Article:
Hautbergue, G.M. orcid.org/0000-0002-1621-261X, Cleary, J.D., Guo, S. et al. (1 more author) (2021) Therapeutic strategies for C9orf72 amyotrophic lateral sclerosis and frontotemporal dementia. Current Opinion in Neurology, 34 (5). pp. 748-755. ISSN 1350-7540

https://doi.org/10.1097/WCO.0000000000000984

REVIEW

OPEN

Therapeutic strategies for *C9orf72* amyotrophic lateral sclerosis and frontotemporal dementia

Guillaume M. Hautbergue[a,b,c,*], *John D. Cleary*[d,e,f,*], *Shu Guo*[d,e,f], *and Laura P.W. Ranum*[d,e,f,g,h]

Purpose of review
An intronic G_4C_2 expansion mutation in *C9orf72* is the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia (C9-ALS/FTD). Although there are currently no treatments for this insidious, fatal disease, intense research has led to promising therapeutic strategies, which will be discussed here.

Recent findings
Therapeutic strategies for C9-ALS/FTD have primarily focused on reducing the toxic effects of mutant expansion RNAs or the dipeptide repeat proteins (DPRs). The pathogenic effects of G_4C_2 expansion transcripts have been targeted using approaches aimed at promoting their degradation, inhibiting nuclear export or silencing transcription. Other promising strategies include immunotherapy to reduce the DPRs themselves, reducing RAN translation, removing the repeats using DNA or RNA editing and manipulation of downstream disease-altered stress granule pathways. Finally, understanding the molecular triggers that lead to pheno-conversion may lead to opportunities that can delay symptomatic disease onset.

Summary
A large body of evidence implicates RAN-translated DPRs as a main driver of C9-ALS/FTD. Promising therapeutic strategies for these devastating diseases are being rapidly developed with several approaches already in or approaching clinical trials.

Keywords
C9ORF72-ALS/FTD, gene therapy, small molecule inhibitors, symptomatic management, therapeutic strategies

INTRODUCTION

A GGGGCC hexanucleotide repeat expansion in the first intron of *C9ORF72* causes the most common forms of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1,2], genetically linking these two clinically distinct adult-onset neurodegenerative disorders. *C9ORF72* ALS, FTD or both ALS and FTD can occur in individual patients and within families [3]. C9-ALS/FTD patients typically have hundreds to thousands of G_4C_2•G_2C_4 repeats, while shorter tracts of 2–24 repeats are present in unaffected people [1,2]. ALS is characterized by motor neuron loss, muscle atrophy, progressive paralysis and usually death within 2–5 years of onset [4]. In FTD, degeneration of neurons in the frontal and anterior temporal lobes can result in personality changes such as apathy, loss of empathy, disinhibition and executive function deficits [4]. Therapeutic options are limited and there are no current treatment options that substantially change the course of *C9ORF72* ALS or FTD. The standard of care includes the antiglutamatergic drug riluzole for ALS [5] and the antidepressant fluoxetine or a related compound for FTD [6]. In 2017, the free-radical scavenger drug edaravone was approved for use in ALS patients [7]. Unfortunately, none of these

[a]Sheffield Institute for Translational Neuroscience (SITraN), Department of Neuroscience, University of Sheffield, [b]Neuroscience Institute, [c]Healthy Lifespan Institute (HELSI), University of Sheffield, Western Bank, Sheffield, UK, [d]Center for NeuroGenetics, [e]Department of Molecular Genetics and Microbiology, College of Medicine, [f]Genetics Institute, [g]McKnight Brain Institute and [h]Norman Fixel Institute for Neurological Diseases, University of Florida, Gainesville, Florida, USA

Correspondence to Laura P.W. Ranum, PhD. E-mail: ranum@ufl.edu

*Guillaume M. Hautbergue and John D. Cleary contributed equally to this article.

Curr Opin Neurol 2021, 33:000–000

DOI:10.1097/WCO.0000000000000984

KEY POINTS

- Hexanucleotide repeat expansions in the *C9ORF72* gene are the most common known genetic cause of ALS and FTD, a spectrum of debilitating and incurable neurodegenerative diseases.
- Repeat associated non-AUG (RAN) translation, which leads to the production of dipeptide repeat proteins, is a major driver of neuronal injury and disease.
- A number of therapeutic strategies aimed at reducing the impact of expansion transcripts, RAN proteins, nuclear transport deficits or stress granule biology have shown efficacy in preclinical models of disease.
- Approaches aimed at modifying environmental factors and lifestyle are promising complementary avenues for improving the primary care of patients.
- Intense research efforts have resulted in several clinical trials and others are expected to start soon.

treatments improve motor or cognitive deficits; however, riluzole and edaravone have been shown to modestly slow disease progression in some ALS patients.

Similar to other microsatellite expansion disorders [8,9], the *C9ORF72* G_4C_2•G_2C_4 mutation is bidirectionally transcribed and sense G_4C_2 and antisense G_2C_4 expansion transcripts form RNA foci and produce repeat associated non-AUG (RAN) proteins [10–15]. Proposed disease mechanisms include toxic effects of the expansion RNAs [1,16]; toxic effects of sense (poly-GA, GR, GP) and antisense (poly-PR, PA, GP) dipeptide RAN proteins [10,12–14] and haploinsufficiency of the *C9ORF72* protein [17,18]. Overall, RAN proteins, particularly GA, GR and PR, have been shown to be toxic in a number of cell culture and animal models (for reviews [19,20]).

The discovery of the *C9ORF72* expansion as the most common genetic cause of ALS and FTD [1,2] has fuelled an interdisciplinary worldwide research effort to understand the mechanisms and develop therapies for this disorder. *C9ORF72* expansions cause nearly 40% of familial and 6–8% of sporadic ALS cases and nearly 18% of familial and 6% of sporadic FTD cases [21,22▪] in European populations but are relatively rare in Asia [23]. The relatively large numbers of C9-ALS/FTD patients worldwide, combined with multiple emerging therapeutic strategies have positioned C9-ALS/FTD well for breakthrough therapy development. Emerging therapeutic strategies include targeting and removing the expanded repeats; degrading, or preventing expression of expansion transcripts; reducing toxic RAN proteins; and modulating downstream affected pathways including nucleocytoplasmic transport and stress granules (Fig. 1). These strategies and additional efforts to understand key molecular and physiological changes that trigger disease may provide insights that will lead to better disease management and help stratify the inclusion of the most informative patients for clinical trials.

