REVIEW

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C9orf72 amyotrophic lateral sclerosis and frontotemporal dementia: gain or loss of function?

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Purpose of review

The molecular mechanisms that underlie chromosome 9 open reading frame 72 (*C9orf72*)-associated amyotrophic lateral sclerosis and frontotemporal dementia are rapidly emerging. Two potential disease mechanisms have been postulated – gain or loss of function. We provide an overview of recent advances that support or oppose gain-of-function and loss-of-function mechanisms.

Recent findings

Since the discovery that a noncoding repeat expansion in *C9orf72* was responsible for chromosome 9-linked amyotrophic lateral sclerosis and frontotemporal dementia in 2011, a plethora of studies have investigated clinical, pathological and mechanistic aspects of the disease. Loss of function is supported by reduced levels of *C9orf72* in patient brain and functional work, revealing a role of the C9orf72 protein in endocytic and autophagic pathways and motor function. Gain of function is supported by the presence in patient brain of both repeat RNA and protein aggregates. Repeat RNA aggregates termed RNA foci, a hallmark of noncoding repeat expansion diseases, have been shown to sequester proteins involved in RNA splicing, editing, nuclear export and nucleolar function. Repeat-associated non-ATG dependent translation gives rise to toxic dipeptide repeat proteins that form inclusions in patient tissue. Antisense oligonucleotides targeting *C9orf72* have shown promise for combating gain-of-function toxicity.

Summary

Rapid progress is being made towards understanding this common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. Overall, the weight of data currently sits in favour of gain of function as the most important disease mechanism, which has important implications for the development of effective and targeted therapies.

Keywords

amyotrophic lateral sclerosis, *C9orf72*, frontotemporal dementia, gain or loss of function

INTRODUCTION

A noncoding repeat expansion in *C9orf72* is a common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTD) (C9FTD/ALS) [1,2]. Disease may occur through loss of function of *C9orf72*, or two distinct gain-of-function mechanisms: first, the formation of repeat RNA aggregates, termed RNA foci, in neuronal nuclei that sequester important RNA-binding proteins and second, the generation of toxic, dipeptide repeat (DPR) proteins, due to the repeat RNA mediating its own translation (Fig. 1) [3]. In this review, we will cover recent findings concerning the role of each of these mechanisms in C9FTD/ALS and their relevance for developing therapeutics. Key findings relating to loss or gain of function are summarized in Table 1 [4,5▪–7▪,8▪▪,9▪–11▪,12–14,15▪,16▪,17▪▪,18, 19▪–26▪,27▪▪,28▪▪,29▪–31▪].

GAIN AND LOSS OF FUNCTION IN NONCODING REPEAT EXPANSION DISORDERS

Noncoding repeat expansions have been commonly assigned into two groups: those which cause loss of function of the protein in which the mutation resides (fragile X syndrome and Friedreich's ataxia),

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KEY POINTS

- Evidence exists supporting a role for both gain-of-function and loss-of-function mechanisms in C9FTD/ALS.
- The weight of evidence currently suggests gain of function is the primary disease mechanism.
- Loss of function is more likely to modulate the disease phenotype than be a primary cause of disease.
- Therapeutics that target gain-of-function mechanisms, such as ASOs, are a promising avenue for therapy.

and those in which a gain-of-function mechanism has been identified because of repeat RNA species [myotonic dystrophy, fragile X-associated tremor/ataxia syndrome (FXTAS), Huntington's disease-like 2 and spinocerebellar ataxia types 8, 10, 12 and 31]. In the fragile X mental retardation protein (*FMRP*) gene, CGG repeat size determines the clinical syndrome; expansions of 55–200 repeats result in the gain-of-function disease FXTAS [32,33], whereas expansions of more than 200 repeats lead to hypermethylation of the *FMRP* gene, which silences transcription, leading to a loss of FMRP function and fragile X syndrome [34]. There is no evidence for such a bimodal mechanism in C9FTD/ALS, indeed, a large study found no effect of repeat size on clinical presentation [35▪]. The first mechanism attributed to the gain-of-function noncoding repeat expansion diseases was toxic functions of the repeat RNA [36]. Recently, a novel mechanism was identified whereby expanded CAG repeats are translated in the absence of an ATG initiation codon, termed repeat-associated non-ATG dependent (RAN) translation [37]. RAN translation has now been found to be common to several noncoding repeat expansions, including C9FTD/ALS [38], increasing the spectrum of potential mechanisms in disease.

