frontotemporal dementia (FTD): a neurodegenerative disease with a widespread affection of neurons in the frontal and temporal cerebral cortex, resulting in cognitive and language deficits or changes in personality and behavior.
Oligogenic: several mutations in different genes are additively or synergistically acting to cause disease in a specific patient.
P bodies: defined structures in eukaryotic cells that are involved in mRNA decay.
Paresis: weakness of voluntarily innervated muscles.
PolyQ ataxin-2: *ataxin-2* with a trinucleotide repeat expansion coding for a polyglutamine stretch that can cause ataxia or represents a risk factor for amyotrophic lateral sclerosis, depending on the length of the repeat.
premanifest phase: the time that precedes the onset of disease, that is, the manifestation of clinical symptoms.
Prion: an infectious agent that is composed of protein material. The term 'prion' is derived from prion and infection. A prion protein can fold in multiple distinct ways, and transmit its conformation to other proteins, leading to a self-propagating pathological folding.
Tau protein: a protein that binds to and regulates assembly of microtubules.

Table 1. Common Functional Pathways of ALS/FTD Genes[a]

Cell biological function(s)	Gene symbol	Protein	Mode of inheritance	Comment	Refs
RNA regulation	*TARDBP*	TDP-43	AD	Cytoplasmic neuronal inclusions of most sporadic and genetic ALS patients and a subset of FTD patients contain TDP-43; TDP-43, FUS, and HNRNPA1/HNRNPA2B1 are similarly structured and contain RNA-binding domains as well as disordered, aggregation-prone domains	[5,6]
	FUS	FUS (fused in sarcoma)	AD	FUS-mutant ALS patients and a subset of FTD patients without FUS mutations present FUS-positive inclusions; FUS mutations are rarely found in FTD; ALS patients without FUS mutations do not develop FUS-positive inclusions	[14,15]
	HNRNPA1/ HNRPA2B1	HNRNPA1/A2B1 (heterogeneous nuclear ribonucleoproteins A1 and A2B1, respectively)	AD	Mutations in the disordered, aggregation-prone domain of the homologous proteins HNRNPA1/HNRNPA2B1 are found in very rare cases of ALS, or overlapping syndromes of ALS, FTD, or myopathy (summarized as 'multisystem proteinopathy')	[17]
	MATR3	Matrin 3	AD	Mutations in MATR3 can be a rare cause of ALS or myopathy (both in an autosomal-dominant manner)	[16]
Autophagy, proteostasis, and vesicle dynamics	*TBK1*	TBK1 (TANK-binding kinase 1)	AD	Heterozygous loss-of-function mutations are associated with ALS and FTD, indicating haploinsufficiency as the major molecular genetic mechanism of toxicity	[11,61]
	OPTN	Optineurin	AD and AR	Optineurin and p62 are both substrates of TBK1 and autophagy adaptor proteins; linked to ALS and FTD	[63]
	SQSTM1	p62	AD		[64]
	C9ORF72	Protein C9orf72	AD	A hexanucleotide repeat expansion in a *C9ORF72* intron is the most frequent ALS/FTD mutation; accumulating evidence suggests that the physiological function of C9ORF72 is related to the initial phase of autophagosome formation	[8,9]
	UBQLN2	Ubiquilin-2 (UBQLN2)	X-linked dominant	Mutations cause X-linked ALS/FTD	[83]
	VCP	Valosin-containing protein	AD	Similar to HNRNPA2B1, heterozygous mutations in VCP can cause ALS, but also	[10]

Transactive response DNA-binding protein 43 kDa (TDP-43): DNA-43 a protein that is encoded by the *TARDBP* gene. It is a DNA- and RNA-binding protein originally identified as a transcriptional repressor binding to chromosomally integrated trans-activation response element DNA. The protein is involved in both coding and noncoding RNA synthesis and post-transcriptional processing and regulation of translation.

Ubiquitin-like systems: two ubiquitin-like conjugation systems control autophagosome biogenesis and autophagy flux. ATG12 and ATG8 genes share a super-fold with ubiquitin but are conjugated by different enzymes. In particular, ATG8 is conjugated to the phosphatidylinositol, a critical step in the biogenesis of autophagosomes.

