JMA JOURNAL
DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

Review Article

# RNA Dysmetabolism and Repeat-Associated Non-AUG Translation in Frontotemporal Lobar Degeneration/Amyotrophic Lateral Sclerosis due to *C9orf72* Hexanucleotide Repeat Expansion

Kohji Mori[1)], Shiho Gotoh[1)], Ryota Uozumi[1)], Tesshin Miyamoto[1),2)], Shoshin Akamine[1)], Yuya Kawabe[1),3)], Shinji Tagami[3),4)], and Manabu Ikeda[1)]

**Abstract:**
Neuropathological features of frontotemporal dementia and amyotrophic lateral sclerosis (ALS) due to *C9orf72* GGGGCC hexanucleotide repeat expansion include early dipeptide repeats, repeat RNA foci, and subsequent TDP-43 pathologies. Since the discovery of the repeat expansion, extensive studies have elucidated the disease mechanism of how the repeat causes neurodegeneration. In this review, we summarize our current understanding of abnormal repeat RNA metabolism and repeat-associated non-AUG translation in *C9orf72* frontotemporal lobar degeneration/ALS. For repeat RNA metabolism, we specifically focus on the role of hnRNPA3, the repeat RNA-binding protein, and the EXOSC10/RNA exosome complex, an intracellular RNA-degrading enzyme. In addition, the mechanism of repeat-associated non-AUG translation inhibition via TMPyP4, a repeat RNA-binding compound, is discussed.

**Key Words:**
Frontotemporal dementia, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, C9orf72, Repeat-associated non-AUG translation, RNA metabolism

## Introduction

Frontotemporal dementia (FTD) is a clinical concept of neurocognitive disorder that encompasses several groups of neurodegenerative conditions characterized by slowly progressive and characteristic alterations or deficit in behavior, executive function, and/or language [(1)]. Recently, the term "frontotemporal lobar degeneration" (FTLD) is often used to define these conditions from a neuropathological/mechanistic aspect. FTLD is heterogeneous in accumulating proteins and is subclassified into several subgroups according to the major contents of the accumulating protein. Among these, FTLD cases with prominent tau pathology are called FTLD-tau, and cases with abundant TDP-43 accumulation are called FTLD-TDP.

These two categories account for roughly 90% of neuropathologically confirmed FTLD cases.

Although the majority of FTLD-TDP cases are sporadic, there are cases caused by genetic mutations. The most frequent genetic cause of FTLD-TDP is the hexanucleotide repeat expansion mutation in the intron of the *C9orf72* (chromosome 9 open reading frame 72) gene. In contrast to 2-23 (or up to 30) GGGGCC repeats in non-carriers, hundreds to more than a thousand tandem expanded repeats can be found in the expansion carriers. This mutation has been reported at a low frequency in Japan, although there are some reported cases [(2), (3), (4)]. How these extended repeats cause TDP-43 protein abnormalities, neurodegeneration, and ultimately neurocognitive and motor neuron diseases has been actively debated since the identification of repeat expansion.

TDP-43 is a ubiquitously expressed multifunctional RNA-binding protein and confers splicing, RNA transports, and metabolism. Physiologically, TDP-43 is predominantly present within the nucleus and shuttles between the nucleus and cytoplasm; however, in FTLD-TDP cases including the *C9orf72* mutation, neuronal TDP-43 typically forms aberrant intracytoplasmic inclusions with a concomitant absence of TDP-43 from the nucleus. The higher TDP-43-positive inclusion load correlates well with the severity of neurodegeneration. Thus, TDP-43 appears to be more akin to a downstream executor of neuronal cell death.

[1)] Psychiatry, Osaka University Graduate School of Medicine, Suita, Japan. [2)] Seifukai Ibaraki Hospital, Ibaraki, Japan. [3)] Minoh Neuropsychiatric Hospital, Minoh, Japan. [4)] Health and Counseling Center, Osaka University, Toyonaka, Japan
Corresponding author: Kohji Mori, kmori@psy.med.osaka-u.ac.jp
JMA J. 2023;6(1):9-15
Received: August 17, 2022 / Accepted: September 26, 2022 / Advance Publication: December 23, 2022 / Published: January 16, 2023