LOSS OF FUNCTION OF C9ORF72 PROTEIN

The main support for a loss-of-function mechanism in C9FTD/ALS is numerous reports of decreased transcript levels of all three *C9orf72* mRNA variants

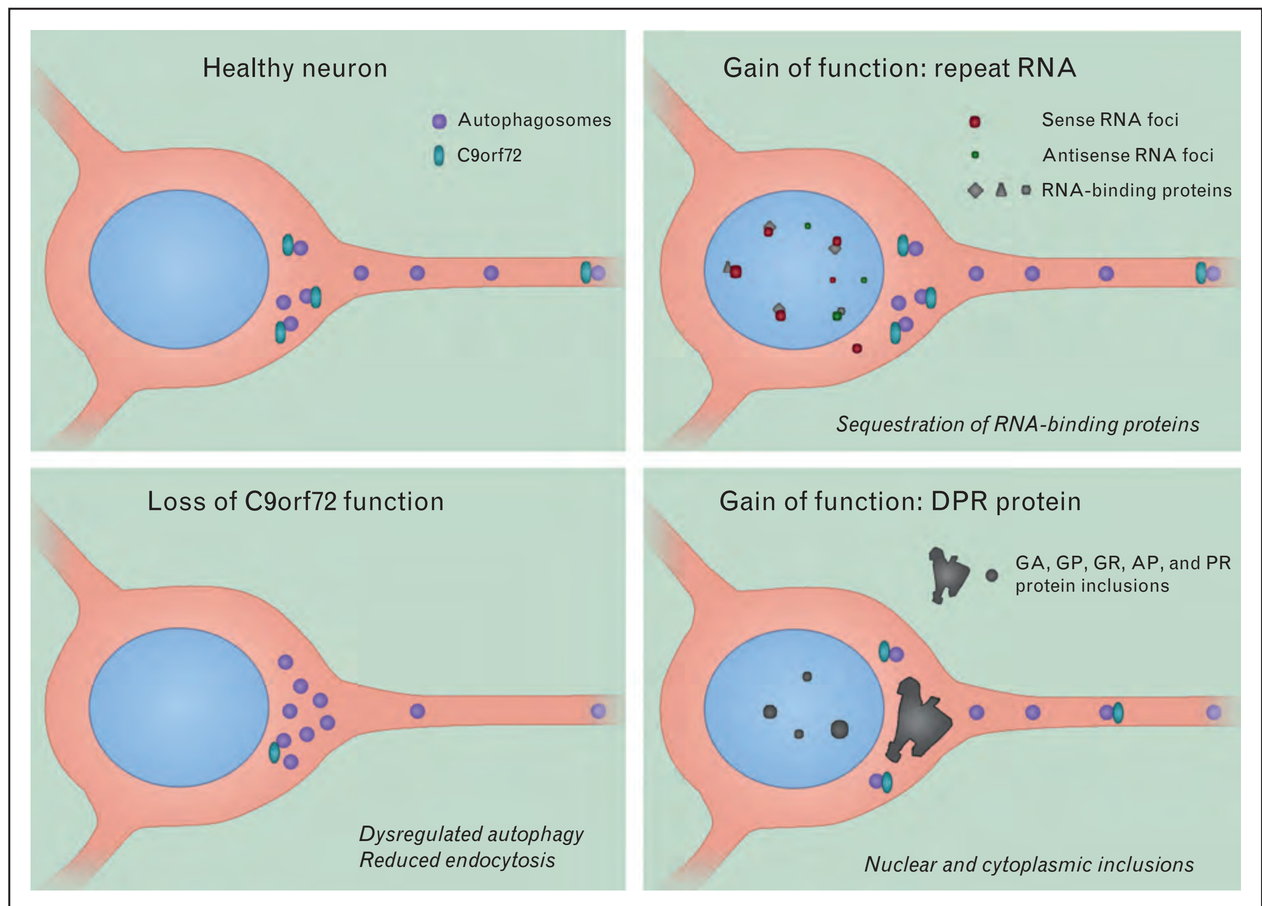

FIGURE 1. Potential mechanisms of disease in C9FTD/ALS. AP, alanine-proline; DPR, dipeptide repeat; GA, glycine-alanine; GP, glycine-proline; GR, glycine-arginine; PR, proline-arginine.

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Table 1. Summary of the key evidence for gain-and loss-of-function mechanisms in C9FTD/ALS

	For (+) or against (−) hypothesis	Study
Loss-of-function		
Reduced mRNA and protein expression		
All mRNA isoforms reduced in C9FTD/ALS patient tissue and iPSC neurons	+	[1,4,5▪–7▪,8▪▪]
Reduced C9orf72 protein in C9FTD/ALS patient brain	+	[9▪]
Hypermethylation of *C9orf72* promoter reduces transcript levels and correlates with shorter disease duration	+	[10▪]
Hypermethylation of *C9orf72* promoter reduces transcript levels, increasing resistance to cellular stress and reducing RNA foci and DPR proteins	–	[11▪]
Models of loss of protein		
Dysregulation of cellular trafficking associated with reduction of C9orf72 protein	+	[12–14]
C9orf72 orthologue knockdown in zebrafish has a motor phenotype	+	[15▪]
C9orf72 orthologue knockout in *Caenorhabditis elegans* has a motor phenotype	+	[16▪]
C9orf72 orthologue knockdown in mice has no motor phenotype	–	[17▪▪]
Genetics		
Lack of coding mutations in C9orf72 protein in C9ALS	–	[18]
Homozygous C9FTD case not as severe clinically and pathologically as pure loss-of-function diseases	–	[5▪]
