

REVIEW ARTICLE

C9orf72 hexanucleotide repeat expansion in amyotrophic lateral sclerosis and frontotemporal dementia: Molecular pathogenesis and therapeutic implications

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Abstract

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are neurodegenerative disorders that share clinical, pathological, and genetic overlap, most notably through mutations in the *C9orf72* gene. Since its discovery in 2011, this mutation has emerged as a key driver of neurodegeneration through a complex interplay of toxic gain-of-function and loss-of-function mechanisms. The repeat expansion disrupts RNA metabolism, proteostasis, and nucleocytoplasmic transport, while impairing autophagy and endolysosomal trafficking due to reduced *C9orf72* protein expression. Concurrently, bidirectional transcription of repeat-containing RNA generates RNA foci and dipeptide repeat proteins via repeat-associated non-AUG translation, sequestering RNA-binding proteins and inducing cellular stress. These processes converge on neuroinflammation, mitochondrial dysfunction, and TDP-43 proteinopathy—culminating in a rapidly progressive clinical phenotype with frequent cognitive involvement. This review synthesizes recent mechanistic, clinical, and therapeutic advances in *C9orf72*-associated ALS/FTD. It highlights the growing relevance of biomarkers such as neurofilament light chain and poly-GP, the failure of antisense oligonucleotide therapy (BIIB078) to modify TDP-43 pathology despite adequate CNS penetration, and the promise of emerging approaches, including allele-specific CRISPR-Cas9 editing, autophagy modulation, and inflammasome inhibition. Future research must integrate multi-omics datasets, patient-derived organoids, and advanced preclinical models to clarify the relative contributions of gain- and loss-of-function mechanisms. Bridging these insights with precision medicine frameworks offers the most compelling path toward mechanism-driven interventions capable of altering the natural course of *C9orf72*-related neurodegeneration.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by degeneration of upper and lower motor neurons, leading to muscle weakness, paralysis, and ultimately respiratory failure.¹ The global incidence of ALS is approximately 1–2 cases per 100,000 person-years, with a median survival of 3–5 years

from symptom onset.^{1,2} Although most cases are sporadic, approximately 5–10% are familial and linked to defined genetic mutations.³

Frontotemporal dementia (FTD) is a clinically and pathologically heterogeneous neurodegenerative disorder marked by progressive deterioration in behaviour, executive function, and language. FTD represents one of the most common causes of early-onset dementia, with symptom onset typically before the age of 65.⁴ Neuropathologically, ALS and FTD converge through shared mechanisms, most notably TDP-43 proteinopathy, supporting their classification as a disease continuum rather than distinct entities.⁵

A major breakthrough in understanding this continuum was the discovery of the GGGGCC hexanucleotide repeat expansion in the *C9orf72* gene, which represents the most frequent genetic cause of both ALS and FTD.⁶ This

expansion occurs in the first intron of the gene and gives rise to a complex pathogenic cascade involving both toxic gain-of-function mechanisms—mediated by repeat RNA and dipeptide repeat (DPR) proteins—and loss-of-function effects resulting from reduced *C9orf72* expression.^{7,8}

Clinically, *C9orf72*-associated disease is characterized by earlier onset, rapid progression, and a high prevalence of cognitive and behavioral impairment compared with non-*C9orf72* ALS.⁹ These features underscore the importance of understanding the molecular and cellular consequences of the repeat expansion to enable rational therapeutic development.

This review synthesizes current knowledge on the molecular pathogenesis, clinical phenotype, disease models, and therapeutic strategies targeting *C9orf72*-associated ALS and FTD, with emphasis on translational relevance and emerging precision-medicine approaches (Table 1).