TARGETING EXPANSION TRANSCRIPTS FOR DEGRADATION

Sense *C9ORF72*-repeat transcripts

Almost immediately following the discovery of the *C9ORF72* expansion mutation, efforts to develop antisense oligonucleotide (ASO) drugs to knockdown the repeat expansion RNAs began. These nucleic acid based drugs are chemically modified, and in some applications take advantage of the nuclear RNase H1 pathway to degrade double-stranded sequences that form when ASOs bind to targeted gene transcripts [24▪▪]. For *C9ORF72*, specific ASOs were shown to selectively reduce sense G_4C_2 RNA foci in patient cells without reducing the levels of *C9ORF72* mRNA [11,16]. BAC transgenic mice treated with single-dose ASOs that selectively target sense expansion RNAs but not mRNAs encoding *C9ORF72* protein, decreased sense RNA foci and sense DPRs and improved behavioural abnormalities [25]. Together, these results paved the way for a phase I clinical trial to test the safety, tolerability and pharmacokinetics of the Ionis/Biogen BIIB078 ASO in adults with *C9ORF72* ALS (NCT03626012).

More recently, stereopure ASOs were shown to increase RNAse H activity *in vitro* and *in vivo* compared with stereorandom ASOs [26]. A lead candidate stereopure ASO showed selective degradation of sense G_4C_2 expansion containing transcripts without reducing the variant 2 isoform which lacks the repeat. Treatment of BAC transgenic mice with these ASOs reduced RNA foci and RAN GP proteins but not *C9ORF72* protein levels. In addition, these oligonucleotides selectively protected iPSC-derived motor neurons harbouring *C9ORF72*-expansion mutations from glutamate-induced toxicity [27▪▪]. In an alternative approach, miRNAs targeting sense *C9ORF72*-repeat transcripts using adeno-associated virus serotype 5 (AAV5) delivery, reduced levels of sense expansion transcripts and RNA foci in FTD iPSC-derived frontal brain-like neurons and BAC transgenic mice [28▪▪]. Interestingly, AAV-mediated miRNA-depletion of SOD1 was recently reported in two patients with SOD1-ALS [29▪▪], providing proof-of-concept data that intrathecally delivered microRNAs can be used as a potential treatment strategy for ALS.

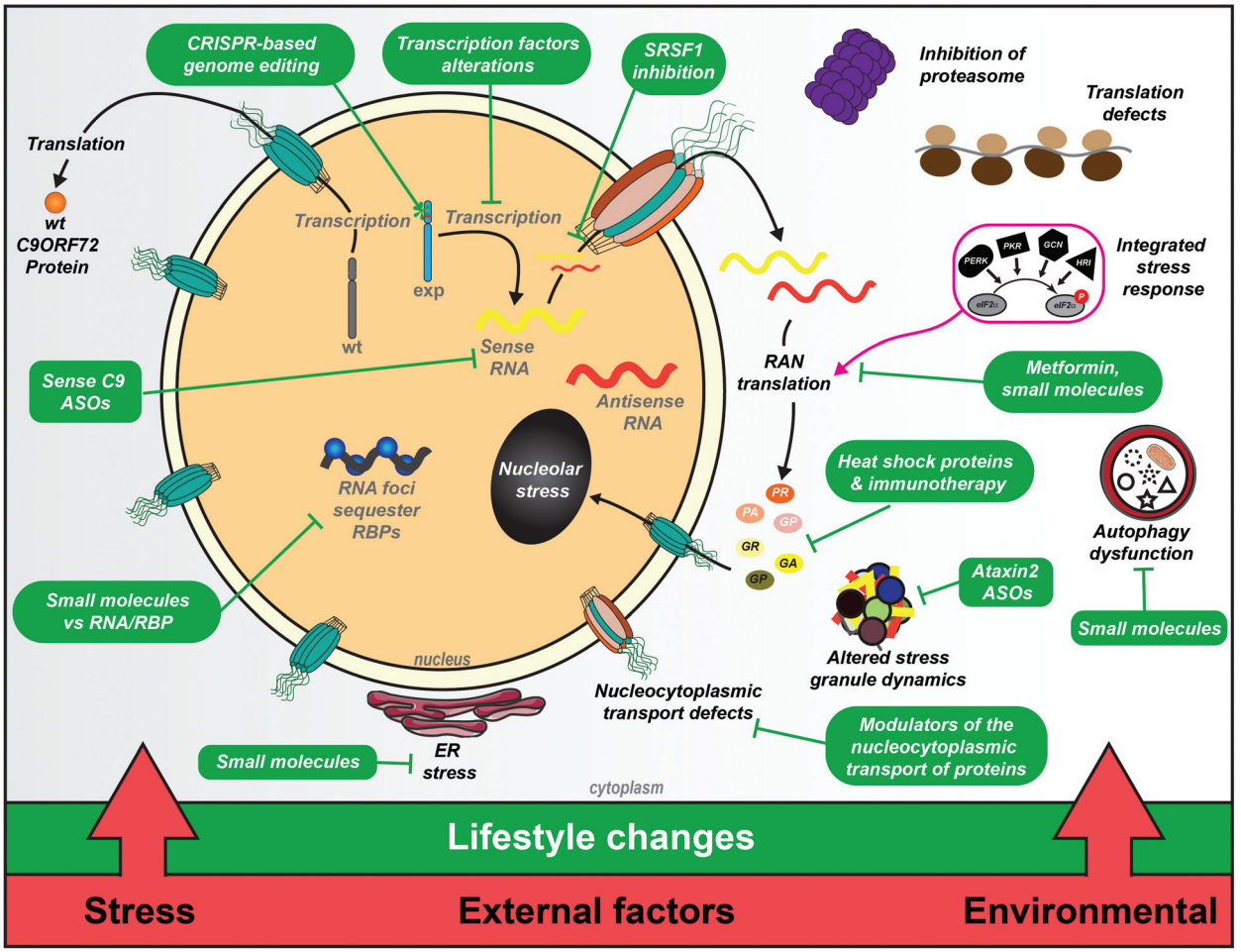

FIGURE 1. Cellular consequences and therapeutic approaches for *C9ORF72*-ALS/FTD. C9-ALS/FTD affects a wide variety of downstream cellular pathways with different therapeutic approaches (green boxes) targeting pathogenic C9 RNAs and RAN DPRs as well as downstream pathways. External factors (bottom red bar) can also influence cellular events and combined with lifestyle changes (bottom green bar) may affect disease onset and progression. Some content modified from Servier Medical Art (smart.servier.com) under creative commons license.