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

## Mechanism of Repeat RNA Production in *C9orf72* FTLD/ALS

Aberrant DNA repeat expansion is a genetic cause and thus is unquestionably upstream of the pathological cascade. First, the DNA repeat is transcribed into an RNA repeat. As the expanded repeat is located in the intron (or promoter) of the *C9orf72* gene, the mature *C9orf72* RNA transcript does not contain the repeat region and thus none of mature C9ORF72 protein contains the translated repeat sequence. Then, how does the repeat cause neurodegeneration? One hypothetical mechanism is the repeat RNA toxicity. The repeat-containing region in *C9orf72* is bidirectionally (sense direction and antisense direction) transcribed; accordingly, the GGGGCC repeat RNA transcript and CCCCGG repeat RNA transcript are generated from the same DNA GGGGCC repeat expansion. These repeat RNA sequences can sequestrate RNA-binding proteins that preferentially bind to the repeated RNA sequence. These RNA/protein complexes may aggregate, and form intracellular structures called RNA foci. RNA foci are a neuropathological hallmark in *C9orf72* repeat expansion carriers and can be found not only in neurons but also in non-neuronal cells (astrocytes, microglial cells, fibroblasts, transformed lymphoblasts, etc.). Although still controversial [5], several studies have revealed a correlation between RNA foci with neurodegeneration in *C9orf72* FTLD/ALS cases [6, 7]. Moreover, a zebrafish model demonstrated clear repeat RNA toxicity [8].

## Mechanism of DPR Production in *C9orf72* FTLD/ALS

The other disease hallmark of *C9orf72* FTLD/ALS cases is the accumulation of dipeptide repeat proteins (DPRs) [9]. Transcribed intronic repeat RNA not only forms RNA foci but is also translated through repeat-associated non-AUG (RAN) translation [10]. RAN translation can occur in all possible reading frames and thus results in the production of five different DPRs from the bidirectionally transcribed repeat RNA [9, 11, 12, 13, 14]. Conventional translation requires a "start translation here" signal during ribosomal scanning from the 5′- to 3′-side of an RNA molecule. The start signal is usually an AUG (encoding methionine) initiation codon with a surrounding sequence that matches the Kozak rule well. Repeat-associated non-AUG translation (RAN translation) [10] is recognized as an unconventional translation event in which translation of the repetitive sequence occurs in the absence of an AUG initiation codon.

It is believed that there are at least two types of RAN translations: those initiated from near cognate codons (for example, one base mismatch) and those initiated from specific RNA structures such as RNA hairpins.

The start site of RAN translation in the poly-Gly-Ala (GA) reading frame of the *C9orf72* repeat expansion has been reported as a CUG codon in the 5′-flanking region of the GGGGCC repeat [15]. It has also been suggested that this CUG codon-dependent RAN translation of poly-GA is involved in the production of poly-Gly-Pro (GP) and poly-Gly-Arg (GR) through translational frameshift [16, 17]. Conversely, another group has ruled out the production of poly-GP and poly-GR by frameshifting [18]. Additionally, the presence of an AUG-initiated upstream open reading frame (uORF) of 180 bases spanning exon 1 and intron 1 of the *C9orf72* gene located in the poly-GP reading frame was reported [19]. This uORF ends just before the GGGGCC repeat and translation of the uORF suppresses RAN translation in poly-GA and poly-GP frames, adding further complexity to the regulation of *C9orf72* RAN translation.

The molecules that mediate RAN translation are still little known. RPS25, a factor previously known to be associated with Internal Ribosome Entry Site-dependent translation, was reported to activate RAN translation [20]. The RNA helicase DHX36 unwinds the G-quadruplex structure of GGGGCC repeats and promotes RAN translation, whereas DDX3X conversely represses the RAN translation of GGGGCC repeats in an RNA helicase activity-dependent manner [21, 22]. Cellular stress has also been reported to enhance RAN translation via the phosphorylation of eIF2α [15, 23]. Double-stranded RNA-dependent protein kinase (PKR), which is activated by repeat RNA, also enhances RAN translation via the phosphorylation of eIF2α. Conversely, the anti-diabetic drug metformin, a PKR inhibitor, inhibits RAN translation [24]. These RAN translation regulators are attracting attention as potential target molecules for disease-modifying drugs in *C9orf72* FTLD/ALS via the inhibition of RAN translation.

## Mechanisms of DPR-mediated Neurodegeneration

The neurotoxicity of DPR generated by RAN translation has been shown in multiple disease models. The most abundant DPR poly-GA aggregates adsorb large amounts of stacked proteasomes, which impairs intracellular proteostasis [25]. In contrast, poly-GP and poly-Pro-Ala (PA) have been shown to have no apparent toxicity [26].