Table 1. Global and regional incidence and prevalence of amyotrophic lateral sclerosis

Region/Country	Incidence (per 100,000 person-years)	Prevalence (per 100,000)	Notes/Source
Global (meta-analysis)	0.8–2.5	4.5–8.0	Higher in Europe ^{10–13}
Europe (e.g., Italy)	3.0–3.8	10–16 (projected 2040)	Increasing due to aging ¹³
United States	~1.5–2.0	9.7–11.2 (2024–2040)	~30,000 cases projected by 2040 ^{13,14}
China	Rising	Significant increase expected	42.7% rise in cases by 2040 ¹³

Note: Data synthesized from Refs.^{10–14}

2. Epidemiology and disease burden

The *C9orf72* hexanucleotide repeat expansion constitutes a major global health burden, representing the most prevalent genetic mutation in both ALS and FTD worldwide.^{6,15–17} Large-scale epidemiological studies have established that expansion accounts for approximately 20–67% of familial ALS cases, depending on the population studied, with more consistent estimates of 39–40% in European and North American cohorts.^{6,18–21}

In familial FTD, the mutation has a similarly high prevalence rate, contributing to 12–50% of cases worldwide, with consistent reports of approximately 25% across multiple populations.^{6,18,20–22} Finland represents a notable outlier with exceptionally high rates, where the expansion explains nearly 50% of familial frontotemporal lobar degeneration (FTLD) cases.²²

The expansion also contributes significantly to sporadic forms of both diseases, accounting for 5–15% of sporadic ALS cases and 2–16% FTD cases across different populations.^{6,17,18,20,23} In the United States specifically, the mutation accounts for approximately 10% of all ALS

diagnoses and 10–15% of all FTD diagnoses.²¹

Population-level studies indicate that *C9orf72* expansions occur in approximately 0.14% (1 in 700) of the general population, with expanded alleles found in 0.2–0.3% of unaffected individuals in large cohort studies.^{17,19} The mutation demonstrates high but incomplete penetrance, being non-penetrant in individuals younger than 35 years, 50% penetrant by age 58, and almost completely penetrant by age 80.^{6,24}

2.1. Ethnic and geographic variability

The *C9orf72* expansion shows dramatic ethnic and geographic variation in prevalence, with the most pronounced differences observed between Asian and European populations. In Caucasian populations, the expansion serves as the leading genetic cause of both ALS and FTD, accounting for 21.7–47.0% of familial ALS cases and 13.8–48.1% of familial FTD cases.^{6,25} In contrast, the mutation is extremely rare in Asian populations, explaining only around 0.4% of ALS cases in Japan and 3.5% of familial ALS cases in Chinese populations.^{26,27} The rarity in Asian populations is further emphasized by the

identification of only two Chinese cases of *C9orf72*-related FTD reported to date.^{28,29}

Within European populations, notable geographic clustering occurs, with higher prevalence rates observed in northern European and genetically isolated populations.³⁰ Finland represents the most extreme example, where the *C9orf72* mutation reaches an exceptional prevalence rate of 46% of all familial ALS cases, 21.1% of sporadic ALS cases, and 29.3% of familial FTD cases.³⁰ Similarly elevated rates are found in other isolated populations such as Sardinia.³⁰

The ethnic variability extends beyond the simple presence or absence of the mutation to influence the characteristics of normal repeat lengths. Asian populations display shorter normal repeat lengths compared to Caucasians, and the length of hexanucleotide repeats appears to be influenced by both ethnicity and underlying haplotype.³¹ Despite this ethnic variation, meta-analyses demonstrate that *C9orf72* repeat expansions are positively associated with ALS risk in both Caucasian and Asian populations, though with markedly different effect sizes (odds ratio: 57.56 vs. 6.35).³²

While the mutation is predominantly found in European populations, *C9orf72* expansions have been identified across diverse ethnic groups, including Middle Eastern, African American, and Asian patients.^{30,33} In Latin American populations, such as Argentina, the expansion accounts for 18.2% of FTD cases and shows familial clustering like European populations.^{6,34}

3. Background

3.1. The *C9orf72* gene and its normal function

The *C9orf72* gene, located on chromosome 9p21.2, codes for a protein that plays an important role in maintaining cellular homeostasis through several mechanisms.³⁵ In healthy neurons and glial cells, *C9orf72* regulates the degradation and recycling of cellular components, contributing to the removal of misfolded proteins and damaged organelles.³⁵