Table 1. (continued)

Cell biological function(s)	Gene symbol	Protein	Mode of inheritance	Comment	Refs
				FTD, as well as inclusion body myopathy or Paget's disease of the bone (collectively also termed 'multisystem proteinopathy')	
	VAPB	Vesicle-associated membrane protein-associated protein B/C	AD	Involved in the endoplasmic reticulum unfolded protein response	[92]
	ALS2	Alsin	AR	ALS2/alsin is a guanine nucleotide exchange factor for the small GTPase Rab5 and involved in macropinocytosis-associated endosome fusion and trafficking; linked to juvenile ALS	[93,94]
	CHMP2B	Charged multivesicular body protein 2b	AD	CHMP2B has been linked to both FTD and ALS syndromes. CHMP2B is a component of the endosomal/lysosomal pathway, and probably required for the fusion process between autophagosomes and endosomal compartments or lysosomes	[65,66]
Cytoskeletal dynamics	*PFN1*	Profilin 1	AD	Regulates actin cytoskeleton dynamics	[85]
	DCTN1	Dynactin subunit 1	AD	Required for the cytoplasmic dynein-driven retrograde movement of vesicles and organelles along microtubules. Dynein–dynactin interaction is also required for axonal transport of vesicles and organelles	[84]
	NEFH	Neurofilament heavy polypeptide	AD	Involved in axonal transport	[86]
	MAPT	Tau protein	AD	Regulates microtubule assembly and stabilization; one of the three most frequent FTD genes, rarely causes ALS	[95–97]
	TUBA4A	Tubulin A 4 alpha	AD	Linked to ALS, identified by exome sequencing and association analysis	[88]
DNA damage repair	*FUS*	FUS	AD	ALS-associated FUS mutations lead to impaired DNA damage responses	[51]
	NEK1	Never in mitosis A-related kinase 1	AD, AR	Heterozygous loss-of-function mutations are associated with ALS; homozygous loss-of-function mutations are associated with short-rib thoracic dysplasia, a group of autosomal recessive	[61,89]

Table 1. (continued)

Cell biological function(s)	Gene symbol	Protein	Mode of inheritance	Comment	Refs
				ciliopathies characterized by a constricted thoracic cage, short ribs, and shortened tubular bones	
	C21ORF2	Protein C21orf2	Risk factor, AD?	Risk factor recently identified in a large ALS cohort genome-wide association study; NEK1 requires interaction with C21ORF2 for DNA damage repair	[90]
	SPG11	Spatacsin	AR	Mutations in SPG11 can cause juvenile ALS or hereditary spastic paraplegia (both in a recessive mode of inheritance)	[91]
Other established frequent ALS/FTD genes that cannot be grouped in the above topics	*SOD1*	Superoxide dismutase 1	AD	Antioxidant enzyme; second most frequent ALS gene, mutations cause almost exclusively ALS; most likely toxic gain-of-function principle; most SOD1 mutations lead to protein misfolding and tendency to aggregate; SOD1-mutant patients develop SOD1-positive cytoplasmic aggregates	[98]
	GRN	Granulin	AD, AR	Secreted protein, possible cytokine/growth factor-like activity; most likely loss-of-function mechanism; amongst the two most frequently mutated genes in FTD patients (besides *C9ORF72*)	[99,100]

[a]Abbreviations: ALS, amyotrophic lateral sclerosis; AD, autosomal-dominant; AR, autosomal-recessive; C9ORF72, chromosome 9 open reading frame 72; FTD, frontotemporal dementia; HNRNPA1, heterogeneous nuclear ribonucleoprotein A1; MATR3, matrin 3; NEK1, NIMA-related kinase 1; SOD1, superoxide dismutase 1; TARDBP, TAR DNA-binding protein 43 gene; TDP-43, transactive response DNA-binding protein 43 kDa.

Protein Aggregation and Prionlike Activity: A Possible Underlying Cause of ALS

Similar to other neurodegenerative diseases, for example, AD or Parkinson's disease, ALS and FTD patients display intracytoplasmic protein aggregates. FTD-associated protein aggregates are often positively stained for the **tau protein**. Nevertheless, in the case of ALS and the majority of FTD cases, the constituents of these deposits had remained elusive until 2006. Neumann *et al.* [5] subsequently showed that aggregates representing a neuropathological hallmark of most ALS patients were mainly composed of hyperphosphorylated TDP-43 protein. Similarly, many tau-negative FTD patients were found to develop TDP-43 pathology [5]. Moreover, it soon turned out that mutations in the disordered, **prion**like domain of TDP-43 (coded by the gene *TARDBP*) could cause genetic forms of ALS and FTD [6,7]. Thus, although only a small percentage of ALS/FTD patients carry *TARDBP* mutations, these genetic studies proved that TDP-43 was involved in disease causation and did not merely represent a neuropathological marker.