Inhibiting transcription of C9-expansion transcripts

Several different strategies are being actively pursued to decrease the transcription of C9-repeat expansion transcripts. For example, SUPT4H, SUPT5H and RNA polymerase II associated factor 1 complex (PAF1C) are transcription factors that play important roles in the elongation of RNAs containing expanded repeats [30,31]. Decreased expression of SPT4 encoded by *SUPT4H* was shown to decrease the levels of sense and antisense *C9ORF72* expansion transcripts and GP-RAN proteins in *Caenorhabditis elegans, Drosophila* and iPSC-derived models of *C9ORF72*-ALS/FTD and to ameliorate neurodegenerative phenotypes in *Drosophila* [32]. Similarly, depletion of PAF1C reduced expression of G_4C_2 expansion RNAs and poly (GR) in a *Drosophila* model [31]. Although interesting, the therapeutic potential of these strategies may be limited by off-target effects [33▪].

In other approaches, CRISPR-Cas9 deletion of the promoter region driving expression of the *C9ORF72* repeat-containing transcript isoforms 1 and 3 led to the efficient reduction in expression levels of all three sense DPRs in C9-ALS patient-derived motor neurons [34▪]. Pinto *et al.* [35] showed targeting *C9ORF72* expansions at the DNA level using deactivated Cas9 (dCas9) efficiently inhibits transcription and reduced the levels of GP RAN protein in reporter cells. Similarly, use of RNA-targeting deactivated-Cas9 (RCas9) allows degradation of *C9ORF72* expansion transcripts in cell models [36], although it is possible that this strategy also blocks transcription by binding of the dCas9-PIN-gRNA to the DNA repeats as in dCas9 strategy.

Although there is great hope that decreasing the levels of G_4C_2 expansion transcripts will be sufficient to improve disease, several studies suggest that it will be important to increase efforts to also target antisense transcripts. For example, antisense RNA foci preferentially accumulate in regions of disease in a *C9ORF72* BAC transgenic mouse model suggesting antisense expansion RNAs or RAN proteins may be more toxic than their corresponding sense products [37]. Additionally, ASOs that target sense *C9ORF72* expansion transcripts did not correct widespread transcriptomic defects found in patient-derived cells [11], suggesting strategies targeting both transcripts may offer the best outcomes.

IMMUNOTHERAPY STRATEGIES TARGETING RAN PROTEINS

There is strong evidence that RAN DPRs, particularly GA, GR and PR, are one of the main drivers of disease (for reviews [19,20]) and hence decreasing their levels is an attractive therapeutic approach. Several immunotherapy approaches have focused on the GA RAN proteins. In patient fibroblasts and primary neurons, α-GA antibodies reduced GA aggregate formation and blocked aggregate seeding activity of cerebellar extracts from *C9ORF72* autopsy tissue [38]. Vaccination of GA-overexpression mice with ovalbumin-$(GA)_{10}$ peptides elicited the production of α-GA antibodies, lowered GA protein levels and prevented microglial activation and motor deficits [39▪▪]. Using BAC transgenic mice that express sense and antisense transcripts and multiple types of RAN proteins, Nguyen *et al.* [40▪▪] showed passive immunotherapy with α-GA antibodies improved behavioural deficits, increased survival and decreased neuroinflammation and motor neuron loss. These peripherally injected antibodies crossed the blood brain barrier and co-localized with GA protein aggregates. Glycosylation of the Fc antibody region was important for cell entry and GA proteins were reduced in a TRIM-21-, proteosome-, and autophagy-dependent manner [40▪▪]. In addition to reducing GA, the α-GA1 treatment surprisingly also reduced GP and GR proteins, likely through increased proteosome function. No changes in sense or antisense RNA levels or foci were observed in α-GA1 treated mice providing strong support that RAN proteins and not RNA gain of function effects drive C9-ALS/FTD [40▪▪].

DECREASING RAN PROTEIN LEVELS

Several groups have shown that activation of the integrated stress response (ISR) and increased p-eIF2α levels increase RAN translation [41–44,45▪▪]. Zu *et al.* [45▪▪] showed G_4C_2 and other repeat expansion RNAs activate ISR protein kinase R (PKR) and that PKR inhibition dramatically reduces RAN protein levels. Zu *et al.* [45▪▪] went on to show inhibition of PKR using AAV-delivered dominant negative PKR-K296R or the FDA-approved drug metformin, decreased p-PKR and RAN protein levels and improved behaviour and neuropathology in C9 BAC transgenic mice without changing sense or antisense transcript levels. There is an active clinical trial to test the safety of metformin in *C9ORF72* ALS patients and its effects on RAN protein levels (NCT04220021). Inhibition of the SRSF1-dependent nuclear export of both sense and antisense *C9ORF72*-repeat transcripts and subsequent RAN translation was also reported as a promising gene therapy approach in preclinical models including patient-derived motor neurons and *Drosophila* models of disease [46].

Other strategies to reduce RAN protein levels include stimulating their clearance. For example, heat shock protein family B member 8 (HSPB8) has been shown to promote autophagy-mediated removal of several misfolded C9 RAN proteins from motor neurons [47]. Although the therapeutic potential of this approach is uncertain, clearance of protein aggregates could have applications for a wide variety of neurodegenerative diseases. Taken together, these data support the therapeutic potential of targeting RAN translation and RAN proteins for C9-ALS/FTD as well as other RAN protein associated disorders.