Firstly, it is essential for autophagy, as it activates small GTPases and enables the formation of the homotypic fusion and protein sorting complex to promote autophagosome-lysosome fusion, subsequently generating the autolysosome necessary for autophagic flux.³⁶ In addition, *C9orf72* is involved in endolysosomal trafficking by modulating Rab family GTPases; loss-of-function or toxic gain-of-function results in defects in endosome-lysosome transport.³⁷ Moreover, *C9orf72* participates in immune regulation within microglia by modulating synaptic pruning and pro-inflammatory signalling.³⁸ *C9orf72* encodes two isoforms, with the long neuronal isoform being critical

for endolysosomal trafficking; its loss amplifies repeat-induced toxicity.³⁹ The high evolutionary conservation of *C9orf72* underscores its essential role, while pathogenic repeat expansions show variable penetrance, and the frequency of the expansion is particularly high in European populations.⁴⁰

It is important to clarify the reported variability in repeat length thresholds across studies. In healthy individuals, the GGGGCC repeat typically ranges from 2 to fewer than 30 units^{4,31,41,42}, with the majority of alleles containing ≤ 11 repeats. Some population-based studies report rare non-pathogenic alleles extending up to 45 repeats.⁴³

This variability reflects differences in repeat-sizing methodologies, assay resolution, and population-specific genetic background. These intermediate repeat lengths are considered non-pathogenic and do not result in clinical disease.³³ In contrast, pathogenic expansions in ALS and FTD consist of hundreds to thousands of repeats, clearly distinguishing physiological variation from disease-causing expansions.⁴⁴ This hexanucleotide repeat expansion—the most common genetic cause of ALS—is detected in approximately 25–40% of familial ALS cases and 5–10% of sporadic ALS cases.⁴⁵

3.1.1. Genetic and transcriptional mechanisms

C9orf72 repeat expansions exert dual pathogenic effects through gain-of-function and loss-of-function mechanisms.⁴⁶ Expanded repeats reduce *C9orf72* expression through epigenetic silencing, including CpG island hypermethylation and histone H3K9 trimethylation.⁴⁷ This loss-of-function disrupts vesicular trafficking and autophagy, processes in which *C9orf72* acts as a Rab1a effector recruiting the ULK1 complex to initiate autophagosome formation.⁴⁸ Deficiency leads to autophagic blockade, accumulation of p62 and ubiquitinated inclusions, and impaired proteostasis.⁴⁸

Conversely, toxic gain-of-function arises from bidirectional transcription of expanded repeats, generating sense and antisense RNAs that form nuclear RNA foci.⁴⁹ These foci sequester RNA-binding proteins such as matrin-3, with colocalization observed in a majority of affected neurons.⁴⁹ This sequestration disrupts RNA splicing, nucleocytoplasmic transport, and stress granule dynamics.⁴⁹

Emerging evidence suggests RNA-editing mechanisms may modulate toxicity.⁴⁹ ADAR-mediated A-to-I editing of G4C2 repeats reduces RNA toxicity in preclinical models, although validation in human systems remains limited.⁵⁰ Collectively, epigenetic silencing and RNA-mediated toxicity converge to disrupt transcriptional homeostasis at the *C9orf72* locus.

3.1.2. Dipeptide repeat proteins and cellular toxicity

Repeat-associated non-AUG (RAN) translation of expanded sense and antisense transcripts generates five DPR proteins: poly-GA, poly-GP, poly-GR, poly-PA, and poly-PR.⁵¹ These DPRs accumulate in neuronal and glial cytoplasm and nuclei, exerting widespread cytotoxic effects.⁵² Arginine-rich DPRs (poly-GR and poly-PR) are particularly neurotoxic, disrupting mitochondrial oxidative phosphorylation, inhibiting ribosomal translation, impairing proteasomal and autophagic flux, and altering nucleocytoplasmic transport.^{51,52}

The accumulation of DPR further promotes cytoplasmic mislocalization of TDP-43, linking *C9orf72*-mediated toxicity to canonical ALS proteinopathy.^{48,53} The convergence of DPR toxicity, impaired proteostasis, and TDP-43 dysfunction drives cellular collapse and neuronal death.^{48,53}