Cellular organelles, such as the endoplasmic reticulum, lysosomes, mitochondria, and nucleus, are intracellularly compartmentalized by lipid bilayers, as described in textbooks. Unlike these classical organelles, intracellular structures, such as the nucleolus, centrosome, spliceosome, and stress granules, do not have such membrane partitions of lipid bilayers. Liquid-liquid phase separation is a mechanism commonly used in cells as a principle for ordering the formation and maintenance of such non-membranous structures. Poly-GR and poly-Pro-Arg (PR), that is, DPRs containing arginine residues within the repeat motif, preferentially disrupt the behavior of this liquid-liquid phase separation, leading to the impaired ar-

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

chitecture and function of the membrane-less organelles, including stress granules [27], [28], chromatin [29], nuclear membrane pores, and nucleoli [27], [30], [31].

A major physiological function of nucleoli is the production of cellular translational machinery, the ribosome. Poly-GR and poly-PR accumulate on nucleoli and inhibit ribosomal (r)RNA production [27] in part through the inhibition of small nucleolar RNA maturation [32]. In addition, poly-GR and poly-PR directly inhibit translation through their interaction with ribosomes [33], [34], [35]. Thus, each DPR is thought to impair cellular function through different mechanisms, eventually leading to neuronal death.

## Identification of hnRNPA3, the Repeat RNA Suppressor

There is debate about whether the repeat RNA itself is neurotoxic because of the difficulty in fully separating repeat RNA toxicity from DPR toxicity [36]. Even so, an association between RNA foci and the abnormal localization of TDP-43 has been noted in autopsy cases [6], [7]. We previously established an *in vitro* RNA-binding assay to purify RNA-binding proteins that selectively bind to GGGGCC repeat RNA, followed by their identification by mass spectrometry [37]. We then performed secondary immunohistochemical screening for these candidate proteins using the hippocampal tissue of patients with *C9orf72* FTLD/ALS and compared them with control tissue. From this, we noticed that hnRNPA3 is present in the cell nucleus in healthy tissues, whereas in tissues of patients with *C9orf72* FTLD/ALS, nuclear staining was variably lost, and hnRNPA3-positive inclusion pathology was observed in the hippocampal dentate gyrus [37].

So, how does hnRNPA3 affect the pathogenesis of *C9orf72* FTLD/ALS? To answer this question, we performed functional analysis of hnRNPA3 in the context of *C9orf72* FTLD/ALS. siRNA-mediated knockdown of hnRNPA3 in cells exogenously expressing GGGGCC repeats increased the accumulation level of GGGGCC repeat RNA. An RNA-binding domain mutant of hnRNPA3 lacked the ability to rescue the phenotype. Conversely, overexpression of hnRNPA3 decreased the expression level of repeat RNA. Thus, hnRNPA3 repressively regulates the repeat RNA expression level and this inhibits the DPR expression level. These findings were confirmed from analyses using patient-derived fibroblasts and primary cultured rat neurons, as well as from analyses of patient brains. These results indicate that hnRNPA3 suppresses repeat RNA expression levels by promoting repeat RNA metabolism. When hnRNPA3 is lost and this repression is compromised, there is a marked accumulation of repeat RNA and an increased expression level of DPR [38], [39].

## Mechanism of Repeat RNA Degradation by the RNA Exosome Complex and Its Disruption

As the expanded repeat hinders efficient transcription, the expression levels of mature *C9orf72* mRNA transcripts in cells derived from *C9orf72* mutation carriers are only about half that of those without the repeat expansion. However, repeat RNA, derived from the same RNA transcript, accumulates as RNA foci. As an explanation for this seemingly contradictory phenomenon, we considered the possibility that the degradation of abnormally elongated repeat RNAs is impaired.

As there was no prior knowledge of how GGGGCC repeat RNA is degraded in the cell, we first knocked down molecules constituting several representative RNA-degrading enzyme systems and monitored the expression level of DPR as a readout in preliminary experiments. With this, we found that EXOSC10 plays an important role in the degradation of repeat RNA [32]. The RNA exosome complex is a multimeric protein complex that governs the metabolism of RNA and this EXOSC10 is a nucleolar enriched subunit of the RNA exosome complex. Interestingly, the genetic mutations of other components of the RNA exosome complex have been linked with neurodegenerative phenotypes [40], [41], [42].

The knockdown of EXOSC10 in fibroblasts derived from patients with *C9orf72* FTLD/ALS resulted in intracellular repeat RNA accumulation and increased nuclear RNA foci, suggesting that the EXOSC10/RNA exosome complex is involved in the metabolism of endogenous repeat RNA in patient-derived cells. In cells that expressed poly-GR or poly-PR through RAN translation, EXOSC10 was redistributed diffusely into the nucleus instead of being confined to the nucleolus.