TARGETING THE GENOMIC *C9ORF72* HEXANUCLEOTIDE-REPEATS

Correcting the GGGGCC•GGCCCC repeat expansion mutation should theoretically address all deleterious mutation effects, including effects from sense and antisense RNAs and DPRs. Current gene editing techniques have focused primarily on clustered regular interspaced short palindromic repeats (CRISPR)-associated (Cas) systems, although application to C9-ALS/FTD has so far been limited [48,49▪]. In iPSC-cells, CRISPR/Cas9 editing and homology-directed repair (HDR) replacement of the expansion with a wildtype repeat resulted in restoration of *C9ORF72* gene expression and methylation and reduced intron retention and downstream pathogenic phenotypes [49▪]. In iPSC-derived motor neurons, CRISPR/Cas9 correction abolished GluA1 AMPA receptor (AMPAR) mediated excitotoxicity [48]. Targeting regions outside the repeat or the entire *C9ORF72* gene have also been tested with varying degrees of success. For example, deletion of a portion of the upstream *C9ORF72*

promoter prevents the production of exon 1a expansion containing transcripts and the activation of neurodegenerative pathways [34▪]. Unfortunately, this approach does not prevent expression of antisense RNA and associated antisense DPRs, which likely contribute to disease. Although correcting the expansion mutation seems to be a straight-forward idea, adequate delivery to affected tissues/cell types and the accuracy of emerging CRISPR based approaches will be critical for effective therapy development.

STRESS GRANULES AND NUCLEOCYTOPLASMIC TRANSPORT

TDP43 plays important roles in transcriptional regulation, alternative splicing of pre-mRNAs, axonal transport of mRNAs, translational regulation and miRNA processing. TDP-43 also associates with stress granules [50], which constitute dynamic membrane-less organelles that promote cell survival by halting translation of nonessential mRNAs in response to cellular stress [51]. Stress granules are composed of RNA and RNA-binding proteins with low complexity domains (LCDs) that mediate liquid-liquid phase separation (LLPS). Mutations in the LCDs domains of TDP43, ataxin-2 and other RNA-binding proteins involved in ALS/FTD stimulate their self-assemblies leading to the formation of persistent cytoplasmic stress granules that leave aggregated proteins that may contribute to disease [52]. Interestingly, arginine-rich *C9ORF72* DPRs impair stress granule assembly dynamics by undergoing LLPS, further inducing the phase separation of stress granule proteins [53]. Overexpression of GFP-polyGR proteins impairs stress granules and protein translation in mice deficits [54]. TDP-43 proteinopathy, aggregation of stress granule proteins (G3BP1, ataxin-2), nucleocytoplasmic defects, neuronal loss and motor/cognitive deficits were observed in an AAV-driven overexpression mouse model of expanded G_4C_2 repeats [55]. DPRs also promote nucleocytoplasmic transport disruption by stimulating the recruitment of nucleocytoplasmic transport proteins to stress granules [56]. In fact, many nucleocytoplasmic transport factors are localized to stress granules when exposed to stressors or mutant proteins implicated in ALS pathogenesis, leading to impaired nucleocytoplasmic transport [56].

TARGETING NUCLEOCYTOPLASMIC TRANSPORT DEFICITS

In 2015, Zhang *et al.* [57] demonstrated increased nuclear export in *C9ORF72* ALS iPSN models showed abnormal cytoplasmic RanGTPase accumulation. RanGTPase is important in nucleocytoplasmic protein transport (reviewed in [58]). Abnormal expression and localization of nuclear pore proteins are found in *C9ORF72* autopsy tissue and patient-derived iPSNs [57,59]. Modulating the expression of nuclear pore proteins or transport-associated proteins affects G_4C_2 expansion transcripts and arginine containing RAN protein toxicity [57,60,61]. Overexpression of importin or inhibition of nuclear export with RNA inhibition of (Exportin 1) XPO1 or pharmacologically ablating XPO1 function using KPT-276 rescued *C9ORF72* toxicity in the *C9ORF72* fly model [57]. Another XPO1 inhibitor, KPT-350, designed by Karyopharm Therapeutics and acquired by Biogen (BIIB100), has been used in preclinical studies of many neurological diseases and demonstrated neuroprotective and anti-inflammatory roles [62,63,64▪,65].

TROPHIC SUPPORT SUPPLEMENTATION

Neurotrophic factors (NTFs), a family of biomolecules that support neuronal growth, survival and differentiation, have been explored for decades as therapeutic strategies for neurodegenerative diseases [66▪], including ALS. Various NTFs have been tested in preclinical rodent models of SOD1-ALS, including brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF). Small molecule agonist of the BDNF receptor [67], VEGF injections [68,69], lentiviral and AAV-mediated delivery of NTFs [70–73] and stem cell therapy of NTF secreting cells [74–82] have shown promise in SOD ALS models. Despite these promising results, including several ongoing clinical trials [66▪], there have been no direct studies looking at NTF therapeutics in C9-ALS/FTD. Although some NTF clinical trials in ALS likely include C9-ALS/FTD participants, it will be important, given the unique disease mechanisms of C9-ALS/FTD, to directly examine NTF specifically in the C9 context.

PREVENTION AND FUNCTIONAL MANAGEMENT

For C9-ALS/FTD, there is a relatively long period of apparent good health prior to disease onset, which most often occur in the fourth or fifth decade of life, and extending this period of good health has been gaining considerable attention. Both preclinical animal studies and human studies demonstrate that moderate exercise regimens improve functionality and ameliorate disease symptoms for ALS in general (reviewed in [83]). However, the role of exercise is complex. In a retrospective study, patient-reported