Additional similar proteins genetically linked to both ALS and FTD were soon discovered, for instance, those encoded by the genes *FUS* [14,15], *MATR3* [16], or *HNRNAPA1* and

Key Figure

The Main Pathways of ALS/FTD Genes Are Functionally Connected

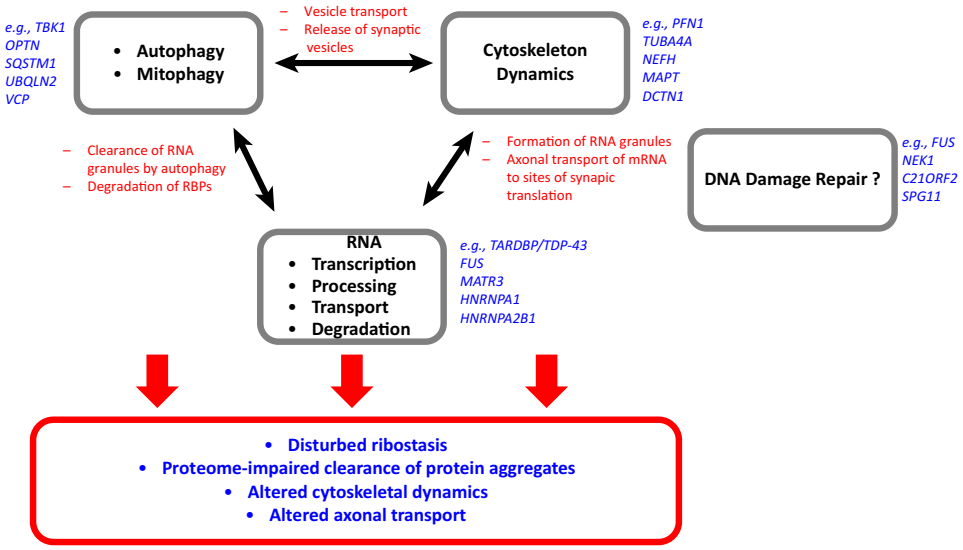

Figure 1. The identification of ALS/FTD genes has revealed a few common functional pathways that regulate autophagy, cytoskeleton, and RNA metabolism, depicted in this schematic diagram. In addition, ALS-related pathways are further connected by their role in cell biology. The relevance of an impaired DNA damage response for ALS/FTD, as suggested by mutations in *FUS*, *NEK1*, and other DNA damage repair genes, remains to be clarified. These pathways can lead to detrimental biological outcomes for a neuronal cell, as shown in the red box. Abbreviations: ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; NEK1, NIMA-related kinase 1; RBPs, RNA-binding proteins.

HNRNPA2B1 [17] (Table 1). A common property of these proteins is their role in the binding, biogenesis, and processing of RNA. From a structural point of view, most of these proteins contain RNA-binding domains in combination with unstructured, aggregation-prone protein domains. The latter protein domain is most likely responsible for the tendency of these proteins to form high-molecular, oligomeric species, and finally, aggregates within neurons [18]. Moreover, disease causing mutational hot spots have been observed within the aggregation-promoting domains of TDP-43 or fused in sarcoma protein (FUS) [6,7,17,19–22], providing genetic support for the importance of these disordered, 'prionlike' protein domains. With regard to FUS, an even more pronounced enrichment of mutations has been documented in the C-terminal nuclear localization sequence [15,23]. The eventual consequences are similar to those involving mutations within aggregation-prone domains of RNA-binding proteins (RBPs), in that a nucleocytoplasmic redistribution of FUS protein leads to a concentration-dependent cytoplasmic aggregation of mislocalized FUS in patients [24].

While mutations in several different RBPs can cause ALS or FTD, it remains unclear why almost all ALS patients, including sporadic cases or patients harboring a mutation in a gene other than *TARDBP*, display TDP-43-positive aggregates. Rare exceptions include SOD1 [25] or FUS [26] positive cytoplasmic deposits in *SOD1*- or *FUS*-mutant ALS patients, respectively. Surprisingly, rarely do FTD patients display FUS aggregates, though this occurs in the absence of FUS mutations and is caused by so far unknown factors [27]. Thus, a specific mutation in a disease-related gene is not the only factor that determines ALS/FTD neuropathology. A better

understanding of the relationship between genetics, disease phenotype, and neuropathology may help to understand why some patients develop ALS or FTD. In this respect, it is interesting that the FUS protein is hypomethylated in FTD patients with FUS-inclusion pathology, while in postmortem material from ALS patients carrying FUS mutations, respective FUS aggregates show FUS protein methylation close to the transportin binding site [28,29]. The resulting changes in transportin binding to FUS are linked to an altered nuclear import/export balance and cytoplasmic aggregation of FUS in both disease conditions, but by distinct pathomechanisms [28]. This finding suggests that post-translational protein modifications may possibly steer the disease toward either an ALS or FTD phenotype.