Gain-of-function: RNA		
RNA foci		
Sense and antisense foci identified in C9FTD/ALS patient tissue and iPSC neurons	+	[1,6▪,8▪▪,17▪▪,19▪–22▪]
Sense foci burden correlates with age-at-onset in C9FTD	+	[19▪]
Sequestration of RNA-binding proteins		
ADARB2 – siRNA reduces sense RNA foci in C9ALS iPSC neurons and exacerbates glutamate-induced toxicity in control iPSC neurons	+	[8▪▪]
hnRNP A1 and pur-alpha – colocalization with sense RNA foci in iPSC motor neurons	+	[22▪]
hnRNP A3 – identified in neuronal cytoplasmic and intranuclear inclusions	+	[23▪]
hnRNP H – colocalization with sense RNA foci in patient brain	+	[24▪]
Nucleolin – colocalization with sense RNA foci in patient brain and cells, indications of nucleolar stress	+	[25▪]
Pur-alpha – overexpression rescues GGGGCC-dependent degeneration in *Drosophila*	+	[26▪]
Antisense oligonucleotide (ASO) treatment		
ASOs reduce sense RNA foci and reverse transcriptome changes and toxicity in patient fibroblasts and iPSC neurons	+	[8▪▪,17▪▪,22▪]
Gain-of-function: DPR proteins		
Inclusion pathology		
All DPR proteins found in neuronal cytoplasmic inclusions in C9FTD/ALS patient brain and iPSC neurons	+	[6▪,20▪,21▪,27▪▪,28▪▪,29▪]
Poly-GA DPR protein inclusions found prior to TDP-43 inclusions in patient brain	+	[30▪]
Toxicity		
GA DPR protein inclusion distribution does not correlate with neurodegeneration in C9FTD/ALS patient brain	–	[31▪]
Poly-GP and poly-PR DPR proteins made from GGGGCC repeats are toxic to HEK293 cells (other DPR proteins not assessed)	+	[21▪]