exercise history was inversely correlated with age-of-onset in *C9ORF72* but not other forms of ALS [84], although additional studies that examine the impact of specific types of exercise will be important. Targeted training may be key. In a randomized, sham-controlled clinical trial, Plowman *et al.* [85,86▪▪] showed that expiratory muscle-strength training is well tolerated in ALS patients and improved bulbar function in a longitudinal *C9ORF72* case-study and also in larger cohorts of genetically undefined ALS patients. Longitudinal studies suggest that lifestyle modifications (e.g. smoking cessation, maintaining a healthy BMI) at a younger age may lower the risk of developing ALS [87▪▪]. Recent studies have identified *C9ORF72* expansions in 1.6% ($n = 8/487$) of cases with possible idiopathic normal pressure hydrocephalus (iNPH) but not in control cases ($n = 0/432$) aged more than 65 years. Clinically significant shunt response was detected in six out of seven shunted *C9ORF72* expansion carriers. Additional studies are needed to understand the frequency of NPH in *C9ORF72* expansion carriers and the potential utility of shunts to drain excess cerebrospinal fluid in these patients. Patient lifestyle, co-morbid diseases and environmental factors (Fig. 1) can influence cellular events, making it important to study and understand external factors that may influence disease. Dietary studies specific to C9-ALS/FTD are rare, although a larger study focused on ALS in general has demonstrated that increasing fruit and vegetable associated fibre, antioxidants and carotenes was associated with improved function [88]. The results of many of these broader ALS studies are complicated by the complex genetics and phenotypic presentation of ALS, increasing the call for preventive and lifestyle studies that focus on *C9ORF72* or other single ALS mutations.

STRATIFICATION AND EFFICIENCY IN CLINICAL TRIALS

Although there are multiple therapeutic approaches for C9-ALS/FTD in the preclinical pipeline, there are only two C9-ALS/FTD specific clinical trials registered in ClinicalTrials.gov: an ASO-based clinical trial of BIIB078 (Biogen), which targets *C9ORF72* expansion containing transcripts for ASO-induced RNase H-mediated degradation (NCT03626012); and a clinical trial (NCT04220021), to test the safety and tolerability of metformin in *C9ORF72* ALS patients and the drug's ability to decrease RAN protein levels. Several additional trials are open to but not specific to C9-ALS/FTD patients. These include a phase 1 clinical trial of BIIB100 to reduce excessive nuclear export (NCT03945279) in C9 and other ALS patients, will examine the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of the drug. A phase 2 clinical trial examining the safety, tolerability, PK and PD of AL001, a recombinant human antihuman sortilin (SORT1) monoclonal IgG1 antibody in FTD patients with either granulin or *C9ORF72* mutations (NCT03987295). Sortilin is a type I membrane glycoprotein involved in proganulin trafficking that is expressed in the central nervous system [89]. Frontotemporal degeneration can be caused by mutation in the progranulin (*GRN*) gene or the *C9ORF72* hexanucleotide expansion repeat and there are rare patients with mutations in both [90]. More recently, Wave Life Sciences has been reported to seek regulatory approval for WVE-004, an investigational stereopure ASO targeting the expansion transcript of C9-ALS/FTD [27▪▪]. It is interesting to note the current batch of clinical trials, which focus on different pathogenic pathways, could potentially be used together.

CONCLUSION

Despite the mechanistic and clinical complexity of *C9ORF72* ALS/FTD, intense research efforts over the 10 years since the expansion mutation was identified have led to a remarkable number of novel therapeutic approaches in preclinical and clinical trial stages. The breadth and diversity of these approaches provide hope for C9-ALS/FTD patients, who currently have limited therapeutic options focused on supportive care. The pace of research focused on the root causes of this disease has been remarkable and is likely to accelerate and uncover additional new therapeutic targets and treatment strategies that will significantly impact C9-ALS/FTD and the larger family of repeat expansion disorders.

Acknowledgements

L.P.W.R. and G.M.H. express their gratitude to ALS patients and their families for participating in research and for donating biological samples to the Center for NeuroGenetics at the University of Florida or the NIHR Sheffield Biomedical Research Centre: Translational Neuroscience for chronic neurological disorders (IS-BRC-1215-20017).

Financial support and sponsorship

L.P.W.R. gratefully acknowledges funding from the National Institutes of Health (RO1 NS098819, R37NS040389 and PO1-NS058901), Target ALS, ALS Association, Packard Center, Myotonic Dystrophy Foundation, Department of Defense (W81XWH1910654) and Muscular Dystrophy Association for support. GMH thanks the Medical Research Council (MRC)

New Investigator research grant MR/R024162/1 and the Biotechnology and Biological Sciences Research Council (BBSRC) grant BB/S005277/1.

Conflicts of interest

G.M.H. is an inventor on patents granted in the USA (US10801027B2) and Europe (EP3430143B1) for the use of inhibitors of SRSF1 to treat neurodegenerative disorders (WO2017207979A1). L.P.W.R. is an inventor on patents and pending patents related to RAN translation and the use of metformin and PKR inhibition to treat RAN protein disorders. The authors declare no other relationships, conditions or circumstances that present a potential conflict of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

1. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, *et al.* Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011; 72:245–256.
2. Renton AE, Majounie E, Waite A, *et al.* A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011; 72:257–268.
3. Burrell JR, Halliday GM, Kril JJ, *et al.* The frontotemporal dementia-motor neuron disease continuum. Lancet 2016; 388:919–931.
4. Abramzon YA, Fratta P, Traynor BJ, Chia R. The overlapping genetics of amyotrophic lateral sclerosis and frontotemporal dementia. Front Neurosci 2020; 14:42.
5. Bensimon G, Lacomblez L, Meininger V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. N Engl J Med 1994; 330:585–591.
6. Mendez MF. Frontotemporal dementia: therapeutic interventions. Front Neurol Neurosci 2009; 24:168–178.
7. Writing G, Edaravone ALSSG. Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. Lancet Neurol 2017; 16:505–512.
8. Moseley ML, Zu T, Ikeda Y, *et al.* Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. Nat Genet 2006; 38:758–769.
9. Cho DH, Thienes CP, Mahoney SE, *et al.* Antisense transcription and heterochromatin at the DM1 CTG repeats are constrained by CTCF. Mol Cell 2005; 20:483–489.
10. Gendron TF, Bieniek KF, Zhang YJ, *et al.* Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. Acta Neuropathol 2013; 126:829–844.
11. Lagier-Tourenne C, Baughn M, Rigo F, *et al.* Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. Proc Natl Acad Sci U S A 2013; 110:E4530–E4539.
12. Zu T, Liu Y, Banez-Coronel M, *et al.* RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. Proc Natl Acad Sci U S A 2013; 110:E4968–E4977.
13. Ash PE, Bieniek KF, Gendron TF, *et al.* Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron 2013; 77:639–646.
14. Mori K, Weng SM, Arzberger T, *et al.* The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 2013; 339:1335–1338.
15. Zu T, Gibbens B, Doty NS, *et al.* Non-ATG-initiated translation directed by microsatellite expansions. Proc Natl Acad Sci U S A 2011; 108:260–265.
16. Donnelly CJ, Zhang PW, Pham JT, *et al.* RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention. Neuron 2013; 80:415–428.
17. O'Rourke JG, Bogdanik L, Yáñez A, *et al.* C9orf72 is required for proper macrophage and microglial function in mice. Science 2016; 351:1324–1329.
18. Burberry A, Suzuki N, Wang JY, *et al.* Loss-of-function mutations in the C9ORF72 mouse ortholog cause fatal autoimmune disease. Sci Transl Med 2016; 8:347ra93.
19. Balendra R, Isaacs AM. C9orf72-mediated ALS and FTD: multiple pathways to disease. Nat Rev Neurol 2018; 14:544–558.
20. Jiang J, Ravits J. Pathogenic mechanisms and therapy development for C9orf72 amyotrophic lateral sclerosis/frontotemporal dementia. Neurotherapeutics 2019; 16:1115–1132.
21. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol 2020; 27:1918–1929.
22. ■ Roggenbuck J. C9orf72 and the care of the patient with ALS or FTD: progress and recommendations after 10 years. Neurol Genet 2021; 7:e542.

An excellent review on C9ORF72-ALS/FTD genetics, clinical challenges and available care for patients regarding testing and counselling.

23. Ogaki K, Li Y, Atsuta N, *et al.* Analysis of C9orf72 repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis. Neurobiol Aging 2012; 33:2527. e11-6.
24. ■■ Roberts TC, Langer R, Wood MJA. Advances in oligonucleotide drug delivery. Nat Rev Drug Discov 2020; 19:673–694.

A comprehensive review on the use and delivery of advanced oligonucleotide therapeutics including RNA interference, RNase H-mediated degradation, transcription/splicing modulation and genome editing technologies.

25. Jiang J, Zhu Q, Gendron TF, *et al.* Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. Neuron 2016; 90:535–550.
26. Iwamoto N, Butler DCD, Svrzikapa N, *et al.* Control of phosphorothioate stereochemistry substantially increases the efficacy of antisense oligonucleotides. Nat Biotechnol 2017; 35:845–851.
27. ■■ Liu Y, Dodart JC, Tran H, *et al.* Variant-selective stereopure oligonucleotides protect against pathologies associated with C9orf72-repeat expansion in preclinical models. Nat Commun 2021; 12:847.

Latest improved iterations of ASOs targeting all isoforms of pathological sense C9ORF72-repeat transcripts with increased RNase H-mediated activity. Nonviral gene therapeutic approach requiring repeated injections over time.

28. ■■ Martier R, Liefhebber JM, Garcia-Osta A, *et al.* Targeting RNA-mediated toxicity in C9orf72 ALS and/or FTD by RNAi-based gene therapy. Mol Ther Nucleic Acids 2019; 16:26–37.

Use of adeno-associated virus serotype 5 (AAV5) expressing a microRNA targeting C9ORF72 intron-1 upstream of the repeat expansion in patient-derived neurons and a C9ORF72-ALS BAC transgenic mouse model. Viral gene therapeutic approach, which requires a single dose injection.

29. ■■ Mueller C, Berry JD, McKenna-Yasek DM, *et al.* SOD1 suppression with adeno-associated virus and microRNA in familial ALS. N Engl J Med 2020; 383:151–158.

First in man adminstration of adeno-associated virus rh10 expressing a microRNA targeting human SOD1 in two SOD1-linked ALS patients.

30. Liu CR, Chang CR, Chern Y, *et al.* Spt4 is selectively required for transcription of extended trinucleotide repeats. Cell 2012; 148:690–701.
31. Goodman LD, Prudencio M, Kramer NJ, *et al.* Toxic expanded GGGGCC repeat transcription is mediated by the PAF1 complex in C9orf72-associated FTD. Nat Neurosci 2019; 22:863–874.
32. Kramer NJ, Carlomagno Y, Zhang YJ, *et al.* Spt4 selectively regulates the expression of C9orf72 sense and antisense mutant transcripts. Science 2016; 353:708–712.
33. ■ Naguib A, Sandmann T, Yi F, *et al.* SUPT4H1 depletion leads to a global reduction in RNA. Cell Rep 2019; 26:45–53. e4.

siRNA-mediated interference of the RNA polymerase II transcriptional elongation SUPT4H1 subunit highlighted a global genome-wide alteration of RNA expression levels in several human cell models and a limitation of SUPT4H1 depletion as a potential therapeutic approach.

34. ■ Krishnan G, Zhang Y, Gu Y, *et al.* CRISPR deletion of the C9ORF72 promoter in ALS/FTD patient motor neurons abolishes production of dipeptide repeat proteins and rescues neurodegeneration. Acta Neuropathol 2020; 140:81–84.

Therapeutic strategy based on the CRISPS-Cas9 mediated chromosomal deletion of the promoter region of pathological sense V1 and V3 isoforms of C9ORF72-repeat transcripts in patient-derived motor neurons.

35. Pinto BS, Saxena T, Oliveira R, *et al.* Impeding transcription of expanded microsatellite repeats by deactivated Cas9. Mol Cell 2017; 68:479–490. e5.
36. Batra R, Nelles DA, Pirie E, *et al.* Elimination of toxic microsatellite repeat expansion RNA by RNA-targeting Cas9. Cell 2017; 170:899–912. e10.
37. Liu Y, Pattamatta A, Zu T, *et al.* C9orf72 BAC mouse model with motor deficits and neurodegenerative features of ALS/FTD. Neuron 2016; 90:521–534.
38. Zhou Q, Lehmer C, Michaelsen M, *et al.* Antibodies inhibit transmission and aggregation of C9orf72 poly-GA dipeptide repeat proteins. EMBO Mol Med 2017; 9:687–702.
39. ■■ Zhou Q, Mareljic N, Michaelsen M, *et al.* Active poly-GA vaccination prevents microglia activation and motor deficits in a C9orf72 mouse model. EMBO Mol Med 2020; 12:e10919.