3.1.3. Cellular homeostasis and immune dysregulation

Loss of *C9orf72* function extends beyond neurons to immune regulation within the central nervous system. Under physiological conditions, *C9orf72* forms a complex with SMCR8 and WDR41 that regulates Rab GTPases involved in endolysosomal trafficking. Deficiency in this pathway leads to lysosomal dysfunction and accumulation of undegraded substrates, including toxic DPR aggregates.⁵⁴

In microglia, *C9orf72* haploinsufficiency results in aberrant activation, excessive synaptic pruning, and increased release of pro-inflammatory cytokines such as tumor necrosis factor- α and interleukin-1 β .⁵⁵ Activation of NF- κ B signaling and the NLRP3 inflammasome establishes a chronic neuroinflammatory state, creating a non-cell-autonomous mechanism that accelerates motor neuron degeneration.⁵⁵

3.2. Clinical and pathological features of *C9orf72*-associated amyotrophic lateral sclerosis

Longitudinal studies demonstrate that neurofilament light chain (NfL) levels correlate with disease stage and prognosis, with thresholds above 100 pg/mL predicting rapid progression and reduced survival.⁹ Clinically, *C9orf72*-associated ALS is more aggressive, with shorter survival (mean ~2.8 years) and higher prevalence of cognitive impairment than non-*C9orf72* ALS.⁹ Approximately 50% of patients show cognitive deficits, and 10–15% meet diagnostic criteria for FTD.⁴⁶

Neuropathologically, *C9orf72*-ALS is characterized by TDP-43 proteinopathy and p62-positive, TDP-43-negative DPR inclusions distributed across cortical, brainstem, and

spinal regions.⁵⁶ Positron emission tomography (PET) imaging using [¹¹C]PK11195 demonstrates microglial activation, while diffusion tensor imaging reveals corticospinal tract degeneration.⁵⁷

Biomarker studies identify elevated NfL and phosphorylated neurofilament heavy chain (pNfH) levels in cerebrospinal fluid (CSF) and blood as indicators of disease severity and prognosis.^{58–60} While DPR species such as poly-GR and poly-GA show limited diagnostic utility, CSF poly-GP has emerged as a reliable pharmacodynamic biomarker for presymptomatic carriers and therapeutic monitoring.^{58,61}

The failure of the BIIB078 antisense oligonucleotide (ASO) trial highlights challenges in clinical translation, despite effective CNS target engagement.^{61,62} These findings underscore the need for multi-targeted therapeutic strategies addressing both gain- and loss-of-function mechanisms.

4. Disease models and preclinical studies

A variety of preclinical frameworks have been established to explore the molecular and cellular processes driving *C9orf72*-linked ALS and FTD. Human induced pluripotent stem cell (iPSC)-derived motor neurons and three-dimensional brain organoids sourced from patients replicate critical pathological signatures, such as the development of RNA foci, the buildup of DPR proteins, failures in nucleocytoplasmic transport, and early neurodegenerative traits.

These models offer direct insights into human-specific disease pathways and allow for the assessment of potential treatments.^{4,37,61} *In vivo* systems, notably transgenic mice expressing expanded GGGGCC repeats alongside *C9orf72*-knockdown models, have been pivotal in confirming that both toxic gain-of-function mechanisms and loss of normal protein function contribute to disease pathogenesis. Specifically, they have elucidated the roles of DPR toxicity, microglial triggers, immune system imbalances, and defects in the autophagy pathway.^{13,37,38}

Furthermore, viral vector-based techniques permit the accelerated control of repeat expression and support the development of gene-editing strategies, though they might not entirely mirror the natural timeline of the disease.⁶³ Together, these synergistic platforms establish the basis for uncovering biological mechanisms and conducting the initial evaluation of precision medicines (Table 2).