Moreover, these arginine-rich DPRs inhibit endogenous EXOSC10 activity, which leads to the additional accumulation of GGGGCC repeat RNA. These results suggest that poly-GR and poly-PR inhibit the metabolism of repeat RNA by inhibiting the EXOSC10/RNA exosome complex. The accumulation of repeat RNA accelerates poly-GR and poly-PR production through RAN translation, thus further aggravating the pathological processes [32].

## Mechanism of Inhibition of RAN Translation by the Repeat RNA-binding Compound TMPyP4

The selective inhibition of RAN translation and DPR production may lead to the development of a novel therapeutic strategy [43], [44], [45]. GGGGCC repeat RNA is known to adopt a strong tertiary structure called the G-quadruplex in the presence of potassium ions. TMPyP4 (5,10,15,20-Tetrakis-(N-methyl-4-pyridyl)porphine) is a type of porphyrin that has been reported to bind to the G-quadruplex of GGGGCC repeat RNA [46]. We therefore investigated the effect of TMPyP4

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

on RAN translation of *C9orf72* GGGGCC repeats. In a cellular model, TMPyP4 inhibited DPR production by RAN translation, while sparing the repeat RNA expression level, nucleocytoplasmic distribution of repeat RNA, and global translational activity. Though an artificial insertion of the AUG initiation codon just before the repeat strongly enhanced repeat translation through conventional initiation, TMPyP4 strongly inhibited repeat translation even in the presence of the AUG initiation codon, suggesting that TMPyP4 does not specifically inhibit the non-AUG-dependent initiation of RAN translation. This finding led us to the hypothesis that TMPyP4 may inhibit the elongation step of RAN translation rather than non-AUG initiation. If elongation is inhibited, a large number of ribosomes stop on a single repeat RNA, and the complex of repeat RNA and ribosomes can be recovered in the higher-density fraction of sucrose density-gradient centrifugation of cytoplasmic cell lysate. Indeed, in cells treated with TMPyP4, more repeat RNA was found in the poly-ribosomal (highest density) fractions than in untreated cells. Furthermore, TMPyP4 and repeat RNA showed a strong interaction that was resistant to denaturing urea. The tight interaction between TMPyP4 and repeat RNA would physically inhibit ribosomal translocation. These results suggest that TMPyP4 binds tightly to GGGGCC repeat RNA and inhibits RAN translation by blocking the RAN translation elongation [47].

## Association of DPR with TDP-43 Proteinopathy

It has been pointed out that DPR and repeat RNA, as well as TDP-43 aggregates themselves, disrupt nucleocytoplasmic transport mechanisms and nuclear membrane pore function in multiple disease models [48], [49], [50], [51], [52]. In particular, in a mouse model expressing 200 repeated poly-GR, poly-GR was found to cause TDP-43 aggregation in the cytoplasm via the mislocalization of nucleocytoplasmic transport factors and nuclear pore component proteins, pointing to a link between DPR and TDP-43 pathology [53].

The relationship between DPR pathology and clinical phenotypes in human patients remains unclear and requires further investigation. Although TDP-43 inclusion pathology correlates well with neurodegeneration, it has been noted that DPR inclusion pathology does not usually co-localize with TDP-43 inclusion. However, recent detailed reports have pointed to an association between poly-GR load and neurodegeneration [54], [55], [56]. In addition, a small number of cases have been reported with clinical FTD and abundant DPR pathology at autopsy, but little or no TDP-43 pathology [57], [58], [59].

## Loss of Function of C9ORF72 Protein and *C9orf72* FTLD/ALS Pathology

The function of the C9ORF72 protein encoded by the *C9orf72* open reading frame was initially unknown, but its physiological function and role in *C9orf72* FTLD/ALS pathogenesis have gradually become clear. The C9ORF72 protein forms a heterotrimer complex with SMCR8 and WDR41 and functions in the autophagy/lysosome system. As abnormally expanded GGGGCC repeats reduce the efficiency of transcription by RNA polymerase, the expression of C9ORF72 protein is decreased [60], [61]. Importantly, no neurodegeneration is observed in *C9orf72* knockout mice [62], but systemic inflammation, including in the brain, lymph node enlargement, splenomegaly, and shortened lifespan due to autoimmune reactions have been observed [63]. Such lysosomal system dysfunction and systemic inflammation are considered to exacerbate repeat-mediated gain of toxicity [61], [64], [65].