Beyond their role as neuropathological markers and possible toxicity factors contributing to disease initiation, the possible prionlike properties of TDP-43, FUS, and SOD1 aggregates – or respective oligomeric precursors – are being intensively studied. Their potential role in disease propagation is also an important focus. The term 'prionlike' refers to the hypothesis that these protein aggregates (or their lower molecular weight and soluble oligomers) can be transmitted to neighboring cells and seed aggregation of proteins endogenously produced in the target cell. This principle is reminiscent of prion domains in yeast proteins and the templating by pathological protein conformations of PrPsc, the pathological agent in **Creutzfeldt–Jakob disease** [30]. This concept has important clinical implications in that it may also help explain the continuous propagation of symptoms from a focal site at disease onset [31]. For example, weakness often starts in the upper limb of an ALS patient, and spreads to adjacent sites, for example, the contralateral limb or the ipsilateral lower limb, when disease progresses [31]. Postmortem examination of ALS patient brain tissue is in agreement with a comparable spreading of disease at the neuropathological level as well [32]. Most recent studies provide experimental support for the concept that TDP-43 [33,34] or SOD1 [35] oligomers are released by neurons and transmitted to a neighboring neuron where they further induce oligomer/aggregate production and toxicity. However, despite accumulating data supporting an intercellular transmission of ALS-associated, misfolded proteins, alternative mechanisms for intercellular ALS disease spreading have to be discussed. Hypothetically, these could include, for example, exosomal transmission of RNA or extending inflammatory processes.

Defects in RNA Processing and Liquid-Phase Separation of RBPs

Taking together the genetic, neuropathological, and experimental data mentioned above, abundant evidence connects altered solubility and function of RBPs to the initiation and propagation of ALS and FTD. Disturbances of mRNA transcription, splicing, transport [36,37] as well as impaired expression of noncoding RNAs [38,39] have been repeatedly described, and are most likely a consequence of the disturbed function of RBPs [40]. However, the exact mechanisms and definition of subcellular compartments responsible for the observed disruption of RNA regulation in neurodegeneration have remained largely elusive until very recently, when the pathological role of RBPs could be linked to their physiological function as regulatory components of intracytoplasmic RNA granules, for example, stress granules [41,42].

Why are defects in these RBPs so intimately linked to the onset of disease? Most of the RBPs associated with ALS/FTD are contained within stress granules [41]. Such granules are similar to **P bodies**, and are cellular structures composed of RBPs and RNA [43]. These RNA/protein granules have central functions in post-transcriptional RNA control, regulating the translation and stability of RNAs. For example, stress granules rapidly form from a variety of cellular stress conditions, such as heat shock or oxidative stress, temporarily assembling nontranslating RNAs and directing cellular resources toward essential survival functions [41].

As recently shown, RNA granules can be regarded as nonmembranous compartments or organelles consisting of liquidlike protein phases in the cytoplasm [44–47] (Box 3). It is known

Box 3. Liquidlike Phase Transition Regulates RNA Granule Formation

An important step forward in understanding why RNA-binding proteins aggregate in these types of neurodegenerative diseases has come from findings showing that stress granules containing proteins implicated in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) belong to a class of liquidlike compartments formed in the nucleus and cytoplasm of neurons [48,49]. These compartments usually consist of proteins and RNA, and are formed by phase separation from cytoplasm. Indeed, stress granules containing the ALS- and FTD-associated proteins FUS (fused in sarcoma protein) or HNRNPA1 (heterogeneous nuclear ribonucleoprotein A1) also form in liquidlike compartments, as supported by *in vitro* experiments [48,49]. Initially, both FUS and HNRNPA1 were found to form liquidlike drops *in vitro* with similar biophysical properties to FUS and HNRNPA1 drops formed in mammalian cells [48,49]. When these drops were incubated in a test tube, they underwent aberrant phase transitions from a liquid state to an irreversibly aggregated, or 'diseaselike' state *in vitro*.

that the assembly properties and phase dynamics of these RNA granules depend in part on prionlike, self-assembling interaction domains typical of several RBPs, including the ALS- and FTD-associated proteins FUS or heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) [48,49]. As a stochastic event or under stress conditions, the inherent physiological aggregation propensity of these proteins may lead to the formation of self-propagating amyloid fibrils. Although not experimentally proven yet, it is thus plausible that aberrant-phase transitions of RNA granule compartments such as P bodies and stress granules may be critically connected to ALS/FTD pathogenesis. It is likely that they represent 'compartments' of disturbed RNA homoeostasis as well as 'bioreactors', where oligomeric seeds or preaggregates of RBPs form. The depletion of RBPs by stable, cytoplasmic aggregates may further disturb RNA granule dynamics, and thereby RNA homoeostasis. Thus, RNA granules may represent subcellular compartments where RBPs exert their detrimental effects on RNA metabolism that could eventually cause ALS or FTD.

Premanifest Phase of ALS/FTD – Age-Dependent Accumulation of Damage or Gradual Decrease in Compensating Mechanisms?

Both ALS and FTD are characterized by an onset of clinical symptoms later in life, mostly in the sixth or seventh decade. Many ALS/FTD patients, even carriers of an inherited ALS germ-line mutation, are completely healthy until the first symptoms of their fatal disease are recognized. This raises the obvious question of what precipitates the disease after decades of health? Furthermore, which age-related factors might be responsible for the relatively sudden onset followed by a fatal outcome within comparably short disease duration? Part of the answer to this question may be that healthy individuals who might develop ALS later in life do in fact already present distinct, yet subclinical pathomolecular changes [38].