ALS, amyotrophic lateral sclerosis; DPR, dipeptide repeat; FTD, frontotemporal dementia; GA, glycine-alanine; GP, glycine-proline; hnRNP, heterogeneous nuclear ribonucleoprotein; iPSC, induced pluripotent stem cell; PR, proline-arginine.

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in patient-derived cells and tissue [1,4,5▪–7▪,8▪▪]. Reduced levels of *C9orf72* transcripts may be due to hypermethylation of the *C9orf72* promoter or increased histone methylation [7▪,10▪,11▪]. Unexpectedly, lower levels of *C9orf72* transcripts are also identified in ALS/FTD cases without *C9orf72* repeat expansion [15▪], suggesting that loss of *C9orf72* could be part of a common pathway affected in these diseases. Current studies on C9orf72 protein are limited by a lack of specific antibodies. However, one study developed a new C9orf72 antibody which detects a protein of 48 kDa in human cell lines that is specifically reduced following treatment with siRNA targeting *C9orf72* [9▪]. This antibody was used to show reduced C9orf72 protein in frontal cortex, but not cerebellum, of C9FTD/ALS patient brain compared to ALS cases without *C9orf72* repeat expansion [9▪], consistent with findings of reduced transcript levels.

When the mutation was discovered in 2011, C9orf72 protein was of unknown function. However, recent studies show that C9orf72 has a high homology to differentially expressed in normal and neoplasia proteins [13,14]. This family of proteins function as guanine nucleotide exchange factors (GEFs) that activate Rab guanosine 5′-triphosphate (GTP)ase and therefore regulate membrane trafficking [13,14]. Consistent with this, knockdown of *C9orf72* leads to reduced endocytosis and dysregulated autophagy in human neuroblastoma cells [12]. Impaired autophagy and endolysosomal degradation are implicated in neurodegenerative diseases [39], thus these data provide a basis for loss of function playing a role in C9FTD/ALS. However, better antibodies are urgently needed to definitively determine the cellular distribution and function of C9orf72 protein; analysis of innate GEF activity will also provide important insight into C9orf72 function.

Knockdown of the zebrafish orthologue of *C9orf72* (*zC9orf72*) with antisense morpholino oligonucleotides leads to axonopathy and motor deficits, which can be rescued by expression of human *C9orf72* [15▪]. Homozygous knockout of the worm orthologue of *C9orf72* (alfa-1) also results in motor phenotypes [16▪]. Together, these data suggest that the loss of C9orf72 protein can lead to motor abnormalities, which argues for a role of loss of function in C9FTD/ALS. Conversely, intracerebroventricular delivery to adult mice of antisense oligonucleotides (ASOs) targeting *C9orf72* leads to knockdown of *C9orf72* throughout the central nervous system but does not result in any motor or behavioural phenotypes [17▪▪]. It is possible that knockdown of *C9orf72* during development has differing effects to knockdown in adults which could explain the discrepancy between the models. Also arguing against a loss-of-function mechanism, no mutations have been found in coding regions of the *C9orf72* gene [18]. Additionally, a rare homozygous *C9orf72* repeat expansion case did not exhibit clinical or pathological features outside the usual disease spectrum as would be expected from reports of homozygous cases in pure loss-of-function diseases [5▪].

Reduced *C9orf72* transcription via cytosine-phosphate-guanine (CpG) hypermethylation correlated with shorter disease duration suggesting *C9orf72* reduction may play a role in disease [10▪]. A second study showed *C9orf72* promoter CpG hypermethylation reduced *C9orf72* mRNA levels, but also decreased RNA foci and RAN protein formation in C9FTD/ALS lymphoblasts and brains. Furthermore, treatment with a demethylating agent increased the vulnerability of C9FTD/ALS lymphoblasts to external stressors, suggesting reduction of *C9orf72* levels may be a protective mechanism [11▪].