## Therapeutic Approach and Utility of DPR as Biomarker

Several attempts are being made to develop a treatment for patients with *C9orf72* FTLD/ALS. One such approach is an antisense oligonucleotide (ASO), which effectively reduces repeat-containing transcripts [66]. Another approach is to identify the RAN translation inhibitor that can selectively inhibit RAN translation, while sparing conventional translation as mentioned in the above section. The other approach includes repeat transcription inhibitors [67].

Biomarkers reflecting disease status are important for diagnosis, disease progression monitoring, and assessment of response to potential treatment. Although RAN translation is considered a rather inefficient event, DPRs can be detected with state-of-the-art highly sensitive assays in the cerebrospinal fluid (CSF) of patients or carriers with *C9orf72* mutations. Especially, poly-GP, which is relatively abundant and has less aggregation potency, could be detected in cases with *C9orf72* repeat expansion specific manner and is currently used as a target engagement biomarker to demonstrate proof of concept in drug discovery. Poly-GP levels are rather consistent in each patient in repeated measurements [68], [69], [70], [71], [72]. The frequently used assay platforms are based on Meso Scale Discovery ELISA or single-molecule array [68].

Recent highly sensitive assays also enabled poly-GA and poly-GR measurements from the CSF of patients with *C9orf72*[69]. CSF poly-GA and poly-GR levels did not correlate with clinical phenotypes, but a patient with *C9orf72*-ALS treated with an ASO targeting the repeat-containing C9orf72 transcript showed decreased CSF poly-GA, poly-GP, and poly-GR levels.

## Summary and Prospects

Extensive efforts to elucidate the pathogenesis of *C9orf72* FTLD/ALS have so far suggested that autophagic dysfunction due to haploinsufficiency of the C9ORF72 protein enhances primary gain of toxicities from repeat RNA and DPR. Inter-

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

estingly, this mutation is known to cause psychiatric symptoms more frequently than FTD because of other genetic causes or sporadic FTD [73], [74], [75]. By analyzing the disease mechanism of this particular mutation in detail, we hope to contribute to develop more accurate and early diagnosis and disease-modifying therapy. More specifically, we hope to extend the knowledge gained from the analysis of hereditary FTD to the pathophysiology of more frequent sporadic FTD.

## Article Information

This article is based on the study, which received the Medical Research Encouragement Prize of The Japan Medical Association in 2021.

### Conflicts of Interest

None

### Sources of Funding

K.M. is supported by the JSPS KAKENHI grant numbers JP20H03602, JP20H05927, and JP22K19492; JST FOREST Program under grant number JPMJFR200Z; and SENSHIN Medical Research Foundation and Takeda Science Foundation. S.G. is supported by a JSPS Research Fellowship for Young Scientists JP22J12248. S.A. is supported by KAKENHI JP21K15682. S.T. is supported by KAKENHI JP22K07559. M.I. is supported by AMED grant number JP21ek0109510h0001.

### Acknowledgement

We apologize that we could not cite all relevant original papers due to space limitations.