5. Biomarkers

5.1. Dipeptide repeat proteins as biomarkers

Dipeptide repeat proteins, particularly poly-GP, poly-GA,

Table 2. Preclinical models used to study C9orf72-associated ALS and FTD

Model type	System	Pathological features recapitulated	Key insights gained	Limitations	References
iPSC-derived motor neurons	Human iPSCs from C9orf72-ALS/FTD patients	RNA foci, DPR accumulation, nucleocytoplasmic transport defects, neuronal vulnerability	Demonstrated bidirectional transcription, DPR toxicity, and rescue via ASOs and CRISPR-mediated repeat excision	Limited maturation; lack of immune components	4,37,61
iPSC-derived brain organoids	3D human cortical organoids	Neuronal network disruption, repeat-dependent toxicity, early neurodegenerative phenotypes	Captures human-specific repeat-length effects and early disease mechanisms	Variability between organoids; limited long-term aging	4,61
Transgenic mouse models (repeat expansion)	BAC or knock-in mice carrying expanded GGGGCC repeats	RNA foci, DPRs, synaptic dysfunction, behavioral abnormalities	Validated gain-of-function toxicity and therapeutic reduction of DPRs	Often lack robust motor neuron degeneration	37,63
C9orf72 knockout mouse models	C9orf72 loss-of-function mice	Microglial activation, immune dysregulation, lysosomal and autophagy defects	Established role of C9orf72 in immune homeostasis and neuroinflammation	Does not model repeat-mediated RNA/DPR toxicity	13,37
Viral vector-based models	AAV-mediated GGGGCC repeat expression in rodents	DPR accumulation, neurotoxicity, motor deficits	Enables rapid modelling of repeat toxicity and testing of gene-editing approaches	Overexpression artifacts; limited disease duration	63

Abbreviations: AAV: Adeno-associated virus; ALS: Amyotrophic lateral sclerosis; ASO: Antisense oligonucleotide; BAC: Bacterial artificial chromosome; DPR: Dipeptide repeat; FTD: Frontotemporal dementia; iPSC: Induced pluripotent stem cell.

and poly-GR, serve as highly specific diagnostic biomarkers for C9orf72-related ALS and FTD that can be detected in CSF even before symptom onset. These proteins show exceptional promise as target engagement biomarkers for clinical trials, as their levels decrease rapidly in response to ASO treatments.

The five DPR proteins generated by RAN translation of the C9orf72 expansion—poly-GP, poly-GA, poly-GR, poly-PR, and poly-PA—represent uniquely specific biomarkers for C9orf72-related diseases.⁶⁴ Among these, poly-GP has emerged as the most extensively validated biomarker due to its relative abundance and reduced aggregation tendency compared to other DPRs.⁶⁵ Highly sensitive immunoassays using platforms such as Meso Scale Discovery ELISA and single-molecule array (Simoa) technology can detect these proteins in CSF with remarkable specificity.^{65,66}

The diagnostic utility of DPRs is exceptional, with poly-GP showing 100% specificity and 100% sensitivity for detecting C9orf72 expansion carriers.^{58,66} Importantly, significant poly-GP levels are detectable in asymptomatic C9orf72 mutation carriers compared to healthy controls and patients with other neurodegenerative diseases, with levels in presymptomatic carriers being similar to symptomatic cases.^{66–68} This finding suggests that DPR production occurs early in the disease process and may contribute to disease pathogenesis.⁶⁹

Recent advances have enabled the detection of additional DPRs beyond poly-GP. Sensitive assays can now measure poly-GA and poly-GR in CSF of C9orf72 patients, with average levels of these proteins being similar between symptomatic and presymptomatic carriers.^{65,66,69,70} Novel antibody combinations have also been developed that can detect DPRs in iPSC-derived motor neurons, expanding the potential applications of these biomarkers.⁷¹

While DPR levels do not correlate with traditional clinical measures such as age at disease onset, disease duration, or rate of functional decline, they serve as excellent target engagement biomarkers for therapeutic trials.^{66,67,70} In a patient with C9orf72-ALS treated with ASO targeting the aberrant C9orf72 transcript, CSF levels of poly-GA and poly-GR decreased approximately 50% within six weeks, demonstrating their utility as pharmacodynamic biomarkers.^{66,70} The stability of DPR levels over time in individual patients further supports their use as reliable biomarkers for monitoring therapeutic response.⁶⁸