On the basis of evidence described above, haploinsufficiency of *C9orf72* may cause defects in endosomal and autophagic processes and motor function. Therefore, the degree of reduction of *C9orf72* may well modulate the disease phenotype. However, clinical data suggest that loss of function is unlikely to be the predominant causative mechanism for neurodegeneration in C9FTD/ALS.

GAIN OF FUNCTION: REPEAT RNA

RNA gain of function is a common mechanism in noncoding repeat expansion diseases [40]. The proposed mode of action in these diseases is via sequestration of essential RNA-binding proteins into aggregates of repeat-containing RNA foci, in the nucleus of affected cells. The most clearly defined molecular mechanism occurs in myotonic dystrophy (DM), a neuromuscular disease caused by CTG or CCTG repeat expansions in the dystrophia myotonica protein kinase (*DMPK*) or zinc finger protein 9 (*ZNF9*) genes (DM type 1 or 2, respectively) [36]. In DM type 1 or 2, RNA foci containing CUG repeat RNA sequester the splicing factor muscleblind-like protein 1, whose loss results in the mis-splicing of a muscle-specific chloride channel that is directly responsible for the myotonia observed in patients [41]. On the basis of this very clear example of RNA gain of function, great effort has focussed on investigating this mechanism in C9FTD/ALS. RNA foci composed of sense and antisense repeat RNA are present in frontal cortex, hippocampus, cerebellum and spinal cord of C9FTD/ALS patients [1,6▪,8▪▪,17▪▪,19▪–22▪]. In support of a role in disease, increased burden of RNA foci in neurons correlates with lower age-at-onset of disease in

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C9FTD cases, with the strongest correlation with sense foci in the frontal cortex, the region most affected in FTD [19▪]. RNA foci were present in several types of glial cells (astrocytes, microglia and oligodendrocytes), but are predominantly a neuronal phenotype [17▪▪,19▪,20▪], which is reflective of relative expression levels of *C9orf72* in these cell types in mouse [42]. This raises the possibility that toxicity could arise from non-cell-autonomous routes. Indeed, astrocytes derived from familial and sporadic ALS patients, including C9ALS cases, can exert toxicity to motor neurons [43].

Although RNA foci can be found in the cytoplasm, the vast majority are localized in the nucleus [17▪▪,19▪]. Several studies have employed biochemical techniques to identify binding partners of the expanded sense repeat *in vitro* using differing methods and sources of protein, reviewed in [44]. Sequestration of some of these proteins into sense RNA foci has been assessed in patient-derived cells and tissue (Table 2). Some inconsistency has been observed between studies, but investigations in larger cohorts will be needed to clarify whether this is due to variation between brain regions or patients or differences due to detection protocols. Splicing factors constituted a large proportion of identified RNA-binding proteins. Of these, the splicing factors heterogeneous nuclear ribonucleoprotein (hnRNP) A1, hnRNP H and serine/arginine-rich splicing factor 2 (SRSF2; also known as SC35) are found to be sequestered into sense RNA foci [22▪,24▪,45▪]. Loss of function of these proteins would be predicted to cause downstream changes in splicing in target mRNAs. All these splicing factors affect splicing of a wide variety of targets, so a major challenge will be to determine whether specific targets are responsible for neurodegeneration and to confirm specific alteration of these targets in C9FTD/ALS patients.