The still ongoing discovery of highly penetrant, autosomal-dominant ALS genes will provide ALS researchers with the opportunity to study first-degree relatives of genetic ALS carriers who are still healthy. One of the first projects studying ALS mutation carriers showed that ALS-associated changes in microRNA profiles were already present many years prior to disease onset [38]. These results demonstrated that a 'fingerprint' of altered RNA homoeostasis could present for an extended duration preceding clinical disease onset. However, neuronal degeneration may require additional, age-related precipitating factors or 'second hits'. Genetic deletion of telomerase and subsequent telomere shortening had been shown to lead to an earlier age of onset in the $SOD1^{G93A}$-transgenic mouse model of ALS, principally linking aging to the onset of motor neuron disease [50]. Another intriguing hypothesis posits that the time-dependent accumulation, for example, of somatic mutations in genomic or mitochondrial DNA damage finally synergize with a pre-existing dysbalance in RNA metabolism [51]. In support of this hypothesis, the ALS-linked genes *FUS*, *NEK1*, and *C21ORF2* have been implicated in DNA damage repair following the induction of DNA double-strand breaks via laser microirradiation or ionizing radiation in human cells [51–53]. Furthermore, ALS-associated mutations in *FUS* have been shown to impair DNA repair in cell lines [51,52] (Table 1).

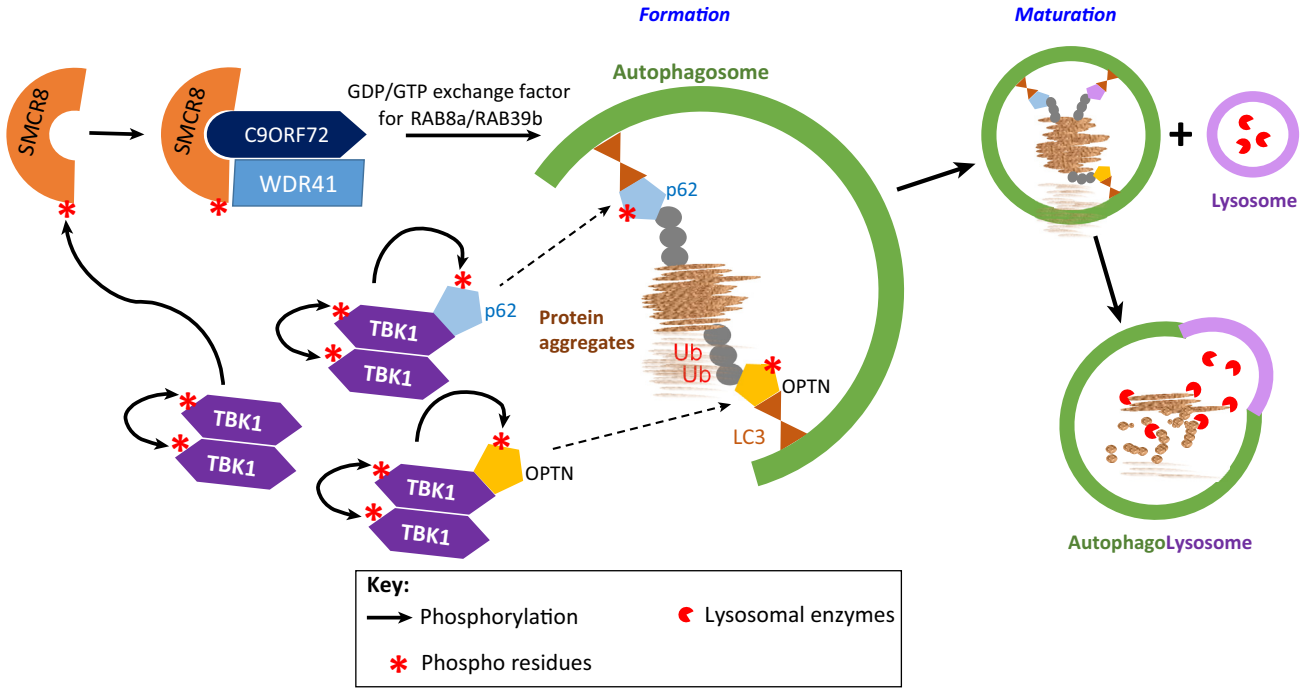

Figure 2. Several ALS/FTD Genes Are Involved in Selective Autophagy. At least four different ALS/FTD genes act in the autophagy degradation pathway that takes part in the clearance of aggregated proteins, but also unnecessary or damaged organelles (mitophagy), stress granules (granulophagy), or invading pathogens (xenophagy). TBK1 is a central upstream component of this pathway, which is activated by autophosphorylation. It phosphorylates the gene products of two further ALS/FTD genes (p62/*SQSTM1* and optineurin/*OPTN*), and regulates the formation of a protein complex between another ALS gene product, C9ORF72, with the proteins SMCR8 and WDR41. The C9ORF72/SMCR8/WDR41 complex acts as a GDP/GTP exchange factor for RAB proteins involved in vesicle trafficking and autophagosome membrane dynamics. Abbreviations: ALS, amyotrophic lateral sclerosis; C9ORF72, chromosome 9 open reading frame 72; FTD, frontotemporal dementia; OPTN, optineurin; SMCR8, Smith–Magenis syndrome chromosome region, candidate 8; TBK1, TANK-binding kinase-1; Ub, ubiquitin.