Blood-based approaches have also shown promise, with studies identifying antibodies against poly-GP/poly-GR DPRs that can distinguish C9orf72-positive from C9orf72-negative patients, achieving an area under the curve of 0.71.⁷² Additionally, poly-GP proteins have been detected in peripheral mononuclear cells of presymptomatic C9orf72 mutation carriers, potentially offering a less

invasive biomarker approach.⁶⁹ These DPR biomarkers are particularly valuable as they represent direct products of the disease-causing mechanism and serve as both diagnostic markers and indicators of target engagement in emerging *C9orf72*-targeted therapies.^{21,24}

5.2. Neurofilament light chain and heavy chain proteins

Neurofilament light chain and pNfH serve as highly validated diagnostic and prognostic biomarkers for *C9orf72*-related ALS and FTD, with elevated levels in both CSF and blood correlating with disease progression and survival. These proteins can be detected before symptom onset in presymptomatic carriers and show promise as pharmacodynamic biomarkers for clinical trials.

Neurofilament proteins, particularly NfL and pNfH, have emerged as the most extensively validated biomarkers for *C9orf72*-related ALS and FTD.⁷³ As structural components of the axonal cytoskeleton, these proteins are released into CSF and blood following axonal damage or degeneration, providing a direct measure of ongoing neurodegeneration.⁷³

The diagnostic performance of neurofilament proteins is exceptional, with NfL showing high sensitivity (85.5–91.3%) and specificity (81.8–91.0%) for distinguishing ALS from healthy controls and other neurological conditions.^{74,75} In *C9orf72* expansion carriers, both NfL and pNfH levels are significantly elevated compared to controls and other ALS patients, with particularly high levels observed in *C9orf72*-positive cases compared to *C9orf72*-negative patients.^{76,77}

The prognostic utility of neurofilament proteins is remarkable, with higher levels consistently associated with faster disease progression rates, shorter survival, and greater cortical volume loss in FTD patients.^{73,74,78} Recent studies demonstrate that NfL levels correlate strongly with disease progression rate ($p = 0.72$) and can predict survival with high accuracy.⁷⁹ pNfH shows particular promise in bulbar-onset ALS, achieving a specificity of 89% in predicting 12-month mortality.⁷⁹

Importantly, neurofilament levels are elevated in presymptomatic *C9orf72* expansion carriers, appearing to rise before symptom onset and presaging the onset of neurodegeneration.^{80,81} Longitudinal studies show that plasma NfL levels can be elevated up to five years before disease onset in both sporadic and familial ALS cases.^{79,82}

The clinical implementation of neurofilament testing has been facilitated by advances in ultrasensitive detection platforms, including single-molecule array (Simoa) technology and clinical-grade platforms such as

Lumipulse and Elecsys.^{79,83} Multiple assay platforms show high correlation ($R^2 = 0.939$ – 0.963) for NfL quantification, with all platforms demonstrating excellent diagnostic performance.⁸⁴

For clinical trial applications, neurofilament proteins offer significant advantages as pharmacodynamic biomarkers. The stability of NfL levels over time in individual patients, combined with their correlation with progression rates, makes them ideal for monitoring therapeutic response.^{73,85} Studies indicate that incorporating baseline serum NfL into clinical trial designs could yield sample size savings of approximately 8% compared to traditional functional rating scales.⁸⁵

Additionally, immune complexes containing neurofilament proteins and antibodies against neurofilament chains show distinct patterns in *C9orf72*-positive patients, with neurofilament-light immune complexes achieving an area under the curve of 0.69 for separating *C9orf72*-positive from *C9orf72*-negative patients.⁷² These findings suggest that the immune response to neurofilament proteins may provide additional biomarker information beyond simple protein concentration measurements.⁷²