Proteins involved in nuclear mRNA export also bind to GGGGCC repeats and are sequestered into foci containing sense RNA transcripts [22▪,45▪]. Aly/REF export factor (ALYREF) functions as an adaptor for mature RNA, transferring it through the nuclear RNA export factor 1 pathway to the nuclear pore for export [46]. Pur-alpha has also been implicated in nuclear export and trafficking in dendritic RNA granules for the regulation of local translation [47,48]. Another RNA-binding protein, hnRNP A3, which binds GGGGCC repeat RNA *in vitro*, but does not colocalize with RNA foci [23▪], is also proposed to function in a similar manner [49]. These proteins may contribute to export of *C9orf72* repeat-containing RNA from the nucleus for C9orf72 and RAN protein translation or degradation. Sequestration of these factors into RNA foci may also prevent export of their other target mRNAs and affect downstream cellular functions.

Other proteins found to interact with GGGGCC repeats were adenosine deaminase, RNA-specific, B2 (ADARB2) [8▪▪] and nucleolin [25▪]. ADARB2 colocalizes with sense RNA foci in C9ALS induced pluripotent stem cell (iPSC)-differentiated motor neurons and patient motor cortex, leading to significant nuclear accumulation of the protein compared with controls. Unexpectedly, knockdown of ADARB2 results in reduced RNA foci in iPSC motor neurons, suggesting a role in foci formation or stabilization. ADARB2 (also known as ADAR3 or RED2) is a member of the ADAR (adenosine deaminase, RNA specific) RNA editing family, but unlike the other members, lacks editing activity [50]. Decrease in another member of this family, ADAR2,

Table 2. Reported sequestration of RNA-binding proteins into sense RNA foci in patient cells and tissue

RNA-binding protein	C9ALS or FTD	Patient-derived cells	Patient tissue	Study
ADARB2	ALS	iPSC-differentiated neurons	Motor cortex	[8▪▪]
ALYREF	ALS	ND	Cerebellum and spinal cord	[45▪]
hnRNP A1	ALS and ALS/FTD	iPSC-differentiated motor neurons		[22▪]
	ALS	ND	Cerebellum	[45▪]
hnRNP H[a]	ALS	ND	Cerebellum	[24▪]
	ALS	Cerebellum and cerebellum and spinal cord		[45▪]
Nucleolin	ALS	ND	Motor cortex	[25▪]
Pur-alpha	ALS and ALS/FTD	iPSC-differentiated motor neurons		[22▪]
SRSF2 (SC35)	ALS	ND	Cerebellum	[24▪]
	ALS	ND	Cerebellum and spinal cord	[45▪]

ADARB2, adenosine deaminase, RNA-specific, B2; ALS, amyotrophic lateral sclerosis; ALYREF, Aly/REF export factor; FTD, frontotemporal dementia; hnRNP, heterogeneous nuclear ribonucleoprotein; iPSC, induced pluripotent stem cell; ND, not determined; SRSF2 (SC35), serine/arginine-rich splicing factor 2 (also known as SC35).
[a]Sequestration of hnRNP H into RNA foci was not observed in C9FTD iPSC-neurons [6▪].

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is thought to underlie excitotoxic loss of motor neurons in ALS via reduced editing of AMPA receptors subunits [51].

GGGGCC RNA and DNA repeats exhibit stable G-quadruplex formation *in vitro* [25▪,52▪,53]. These structures are involved in telomere stability, RNA splicing, transport and degradation and regulation of translation [54–56]. A recent study investigated binding of proteins to GGGGCC RNA hairpins, GGGGCC G-quadruplexes and antisense GGCCCC hairpins [25▪]. The nucleolar protein nucleolin was shown to have specificity for sense G-quadruplex structures, and dispersal of nucleolin staining in the nucleus was observed in C9ALS patient-derived cells and tissue, with sequestration into sense RNA foci visible in the motor cortex. Consequences of impaired nucleolar function were also observed, including decreased RNA processing and an increase in the number of P bodies, which are ribonucleoprotein complexes involved in the degradation of untranslated RNAs. These changes could be recapitulated when GGGGCC repeats were expressed in a cell line, implicating nucleolar stress as a gain-of-function RNA mechanism in C9FTD/ALS.