## References

1. Bang J, Spina S, Miller BL. Frontotemporal dementia. Lancet. 2015;386(10004):1672-82.
2. Ishiura H, Takahashi Y, Mitsui J, et al. C9ORF72 repeat expansion in amyotrophic lateral sclerosis in the Kii Peninsula of Japan. Arch Neurol. 2012;69(9):1154-8.
3. Konno T, Shiga A, Tsujino A, et al. Japanese amyotrophic lateral sclerosis patients with GGGGCC hexanucleotide repeat expansion in C9ORF72. J Neurol Neurosurg Psychiatry. 2013;84(4):398-401.
4. 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(10):2527.e11-6.
5. DeJesus-Hernandez M, Finch NA, Wang X, et al. In-depth clinico-pathological examination of RNA foci in a large cohort of C9ORF72 expansion carriers. Acta Neuropathol. 2017;134(2):255-69.
6. Aladesuyi Arogundade OA, Stauffer JE, Saberi S, et al. Antisense RNA foci are associated with nucleoli and TDP-43 mislocalization in C9orf72-ALS/FTD: a quantitative study. Acta Neuropathol. 2019;137(3):527-30.
7. Cooper-Knock J, Higginbottom A, Stopford MJ, et al. Antisense RNA foci in the motor neurons of C9ORF72-ALS patients are associated with TDP-43 proteinopathy. Acta Neuropathol. 2015;130(1):63-75.
8. Swinnen B, Bento-Abreu A, Gendron TF, et al. A zebrafish model for C9orf72 ALS reveals RNA toxicity as a pathogenic mechanism. Acta Neuropathol. 2018;135(3):427-43.
9. 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(6125):1335-8.
10. 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(1):260-5.
11. Mori K, Arzberger T, Grässer FA, et al. Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins. Acta Neuropathol. 2013;126(6):881-93.
12. Ash PE, Bieniek KF, Gendron TF, et al. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron. 2013;77(4):639-46.
13. 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(6):829-44.
14. Zu T, Liu Y, Bañez-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(51):E4968-77.
15. 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(1):2005.
16. Tabet R, Schaeffer L, Freyermuth F, et al. CUG initiation and frameshifting enable production of dipeptide repeat proteins from ALS/FTD C9ORF72 transcripts. Nat Commun. 2018;9(1):152.
17. McEachin ZT, Gendron TF, Raj N, et al. Chimeric peptide species contribute to divergent dipeptide repeat pathology in c9ALS/FTD and SCA36. Neuron. 2020;107(2):292-305.e6.
18. Almeida S, Krishnan G, Rushe M, et al. Production of poly(GA) in C9ORF72 patient motor neurons derived from induced pluripotent stem cells. Acta Neuropathol. 2019;138(6):1099-101.
19. Spijker HMv, Stackpole EE, Almeida S, et al. Ribosome profiling reveals novel regulation of C9ORF72 GGGGCC repeat-containing RNA translation. RNA. 2021;28(2):123-38.
20. Yamada SB, Gendron TF, Niccoli T, et al. RPS25 is required for efficient RAN translation of C9orf72 and other neurodegenerative disease-associated nucleotide repeats. Nat Neurosci. 2019;22(9):1383-8.
21. Cheng W, Wang S, Zhang Z, et al. CRISPR-Cas9 screens identify the RNA helicase DDX3X as a repressor of C9ORF72