5.3. Cryptic exons and TDP-43-related biomarkers

Cryptic exon inclusion resulting from TDP-43 dysfunction represents a highly specific early biomarker of *C9orf72*-related ALS and FTD, because it generates aberrant transcripts and peptide products that can be detected in biofluids.⁸⁶ Unlike neurofilament proteins that reflect broad neurodegeneration, cryptic exon-encoded peptides provide specificity for TDP-43-related disease and directly measure TDP-43 dysfunction.⁸⁶ This functional relevance makes them particularly valuable as target engagement biomarkers for therapeutics aimed at restoring TDP-43 function.⁸⁶

The most extensively characterized cryptic exon biomarker is derived from the *HDGFL2* gene, which can be detected using specific monoclonal antibodies in both CSF and blood.⁸⁶ Cryptic gene accumulates at significantly higher levels in familial ALS-FTD and sporadic ALS compared to controls, and notably appears elevated earlier than neurofilament light and pNfH proteins in familial disease.⁸⁶ The temporal advantage of cryptic is particularly evident, as it accumulates in CSF at higher levels during early stages of disease, contrasting with neurofilament proteins that increase later.⁸⁶

The biomarker trajectory for cryptic exons versus neurofilament proteins suggests a clinically useful diagnostic paradigm. The ratio of neurofilament proteins to cryptic *HDGFL2* is less than 1 during the presymptomatic

stage and increases to greater than 1 as symptom onset occurs, potentially enabling prediction of phenoconversion in familial ALS.⁸⁶

Additional cryptic exon biomarkers have been identified in other genes critical for neuronal function. Stathmin-2 (STMN2) represents a particularly well-validated target, as TDP-43 dysfunction causes truncated STMN2 accumulation through altered splicing.^{87–89} Truncated STMN2 serves as a biomarker for both ALS and FTD, with levels confined to tissues and disease subtypes marked by TDP-43 inclusions.^{78,87} In FTLD-TDP cases, truncated STMN2 RNA shows noteworthy associations with phosphorylated TDP-43 levels and earlier age of disease onset.⁸⁷

The *UNC13A* gene provides another cryptic exon biomarker with direct genetic relevance to disease risk. TDP-43 depletion induces robust inclusion of a cryptic exon in *UNC13A*, resulting in nonsense-mediated decay and loss of UNC13A protein.⁹⁰ Common intronic UNC13A polymorphisms strongly associated with ALS and FTD risk overlap with TDP-43 binding sites and potentiate cryptic exon inclusion.^{90,91} This creates opportunities for genetic stratification, as UNC13A-derived cryptic peptides enable identification of molecular subtypes linked to TDP-43 pathology.⁹²

The clinical implementation of cryptic exon biomarkers is being facilitated by advanced detection methods, including mass spectrometry approaches that can detect TDP-43-dependent cryptic peptides in *C9orf72*-associated ALS.^{78,93} These biomarkers are especially valuable for precision medicine applications, as they provide both diagnostic information and functional readouts that could guide therapeutic interventions targeting TDP-43 dysfunction.⁷⁸

The integration of cryptic exon biomarkers into clinical practice is supported by their detection in presymptomatic *C9orf72* mutation carriers, positioning them as promising biomarkers for disease prevention trials.²¹ As the field moves toward precision trial design, these TDP-43-dependent biomarkers offer distinct advantages for real-time stratification of patients into molecular subtypes and monitoring of target engagement in therapeutic interventions.⁹²

6. Multi-omics and advanced detection approaches

Advanced multi-omics approaches are revolutionizing *C9orf72* biomarker discovery by integrating high-throughput genomics, proteomics, and transcriptomics data to identify distinct patient subtypes and enable

precision medicine. Ultra-sensitive detection platforms like single-molecule array (Simoa) technology and extracellular vesicle-based liquid biopsy assays are achieving exceptional diagnostic accuracy while enabling real-time monitoring of therapeutic responses.

The integration of high-throughput “omics” approaches has dramatically changed our understanding of ALS, improving comprehension of the complex molecular framework of ALS, discerning unique subtypes, and providing a rational foundation for the discovery of biomarkers and new individualized treatments.⁷⁶ These multi-dimensional perspectives are especially valuable for *C9orf72*-related disease, where the complex pathological mechanisms call for sophisticated analytical strategies to capture the full spectrum of molecular changes.