It is intriguing to speculate whether the heterogeneity seen in C9FTD/ALS could be attributed to differential sequestration of RNA-binding proteins between brain regions and patients, because of protein abundance or availability or RNA foci burden. No protein sequestration into antisense RNA foci has yet been identified, but this could be important as ASOs targeting sense RNA did not reverse all transcriptome changes in C9FTD/ALS-derived fibroblasts [17▪▪]. This suggests antisense transcripts may also cause transcriptional changes, potentially through sequestration of RNA-binding proteins. Repeat RNA may also exert neurotoxic effects through processes other than RNA foci generation. In a fly model of myotonic dystrophy and a cell model of Huntington's disease, expression of expanded repeats led to double stranded RNAs that were processed by the dicer complex into short siRNAs that exert toxicity by silencing complementary CAG or CTG repeat-containing transcripts, respectively [57,58]. It will be interesting to determine whether this pathway contributes to C9FTD/ALS. In a similar manner, antisense transcripts may regulate the levels of sense transcripts, and vice versa, via antisense-mediated RNA degradation. This may explain why sense and antisense RNA foci are rarely found within the same cell [19▪,21▪]. In Huntington's disease, antisense transcripts reduce huntingtin protein expression, partially via dicer [59]. This mechanism could also contribute to the reduced *C9orf72* transcript levels observed in C9FTD/ALS patients.

In summary, the greatest evidence in support for a role of RNA gain of function in C9FTD/ALS are the abundant sense and antisense RNA foci in patient tissue that correlate with clinical phenotypes and can sequester RNA-binding proteins. However, a clear mechanism definitively linking sequestration of specific RNA-binding proteins to disease pathogenesis (as is the case for myotonic dystrophy) is currently lacking.

GAIN OF FUNCTION: DIPEPTIDE REPEAT PROTEINS

The other novel and potentially toxic species in C9FTD/ALS are the DPR proteins formed by RAN translation of the expanded repeat [20▪,21▪,27▪▪,28▪▪,29▪]. DPR proteins are translated from all frames of the GGGGCC repeat resulting in polymers of glycine-alanine (GA), glycine-proline (GP) and glycine-arginine (GR) in the sense frames, and glycine-proline (GP), alanine-proline (AP) and proline-arginine (PR) in the antisense frames. Although poly-GP is translated from both sense and antisense RNA, translation has been found to continue after the repeat expansion (using antibodies against downstream regions), and thus these poly-GP proteins have different carboxy terminal tails that may affect their function [21▪]. All DPR proteins form widespread neuronal cytoplasmic inclusions in patient brain [20▪,21▪,27▪▪,28▪▪,29▪] that frequently colocalize with p62-positive [but not TAR DNA-binding protein 43 (TDP-43)-positive] inclusions [28▪▪,29▪,31▪,60]. Poly-GP, and poly-GA DPR proteins additionally display dot-like neuronal intranuclear inclusions [27▪▪,28▪▪,31▪]. Unlike RNA foci, DPR inclusions appear to be an exclusively neuronal phenotype [27▪▪,31▪,60], possibly reflective of the clearance ability of mitotic cells.

A detailed pathological analysis of C9FTD/ALS cases found that TDP-43, but not poly-GA pathology, correlates with neurodegeneration [31▪], suggesting a lack of pathogenicity of poly-GA inclusions. However, this does not rule out toxicity of soluble GA polymers or other DPR proteins. Interestingly, rare *C9orf72* cases have been reported with DPR but not TDP-43 pathology [28▪▪,30▪]. This suggests DPR proteins can be toxic without invoking TDP-43 dysfunction.