Another piece of evidence on how a predisposition for ALS could become apparent at an advanced age is the gradual, time-dependent decrease in compensating mechanisms. In this context, another common process of several ALS/FTD-associated genes is their involvement in the regulation of autophagy or, more generally, protein quality control (Table 1; Figures 1 and 2). Mechanistically, autophagy denotes a cellular process that involves the expansion of an initiating membrane, the phagophore, which engulfs cytoplasmic material, including proteins, lipids, and organelles, and then closes in, forming a double-membrane vesicle, the autophagosome [54]. The mature autophagosome fuses with lysosomes to give rise to the autolysosomes in which cargoes are degraded; the resulting macromolecules are then released to be reused by the cell (Figure 2). This complex process is governed by a set of conserved proteins that form the autophagy core machinery, involving two **ubiquitin (Ub)-like systems**: The first one conjugates the Ub-like protein ATG12 to ATG5. The resulting ATG12–ATG5 conjugate catalyzes the covalent attachment of the second system, the Ub-like protein ATG8, to autophagic membranes, an essential process enabling phagophores to grow [54].

On the one hand, autophagy is an unspecific bulk degradation pathway that functions as a starvation-induced recycling system to overcome periods of nutrient and/or energy restriction. Correspondingly, autophagy initiation is kept in check by both nutrient- and energy-sensing mechanisms, that is, mechanistic target of rapamycin and 5′-AMP-activated protein kinase [54]. Yet on the other hand, selective autophagy pathways also exist that can specifically target aggregated proteins (aggrephagy), unnecessary or damaged organelles, such as mitochondria

(mitophagy), stress granules (granulophagy), and invading pathogens (xenophagy), thereby acting as an important cytoprotective mechanism (reviewed in [55]). In this context, various stress signals contribute to autophagy induction, yet in many cases the precise molecular mechanisms have not yet been fully elucidated. Key factors of selective autophagy include receptor proteins [such as optineurin (encoded by *OPTN*), p62 (*SQSTM1*), NDP52, and NBR1] that recognize the cargo and hook it to the autophagosomal membrane protein ATG8/LC3 [55]. To this end, autophagy receptors are equipped with an LC3-interacting region and a domain/motif that specifically binds to the cargo. In many cases, this latter domain is an Ub-binding domain and Ub serves as a well-established 'eat-me' signal that is attached to autophagic targets [56]. Recent evidence indicates that upon cargo recognition, autophagy receptors oligomerize and can in fact induce autophagy locally by recruiting the ULK1 complex. Both Ub and LC3 binding can be modulated by TANK-binding kinase-1 (TBK1), which has emerged as a central regulator of diverse selective autophagy pathways [57–60].

Indeed, haploinsufficiency of *TBK1* has been recently described as a cause for both ALS and FTD [11,61,62]. Moreover, the genes coding for optineurin (*OPTN*) [63] and p62 (*SQSTM1*) [64] (both substrates of TBK1) have also been shown to cause ALS/FTD in patients when mutated. Additional ALS/FTD genes, for instance, *VCP* [10] and *CHMP2B* [65,66], have been implicated in vesicle trafficking and autophagy. Intact autophagic activity may thus control the level of aggregation-prone proteins [67], which aggregate and exert their toxicity in a concentration-dependent manner as evidenced by their overexpression, *in vitro* and *in vivo*. Similarly, mitophagy protects neuronal cells from defective mitochondria that represent a major oxidative threat to neuronal function [58–60,68]. However, autophagy decreases with age, which could result in a dysbalance of protein and organelle homoeostasis later in life [69]. Such conceptual or hypothesis-generating insights into ALS have recently been driven by the converging progress in both genetics and cell biology/biochemistry, which is especially evident in the case of autophagy. The identification of *TBK1*, *OPTN*, *C9ORF72*, or *SQSTM1* as ALS disease genes was highly relevant in itself in the context of clinical genetic diagnosis and counseling. However, only the knowledge of selective autophagy mechanisms and the mechanistic role of these genes in the same pathway has, at present, led to novel concepts. Yet, although a network of functionally and genetically linked genes is indicative of disturbed selective autophagy as an important biochemical and cell biological contributor to ALS/FTD pathogenesis, the knowledge regarding causative downstream sequelae of TBK1, optineurin, or p62 dysfunction is still very scarce.