Administration of poly-GA in mice to investigate a vaccine approach as a therapeutic strategy for neuroprotection in mice expressing poly-GA.

40. ■■ Nguyen L, Montrasio F, Pattamatta A, *et al.* Antibody therapy targeting RAN proteins rescues C9 ALS/FTD phenotypes in C9orf72 mouse model. Neuron 2020; 105:645–662. e11.

Development of human anti polyGA antibodies as a novel therapeutic strategy, which improved behaviour, motor neuron survival and extended lifespan in C9ORF72-ALS BAC transgenic mice.

41. Green KM, Glineburg MR, Kearse MG, *et al.* RAN translation at C9orf72-associated repeat expansions is selectively enhanced by the integrated stress response. Nat Commun 2017; 8:2005.
42. Cheng W, Wang S, Mestre AA, *et al.* C9ORF72 GGGGCC repeat-associated non-AUG translation is upregulated by stress through eIF2alpha phosphorylation. Nat Commun 2018; 9:51.
43. Westergard T, McAvoy K, Russell K, *et al.* Repeat-associated non-AUG translation in C9orf72-ALS/FTD is driven by neuronal excitation and stress. EMBO Mol Med 2019; 11:e9423.
44. Sonobe Y, Ghadge G, Masaki K, *et al.* Translation of dipeptide repeat proteins from the C9ORF72 expanded repeat is associated with cellular stress. Neurobiol Dis 2018; 116:155–165.
45. ■■ Zu T, Guo S, Bardhi O, *et al.* Metformin inhibits RAN translation through PKR pathway and mitigates disease in C9orf72 ALS/FTD mice. Proc Natl Acad Sci U S A 2020; 117:18591–18599.

This study showed that PKR activation is a key pathway for RAN translation and PKR inhibition with dominant negative AAV PKR-K296R or repurposed FDA-approved metformin inhibit RAN translation and improve behaviour and neurodegeneration in C9ORF72-ALS BAC mice.

46. Hautbergue GM, Castelli LM, Ferraiuolo L, *et al.* SRSF1-dependent nuclear export inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. Nat Commun 2017; 8:16063.
47. Cristofani R, Crippa V, Vezzoli G, *et al.* The small heat shock protein B8 (HSPB8) efficiently removes aggregating species of dipeptides produced in C9ORF72-related neurodegenerative diseases. Cell Stress Chaperones 2018; 23:1–12.
48. Selvaraj BT, Livesey MR, Zhao C, *et al.* C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca(2+)-permeable AMPA receptor-mediated excitotoxicity. Nat Commun 2018; 9:347.
49. ■ Ababneh NA, Scaber J, Flynn R, *et al.* Correction of amyotrophic lateral sclerosis related phenotypes in induced pluripotent stem cell-derived motor neurons carrying a hexanucleotide expansion mutation in C9orf72 by CRISPR/Cas9 genome editing using homology-directed repair. Hum Mol Genet 2020; 29:2200–2217.

CRISPR-Cas9-mediated chromosomal deletion of C9ORF72-ALS/FTD repeat expansion rescues altered genome-wide gene expression and methylation of the C9ORF72 promoter in patient-derived motor neurons.

50. Khalfallah Y, Kuta R, Grasmuck C, *et al.* TDP-43 regulation of stress granule dynamics in neurodegenerative disease-relevant cell types. Sci Rep 2018; 8:7551.
51. Protter DSW, Parker R. Principles and properties of stress granules. Trends Cell Biol 2016; 26:668–679.
52. Ramaswami M, Taylor JP, Parker R. Altered ribostasis: RNA-protein granules in degenerative disorders. Cell 2013; 154:727–736.
53. Boeynaems S, Bogaert E, Kovacs D, *et al.* Phase separation of C9orf72 dipeptide repeats perturbs stress granule dynamics. Mol Cell 2017; 65:1044–1055. e5.
54. Zhang Y-J, Gendron TF, Ebbert MTW, *et al.* Poly(GR) impairs protein translation and stress granule dynamics in C9orf72-associated frontotemporal dementia and amyotrophic lateral sclerosis. Nat Med 2018; 24:1136–1142.
55. Chew J, Cook C, Gendron TF, *et al.* Aberrant deposition of stress granule-resident proteins linked to C9orf72-associated TDP-43 proteinopathy. Mol Neurodegener 2019; 14:9–15.
56. Zhang K, Daigle JG, Cunningham KM, *et al.* Stress granule assembly disrupts nucleocytoplasmic transport. Cell 2018; 173:958–971. e17.
57. Zhang K, Donnelly CJ, Haeusler AR, *et al.* The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature 2015; 525:56–61.
58. Wente SR, Rout MP. The nuclear pore complex and nuclear transport. Cold Spring Harb Perspect Biol 2010; 2:a000562.
59. Coyne AN, Zaepfel BL, Hayes L, *et al.* G4C2 repeat RNA initiates a POM121-mediated reduction in specific nucleoporins in C9orf72 ALS/FTD. Neuron 2020; 107:1124–1140. e11.
60. Freibaum BD, Lu Y, Lopez-Gonzalez R, *et al.* GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. Nature 2015; 525:129–133.
61. Jovicic A, Mertens J, Boeynaems S, *et al.* Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. Nat Neurosci 2015; 18:1226–1229.
62. Haines JD, Herbin O, de la Hera B, *et al.* Nuclear export inhibitors avert progression in preclinical models of inflammatory demyelination. Nat Neurosci 2015; 18:511–520.
63. Grima JC, Daigle JG, Arbez N, *et al.* Mutant Huntingtin disrupts the nuclear pore complex. Neuron 2017; 94:93–107. e6.
64. ■ Hightower RM, Reid AL, Gibbs DE, *et al.* The SINE compound KPT-350 blocks dystrophic pathologies in DMD zebrafish and mice. Mol Ther 2020; 28:189–201.