DISSECTION OF GAIN-OF-FUNCTION MECHANISMS

We have reviewed evidence for gain-of-function and loss-of-function mechanisms in C9FTD/ALS. However, it still remains to be determined which species and pathways are responsible for neurodegeneration

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and clinical phenotypes in disease. Co-occurrence of sense and antisense RNA foci with DPR, p62 or TDP-43 protein inclusions is only as frequent as expected by chance [8■■,19■–21■,45■], suggesting a lack of interdependence between species and distinct toxic mechanisms. Overexpression of expanded GGGGCC repeats (outside the context of the *C9orf72* gene) can induce RNA foci formation [20■,24■] and DPR protein production [20■,21■,28■■]. Repeats can also exert toxicity in cell lines [21■,24■,25■], flies [26■] and zebrafish [24■], suggesting that gain-of-function mechanisms are sufficient for neurodegeneration. Studies have yet to clearly attribute observed toxicity to repeat RNA or DPR protein species, but one study showed that increasing expression of *C9orf72* repeats specifically in the GP (sense) and PR (antisense) frames can exacerbate toxicity in human HEK293T cells [21■], showing that these DPR proteins can affect cell viability. Impaired degradation through the autophagic system is consistent with the accumulation of p62 and ubiquitin pathology that is abundant in C9FTD/ALS cases [61], and the sensitivity of C9FTD iPSCs specifically to autophagic stressors [6■]. However, it is not clear whether these effects are due to loss of the normal cellular function of C9orf72 or gain of function due to the accumulation of protein aggregates.

THERPAEUTIC TARGETTING OF GAIN-OF-FUNCTION MECHANISMS

There is considerable excitement about the possibility of ASOs for the treatment of C9FTD/ALS. One reason for this is that ASOs should ameliorate both repeat RNA and DPR protein toxicity, and therefore do not need to wait for a better understanding of the contribution of each of these mechanisms to disease pathogenesis. ASOs will not alleviate loss of C9orf72 function, but as discussed above, the weight of evidence currently suggests gain of function is likely to be the primary mechanism to address therapeutically. ASOs targeting sense transcripts reduce sense RNA foci and ameliorate transcriptome changes and toxicity in C9FTD/ALS-derived cells [8■■,17■■,22■]. ASOs targeting antisense transcripts may also be required as it was proposed that antisense RNA-mediated mechanisms were responsible for the proportion of transcriptional changes that remain dysregulated after treatment of patient cells with ASOs targeting sense repeats [17■■]. In addition, antisense DPR proteins have been shown to be toxic to cells [21■]. Given the precedent of a clinical trial for ASOs targeting superoxide dismutase 1 (SOD1) for ALS patients with *SOD1* mutations [62], ASO treatment is currently the most promising prospect for treating C9FTD/ALS. The development of small molecules that specifically bind the secondary structure formed by the GGGGCC repeats is another promising area for therapeutic intervention [52■,63■]. Such small molecules would also be predicted to prevent both repeat RNA and DPR protein mechanisms, with the potential advantage of simpler delivery.

CONCLUSION

In the 3 years since the discovery of the *C9orf72* mutation in ALS and FTD, intense investigations have begun to unfold the mechanisms at play in these diseases. Haploinsufficiency of *C9orf72* may cause defects in endosomal and autophagic processes that lead to dysfunction of the motor system, but clinical data do not support causation of disease. Cellular and animal models inform us that gain-of-function mechanisms from expanded *C9orf72* repeats are sufficient to cause neurodegeneration. As has been found in other diseases classically thought of as either loss-of-function or gain-of-function diseases, it is likely that both mechanisms contribute to different aspects of the heterogeneous phenotypes in these diseases. As the same *C9orf72* mutation appears to result in a spectrum of disease phenotypes, differential regulation of toxic species or the degree of loss of function may play a role in differing disease presentations. Finally, current evidence suggests gain-of-function-based therapies such as ASOs hold promise for C9FTD/ALS.

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Conflicts of interest

There are no conflicts of interest.

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■ of special interest
■■ of outstanding interest

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The study provides proof of principle for RNA G-quadruplex binding small molecules as a therapeutic strategy in C9FTD/ALS.

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