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

(GGGGCC)n repeat-associated non-AUG translation. Neuron. 2019;104(5):885-98.e8.
22. Liu H, Lu YN, Paul T, et al. A helicase unwinds hexanucleotide repeat RNA G-quadruplexes and facilitates repeat-associated non-AUG translation. J Am Chem Soc. 2021;143(19):7368-79.
23. 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(1):51.
24. 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(31):18591-9.
25. Guo Q, Lehmer C, Martínez-Sánchez A, et al. In situ structure of neuronal C9orf72 poly-GA aggregates reveals proteasome recruitment. Cell. 2018;172(4):696-705.e12.
26. Mizielinska S, Grönke S, Niccoli T, et al. C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science. 2014;345(6201):1192-4.
27. Lee KH, Zhang P, Kim HJ, et al. C9orf72 dipeptide repeats impair the assembly, dynamics, and function of membrane-less organelles. Cell. 2016;167(3):774-88.e17.
28. Zhang YJJ, 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(8):1136-42.
29. Zhang YJ, Guo L, Gonzales PK, et al. Heterochromatin anomalies and double-stranded RNA accumulation underlie C9orf72 poly(PR) toxicity. Science. 2019;363(6428):eaav2606.
30. White MR, Mitrea DM, Zhang P, et al. C9orf72 poly(PR) dipeptide repeats disturb biomolecular phase separation and disrupt nucleolar function. Mol Cell. 2019;713-28.e6.
31. Kwon I, Xiang S, Kato M, et al. Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. Science. 2014;345(6201):1139-45.
32. Kawabe Y, Mori K, Yamashita T, et al. The RNA exosome complex degrades expanded hexanucleotide repeat RNA in C9orf72 FTLD/ALS. EMBO J. 2020;39(19):e102700.
33. Kanekura K, Yagi T, Cammack AJ, et al. Poly-dipeptides encoded by the C9ORF72 repeats block global protein translation. Hum Mol Genet. 2016;25(9):1803-13.
34. Moens TG, Niccoli T, Wilson KM, et al. C9orf72 arginine-rich dipeptide proteins interact with ribosomal proteins in vivo to induce a toxic translational arrest that is rescued by eIF1A. Acta Neuropathol. 2019;137(3):487-500.
35. Loveland AB, Svidritskiy E, Susorov D, et al. Ribosome inhibition by C9ORF72-ALS/FTD-associated poly-PR and poly-GR proteins revealed by cryo-EM. Nat Commun. 2022;13(1):2776.
36. Tran H, Almeida S, Moore J, et al. Differential Toxicity of Nuclear RNA Foci versus Dipeptide Repeat Proteins in a Drosophila Model of C9ORF72 FTD/ALS. Neuron. 2015;87(6):1207-14.
37. Mori K, Lammich S, Mackenzie IR, et al. hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations. Acta Neuropathol. 2013;125(3):413-23.
38. Nihei Y, Mori K, Werner G, et al. Poly-glycine-alanine exacerbates C9orf72 repeat expansion-mediated DNA damage via sequestration of phosphorylated ATM and loss of nuclear hnRNPA3. Acta Neuropathol. 2020;139(1):99-118.
39. Mori K, Nihei Y, Arzberger T, et al. Reduced hnRNPA3 increases C9orf72 repeat RNA levels and dipeptide-repeat protein deposition. EMBO Rep. 2016;17(9):1314-25.
40. Wan J, Yourshaw M, Mamsa H, et al. Mutations in the RNA exosome component gene EXOSC3 cause pontocerebellar hypoplasia and spinal motor neuron degeneration. Nat Genet. 2012;44(6):704-8.
41. Boczonadi V, Müller JS, Pyle A, et al. EXOSC8 mutations alter mRNA metabolism and cause hypomyelination with spinal muscular atrophy and cerebellar hypoplasia. Nat Commun. 2014;5:4287.
42. Burns DT, Donkervoort S, Müller JS, et al. Variants in EXOSC9 disrupt the RNA exosome and result in cerebellar atrophy with spinal motor neuronopathy. Am J Hum Genet. 2018;102(5):858-73.
43. Wang ZF, Ursu A, Childs-Disney JL, et al. The hairpin form of r(G4C2)exp in c9ALS/FTD is repeat-associated non-ATG translated and a target for bioactive small molecules. Cell Chem Biol. 2019;26(2):179-90.e12.
44. Simone R, Balendra R, Moens TG, et al. G‐quadruplex‐binding small molecules ameliorate C9orf72 FTD/ALS pathology in vitro and in vivo. EMBO Mol Med. 2018;10(1):22-31.
45. Green KM, Sheth UJ, Flores BN, et al. High-throughput screening yields several small-molecule inhibitors of repeat-associated non-AUG translation. J Biol Chem. 2019;294(49):18624-38.
46. Zamiri B, Reddy K, Macgregor RB, et al. TMPyP4 porphyrin distorts RNA G-quadruplex structures of the disease-associated r(GGGGCC)n repeat of the C9orf72 gene and blocks interaction of RNA-binding proteins. J Biol Chem. 2014;289(8):4653-9.
47. Mori K, Gotoh S, Yamashita T, et al. The porphyrin TMPyP4 inhibits elongation during the noncanonical translation of the FTLD/ALS-associated GGGGCC repeat in the C9orf72 gene. J Biol Chem. 2021;297(4):101120.
48. Chou CCC, Zhang Y, Umoh ME, et al. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. Nat Neurosci. 2018;21(2):228-39.
49. Solomon DA, Stepto A, Au WH, et al. A feedback loop between dipeptide-repeat protein, TDP-43 and karyopherin-α mediates C9orf72-related neurodegeneration. Brain. 2018;141(10):2908-24.
50. Zhang K, Daigle JG, Cunningham KM, et al. Stress granule assembly disrupts nucleocytoplasmic transport. Cell. 2018;173(4):958-71.e17.
51. Freibaum BD, Lu Y, Lopez-Gonzalez R, et al. GGGGCC repeat