Common Denominators of ALS and Their Functional Links

As mentioned in the previous section, based on genetic and neuropathological discoveries, ALS pathogenesis cannot be reduced to only a few functional processes, namely, protein quality control, disturbance of RNA regulation, altered cytoskeletal dynamics, and possibly DNA damage repair. However, most recent evidence points toward a further convergence of these pathways in the causation of ALS: for example, cytoplasmic stress granules have been shown to be cleared by autophagy [70], possibly via a change in the abundance of specific RBPs, which leads to an altered RBP composition and phase transition of RNA granules. Altered autophagy may therefore be critically connected to impaired RNP granule properties and function. This could potentially lead to a unifying model with one main functional pathway of ALS/FTD pathogenesis (Figure 1).

Another, more specific example of how altered RNA homoeostasis, autophagy, and protein aggregation can be genetically linked by a single disease gene is the most frequently mutated gene in ALS/FTD patients: *C9ORF72* [8,9]. This disease gene remained elusive until 2011, when it was discovered that an unexpected hexanucleotide repeat expansion of up to several thousand GGCCCC repeats in the first intron of *C9ORF72* was responsible for more than 70% of familial ALS/FTD cases in some countries [71]. Surprisingly, unconventional repeat-associated non-ATG

translation of this intronic sequence was found to result in dipeptide repeat proteins, which form cytoplasmic aggregates (in addition to TDP-43-positive aggregates also found in *C9ORF72*-mutant patients) [72,73]. These blocked nucleocytoplasmic shuttling of proteins in human and *Drosophila* cells [74,75]. However, the hexanucleotide repeat expansion in the *C9ORF72* gene was also reported to lead to reduced expression of the gene [71]. Thus, toxicity due to the *C9ORF72* mutation could at least partly lead to a loss-of-function phenotype. Indeed, accumulating evidence suggests that the physiological function of the C9ORF72 protein is important in the initial phase of autophagosome formation. Two recent reports have shown that C9ORF72 forms a stable complex with Smith–Magenis syndrome chromosome region, candidate 8 (SMCR8) and WDR41 [76,77] (Figure 2). The C9ORF72/SMCR8/WDR41 complex promotes autophagy by functioning as a GDP/GTP exchange factor for RAB8a and RAB39b, which are involved in the early steps of autophagosome biogenesis [78]. TBK1, a central autophagy modulator that can also cause ALS/FTD when affected by a loss-of-function mutation, regulates this function by phosphorylation of SMCR8 in neurons [76]. Depletion of C9ORF72 in neurons *in vitro* has been reported to impair autophagic activity and result in increased formation of cytoplasmic aggregates [76]. Intriguingly, this consequence of C9ORF72 depletion appeared to synergize with **polyQ ataxin-2** toxicity [76], suggesting a double-hit pathological mechanism in ALS/FTD. Overall, *C9ORF72, TBK1, SQSTM1*, and *OPTN* represent four different ALS/FTD genes that are currently directly connected to an autophagy-regulating network in these conditions.

New Developments in Molecular Diagnosis and Therapy for ALS

To date, a satisfying disease-modifying medical treatment for ALS or FTD is not available. The only neuroprotective drug that has a proven effect on the disease course of ALS is riluzole, a compound inhibiting glutamate release and thereby antagonizing excitotoxicity in neurons [79]. One drawback is that the compound slows down disease progression only by a few months in patients with an average life expectancy of 1 year [79]. Recent genetic and molecular insights into the pathophysiology of ALS and FTD are likely to result in innovative therapeutic approaches. For instance, the direct inhibition of mutant *SOD1* expression in humans by intrathecal delivery of second-generation antisense oligonucleotides with increased biological half-lives and binding affinity to *SOD1* mRNA has been tested, resulting in an effective reduction of SOD1 protein [80]. This could become the first successful example of a genotype-specific therapy in neurodegenerative diseases. Moreover, this type of approach aims to directly reduce the expression of a toxic protein product and to delay disease progression. After successful evaluation in mutant *SOD1*-transgenic rats [81] and in the absence of severe safety issues in a first human pilot study [80], an international multicenter Phase I study is currently being undertaken in several US and European centers (ClinicalTrials.gov identifier NCT02623699)[i].

The observation that incomplete penetrance was shown for several ALS genes may also be of interest for the identification of therapeutic targets. For instance, *C9ORF72* mutation carriers can be detected in healthy control cohorts [82]. Similarly, not all carriers of a pathogenic loss-of-function mutation in *TBK1* develop ALS or FTD, even at an advanced age. Moreover, a surprisingly high number of *TBK1* loss-of-function mutation carriers are found in large control cohorts, for example, as illustrated in the exome sequencing data available from the ExAC data set server (http://exac.broadinstitute.org/). Therefore, it can be postulated that in addition to genetic variants predisposing to illness, protective factors must also exist to prevent disease onset, even in the presence of a known pathogenic dominant mutation. Identification of such protective factors will be a challenge for future studies, but could result in therapeutically highly relevant protective, rather than disease-promoting molecular players. Moreover, novel insights into the mechanisms of ALS will also lead to new paradigms that can be used to screen putative therapeutic compounds. ALS-relevant target parameters, for example, might include the activity of (selective) autophagy, or the modulation in phase transition of RNA granules.