KPT-350, an inhibitor of the nuclear export protein XPO1, which is involved in the nuclear export of proteins and noncoding RNAs including rRNA and U snRNAs, mitigates Duchenne muscular dystrophy pathologies in zebrafish and mouse models of disease.

65. Archbold HC, Jackson KL, Arora A, *et al.* TDP43 nuclear export and neurodegeneration in models of amyotrophic lateral sclerosis and frontotemporal dementia. Sci Rep 2018; 8:4606–4618.
66. ■ Gouel F, Rolland AS, Devedjian JC, *et al.* Past and future of neurotrophic growth factors therapies in ALS: from single neurotrophic growth factor to stem cells and human platelet lysates. Front Neurol 2019; 10:835.

An excellent review on the use of neurotrophic factors and potential regenerative approaches in neurodegenerative disease.

67. Korkmaz OT, Aytan N, Carreras I, *et al.* 7,8-Dihydroxyflavone improves motor performance and enhances lower motor neuronal survival in a mouse model of amyotrophic lateral sclerosis. Neurosci Lett 2014; 566:286–291.
68. Zheng C, Nennesmo I, Fadeel B, Henter J-I. Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. Ann Neurol 2004; 56:564–567.
69. Storkebaum E, Lambrechts D, Dewerchin M, *et al.* Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. Nat Neurosci 2005; 8:85–92.
70. Azzouz M, Ralph GS, Storkebaum E, *et al.* VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. Nature 2004; 429:413–417.
71. Dodge JC, Treleaven CM, Fidler JA, *et al.* AAV4-mediated expression of IGF-1 and VEGF within cellular components of the ventricular system improves survival outcome in familial ALS mice. Mol Ther 2010; 18:2075–2084.
72. Wang Y, Duan W, Wang W, *et al.* scAAV9-VEGF prolongs the survival of transgenic ALS mice by promoting activation of M2 microglia and the PI3K/Akt pathway. Brain Res 2016; 1648:1–10.
73. Kaspar BK, Lladó J, Sherkat N, *et al.* Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. Science 2003; 301:839–842.
74. Xu L, Yan J, Chen D, *et al.* Human neural stem cell grafts ameliorate motor neuron disease in SOD-1 transgenic rats. Transplantation 2006; 82: 865–875.
75. Xu L, Ryugo DK, Pongstaporn T, *et al.* Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. J Comp Neurol 2009; 514:297–309.
76. Hwang DH, Lee HJ, Park IH, *et al.* Intrathecal transplantation of human neural stem cells overexpressing VEGF provide behavioral improvement, disease onset delay and survival extension in transgenic ALS mice. Gene Ther 2009; 16:1234–1244.
77. Knippenberg S, Rath KJ, Böselt S, *et al.* Intraspinal administration of human spinal cord-derived neural progenitor cells in the G93A-SOD1 mouse model of ALS delays symptom progression, prolongs survival and increases expression of endogenous neurotrophic factors. J Tissue Eng Regen Med 2017; 11:751–764.
78. Vercelli A, Mereuta OM, Garbossa D, *et al.* Human mesenchymal stem cell transplantation extends survival, improves motor performance and decreases neuroinflammation in mouse model of amyotrophic lateral sclerosis. Neurobiol Dis 2008; 31:395–405.
79. Uccelli A, Milanese M, Principato MC, *et al.* Intravenous mesenchymal stem cells improve survival and motor function in experimental amyotrophic lateral sclerosis. Mol Med 2012; 18:794–804.
80. Boido M, Piras A, Valsecchi V, *et al.* Human mesenchymal stromal cell transplantation modulates neuroinflammatory milieu in a mouse model of amyotrophic lateral sclerosis. Cytotherapy 2014; 16:1059–1072.
81. Suzuki M, McHugh J, Tork C, *et al.* Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS. Mol Ther 2008; 16:2002–2010.
82. Krakora D, Mulcrone P, Meyer M, *et al.* Synergistic effects of GDNF and VEGF on lifespan and disease progression in a familial ALS rat model. Mol Ther 2013; 21:1602–1610.
83. Tsitkanou S, Della Gatta P, Foletta V, Russell A. The role of exercise as a nonpharmacological therapeutic approach for amyotrophic lateral sclerosis: beneficial or detrimental? Front Neurol 2019; 10:783.
84. Julian TH, Glascow N, Barry ADF, *et al.* Physical exercise is a risk factor for amyotrophic lateral sclerosis: convergent evidence from Mendelian randomisation, transcriptomics and risk genotypes. EBioMedicine 2021; 68:103397.
85. Robison R, Tabor-Gray LC, Wymer JP, Plowman EK. Combined respiratory training in an individual with C9orf72 amyotrophic lateral sclerosis. Ann Clin Transl Neurol 2018; 5:1134–1138.
86. ■■ Plowman EK, Tabor-Gray L, Rosado KM, *et al.* Impact of expiratory strength training in amyotrophic lateral sclerosis: results of a randomized, sham-controlled trial. Muscle Nerve 2019; 59:40–46.

An important study showing simple and well tolerated expiratory muscle training improves respiratory strength and swallowing function in ALS patients.

87. ■■ Westeneng HJ, van Veenhuijzen K, van der Spek RA, *et al.* Associations between lifestyle and amyotrophic lateral sclerosis stratified by C9orf72 genotype: a longitudinal, population-based, case-control study. Lancet Neurol 2021; 20:373–384.

This study identified modifiable disease-promoting lifestyle factors that may provide opportunities to lower risk of developing neurodegenerative disease.

88. Nieves JW, Gennings C, Factor-Litvak P, *et al.* Association between dietary intake and function in amyotrophic lateral sclerosis. JAMA Neurol 2016; 73:1425–1432.
89. Hu F, Padukkavidana T, Vaegter CB, *et al.* Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. Neuron 2010; 68:654–667.
90. Lashley T, Rohrer JD, Mahoney C, *et al.* A pathogenic progranulin mutation and C9orf72 repeat expansion in a family with frontotemporal dementia. Neuropathol Appl Neurobiol 2014; 40:502–513.