DOI: 10.31662/jmaj.2022-0160
https://www.jmaj.jp/

expansion in C9orf72 compromises nucleocytoplasmic transport. Nature. 2015;525(7567):129-33.
52. Zhang K, Donnelly CJ, Haeusler AR, et al. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature. 2015;525(7567):56-61.
53. Cook CN, Wu Y, Odeh HM, et al. C9orf72 poly(GR) aggregation induces TDP-43 proteinopathy. Sci Transl Med. 2020;12(559):eabb3774.
54. Sakae N, Bieniek KF, Zhang YJJ, et al. Poly-GR dipeptide repeat polymers correlate with neurodegeneration and Clinicopathological subtypes in C9ORF72-related brain disease. Acta neuropathol commun. 2018;6(1):63.
55. Saberi S, Stauffer JE, Jiang J, et al. Sense-encoded poly-GR dipeptide repeat proteins correlate to neurodegeneration and uniquely co-localize with TDP-43 in dendrites of repeat-expanded C9orf72 amyotrophic lateral sclerosis. Acta Neuropathol. 2018;135(3):459-74.
56. Quaegebeur A, Glaria I, Lashley T, et al. Soluble and insoluble dipeptide repeat protein measurements in C9orf72-frontotemporal dementia brains show regional differential solubility and correlation of poly-GR with clinical severity. Acta neuropathol commun. 2020;8(1):184.
57. Proudfoot M, Gutowski NJ, Edbauer D, et al. Early dipeptide repeat pathology in a frontotemporal dementia kindred with C9ORF72 mutation and intellectual disability. Acta Neuropathol. 2014;127(3):451-8.
58. Vatsavayai SC, Yoon SJ, Gardner RC, et al. Timing and significance of pathological features in C9orf72 expansion-associated frontotemporal dementia. Brain. 2016;139(12):3202-16.
59. Sampognaro PJ, Vatsavayai SC, Cosme CG, et al. C9orf72-specific phenomena associated with frontotemporal dementia and gastrointestinal symptoms in the absence of TDP-43 aggregation. Acta Neuropathol. 2019;138(6):1093-7.
60. Frick P, Sellier C, Mackenzie IRA, et al. Novel antibodies reveal presynaptic localization of C9orf72 protein and reduced protein levels in C9orf72 mutation carriers. Acta neuropathol commun. 2018;6(1):72.
61. Shi Y, Lin S, Staats KA, et al. Haploinsufficiency leads to neurodegeneration in C9ORF72 ALS/FTD human induced motor neurons. Nat Med. 2018;24(3):313-25.
62. O'Rourke JG, Bogdanik L, Yáñez A, et al. C9orf72 is required for proper macrophage and microglial function in mice. Science. 2016;351(6279):1324-9.
63. Burberry A, Wells MF, Limone F, et al. C9orf72 suppresses systemic and neural inflammation induced by gut bacteria. Nature. 2020;582(7810):89-94.
64. Zhu Q, Jiang J, Gendron TF, et al. Reduced C9ORF72 function exacerbates gain of toxicity from ALS/FTD-causing repeat expansion in C9orf72. Nat Neurosci. 2020;23(5):615-24.
65. Boivin M, Pfister V, Gaucherot A, et al. Reduced autophagy upon C9ORF72 loss synergizes with dipeptide repeat protein toxicity in G4C2 repeat expansion disorders. EMBO J. 2020;39(4):e100574.
66. Tran H, Moazami MP, Yang H, et al. Suppression of mutant C9orf72 expression by a potent mixed backbone antisense oligonucleotide. Nat Med. 2022;28(1):117-24.
67. Czuppa M, Dhingra A, Zhou Q, et al. Drug screen in iPSC-Neurons identifies nucleoside analogs as inhibitors of (G4C2)n expression in C9orf72 ALS/FTD. Cell Rep. 2022;39(10):110913.
68. Wilson KM, Katona E, Glaria I, et al. Development of a sensitive trial-ready poly(GP) CSF biomarker assay for C9orf72-associated frontotemporal dementia and amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry. 2022;93(7):761-71.
69. Krishnan G, Raitcheva D, Bartlett D, et al. Poly(GR) and poly(GA) in cerebrospinal fluid as potential biomarkers for C9ORF72-ALS/FTD. Nat Commun. 2022;13(1):2799.
70. Gendron TF, Chew J, Stankowski JN, et al. Poly(GP) proteins are a useful pharmacodynamic marker for C9ORF72-associated amyotrophic lateral sclerosis. Sci Transl Med. 2017;9(383).
71. Lehmer C, Oeckl P, Weishaupt JH, et al. Poly-GP in cerebrospinal fluid links C9orf72-associated dipeptide repeat expression to the asymptomatic phase of ALS/FTD. EMBO Mol Med. 2017;9(7):859-68.
72. Cammack AJ, Atassi N, Hyman T, et al. Prospective natural history study of C9orf72 ALS clinical characteristics and biomarkers. Neurology. 2019;93(17):e1605-17.
73. Mori K, Ikeda M. Biological basis and psychiatric symptoms in frontotemporal dementia. Psychiatry Clin Neurosci. 2022;76(8):351-60.
74. Snowden JS, Rollinson S, Thompson JC, et al. Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. Brain. 2012;135(3):693-708.
75. Devenney E, Hornberger M, Irish M, et al. Frontotemporal dementia associated with the C9ORF72 mutation: A unique clinical profile. JAMA Neurol. 2014;71(3):331-9.