Importantly, the only established ALS mouse model – based on the overexpression of mutated human SOD1 protein – has had a poor success rate with regard to predicting compounds that may become successful in clinical ALS trials. While dozens of experimental therapies have extended the life of *SOD1*-transgenic mice, none has so far been beneficial in patients when prospectively tested. Several reasons could account for this discrepancy, including the fact that *SOD1* mutations are rare in a general cohort of ALS patients. Consequently, there is an urgent need for innovative animal models in preclinical *in vivo* evaluations of novel therapeutic candidate strategies in ALS. For example, several new ALS/FTD genetic mouse models, *TDP-43* or *TBK1* knockout mice, are currently being analyzed, as they represent recently discovered ALS genes playing a role in RNA or autophagy pathways [6,7,11]. Hopefully, they might bear a broader relevance and improved predictive value in identifying better treatment approaches for ALS and FTD patients.

Concluding Remarks

Results from ALS and FTD human genetic research and related cell biological and biochemical pathways have synergistically resulted in major steps forward in the understanding of these diseases. The results suggest that most ALS/FTD disease genes can be grouped into a few major common pathways critically involved in disease pathogenesis, specifically, RNA dysmetabolism, autophagy, cytoskeleton dynamics, and possibly DNA damage repair. Furthermore, increasing evidence additionally points to functional links between these pathways, and might potentially reveal an ALS 'mega-pathway', predominantly connected to RNA metabolism and autophagy/protein homoeostasis. However, this is currently a theoretical consideration, and an alternate hypothesis is that disruptions in multiple distinct pathways could cause overlapping symptoms in patients. Moreover, although overarching processes have clearly emerged for these conditions, detailed decryption of cell biological defects and biochemical processes still represents major challenges in the field for the next years to come. Nevertheless, with progress, these may constitute the basis in identifying putative therapeutic targets (Box 1 and Outstanding Questions).

Characterization of the presymptomatic phase in ALS/FTD mutation carriers as well as the identification of protective factors that lead to reduced penetrance of dominantly inherited ALS/FTD mutations may provide another opportunity to obtain valuable insights into the earliest steps in ALS/FTD pathogenesis, and consequently, targeting any potential treatment candidates. Moreover, while current experimental ALS paradigms are hardly predictive for the success of new therapies in clinical trials, the most recent discoveries have provided a path to developing innovative models based on pathogenic principles common to most ALS/FTD patients. These may presumably bear higher relevance for sporadic ALS cases as well. Intrathecal antisense-oligonucleotide treatment to knockdown the expression of mutant SOD1 protein in ALS patients represents the first concrete genotype-specific ALS treatment approach that is currently being evaluated in clinical trials. It may prove that translation from human genetics to clinical therapy is feasible in ALS/FTD research.

Outstanding Questions

Which factors determine the neuropathological phenotype of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD)? Is the neuropathology directly linked to mechanisms dictating whether a patient presents ALS or FTD?

What triggers the clinical onset of age-related neurodegenerative disease after decades of health? Is there an age-dependent accumulation of damage or gradual decrease in compensating mechanisms?

What is the common denominator(s) connecting the recently identified functional ALS/FTD gene pathways?

What are the specific biological downstream defects and biochemical processes mediating neurodegeneration in neurons, and stemming from recently identified ALS/FTD disease genes? Which selective autophagy cargoes are relevant for ALS/FTD, and what roles do altered RNA granule dynamics play in disease causation?

Does impaired DNA damage repair play a significant role in ALS/FTD pathogenesis?

Which factors determine cell-type specificity and the development of an ALS versus an FTD phenotype?

What are the protective factors preventing disease outbreak in carriers of dominant mutations?

Can the recent identification of novel ALS/FTD genes lead to new animal models and improved predictive values for preclinical testing of therapeutic compounds?

Acknowledgments

This work was supported in whole or in parts by the Charcot Foundation for ALS Research to J.H.W., by grants from the DFG (DI 931/3-1; SFB1177), the Cluster of Excellence 'Macromolecular Complexes' of the Goethe University Frankfurt (EXC115), LOEWE grant Ub-Net, and LOEWE Centrum for Gene and Cell Therapy Frankfurt. We thank Karin M. Danzer and Albert C. Ludolph for critically reading the manuscript. We thank Simon Alberti for helpful discussions.

Resources

[i] www.clinicaltrials.gov

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