Ultra-sensitive detection platforms have transformed the clinical implementation of *C9orf72* biomarkers. Single-molecule array (Simoa) technology enables ultrasensitive NfL quantification that can stratify therapeutic responders in clinical trials.⁷⁹ Multiple assay platforms show exceptional consistency for neurofilament quantification, with high correlations ($R^2 = 0.939–0.963$) across Simoa, Ella, Lumipulse, and Elecsys platforms.⁹² These clinical-grade platforms are becoming standard practice and show outstanding diagnostic performance, with area under the curve values ranging from 0.889 to 0.912 for ALS diagnosis.⁹²

Advanced liquid biopsy approaches have achieved remarkable diagnostic accuracy through extracellular vesicle analysis. Plasma extracellular vesicles contain quantifiable amounts of TDP-43 and full-length tau, enabling accurate assessment of pathology in frontotemporal dementia and ALS.^{79,94} Liquid biopsy assays detecting TDP-43 fragments in exosomes achieve 94% diagnostic accuracy in presymptomatic *C9orf72* carriers, yielding a minimally invasive approach for early disease detection.⁷⁹

Transcriptomic approaches have revealed global dysregulation patterns that provide novel biomarker opportunities. In *C9orf72* expansion carriers, there is broad dysregulation of transposable elements, which are normally repressed in the human genome and are associated with atrophy of thalamic nuclei relevant to FTD.⁹⁵ Global peripheral activation of transposable elements, including the human endogenous LINE-1 element LIHS, shows association with atrophy of multiple pulvinar nuclei, suggesting that transposable element activation may constitute a functional biomarker of disease progression.⁹⁵

Mass spectrometry-based proteomics has facilitated

direct quantification of disease-relevant proteins without requiring antibodies. Ultra-sensitive mass spectrometry assays successfully quantify *C9orf72* isoform levels in human brain tissue, demonstrating a notable decrease of the *C9orf72* long isoform in the brain of *C9orf72* mutation carriers.^{93,96} These approaches have also enabled the detection of protein products translated from TDP-43 pathology-associated cryptic exon RNAs in the CSF of patients with FTD-ALS, highlighting their potential as peptide-based biomarkers.⁹³

The integration of multiple biomarker modalities is enabling sophisticated patient stratification approaches. NfL facilitates patient stratification based on clinical progression rates, enabling identification of rapid versus slow progressors, while cryptic exon-derived peptides, such as UNC13A-derived peptides, enable genetic stratification by identifying molecular subtypes linked to TDP-43 pathology.⁹² This multi-dimensional approach to biomarker integration provides the foundation for precision trial design by enabling real-time stratification of patients into molecular subtypes.⁹² Advanced imaging integration represents the next frontier in multi-omics approaches for *C9orf72* biomarker development. Increasingly, advanced imaging tools such as PET/magnetic resonance imaging (MRI), sodium imaging, and quantitative susceptibility mapping are being incorporated alongside biofluid biomarkers to provide multi-dimensional monitoring of disease progression.^{92,97} Simultaneous PET/MRI offers the perspective of an integrated platform for reproducible imaging biomarkers on neuronal damage and has the potential to become the new gold standard for characterizing motor neuron disease.⁹⁷

The clinical translation of these multi-omics approaches is facilitated by the development of biomarker panels that integrate multiple molecular readouts. These panels allow for earlier diagnosis, real-time disease monitoring, and adaptive therapeutic trial design.⁸³

Single-cell transcriptomics, CSF exosomal cargo analysis, MRI techniques, and wearable sensor outputs are developing into high-resolution windows of disease progression and onset, providing a comprehensive molecular portrait of *C9orf72*-related neurodegeneration.⁸³

7. Therapeutic strategies

Current therapeutic strategies targeting *C9orf72*-associated ALS and FTD encompass genetic, RNA-based, and downstream pathway-directed approaches, as summarized in Table 3. ASO therapy targeting repeat RNA, including BIIB078, achieved effective reduction of DPR production as reflected by decreased poly-GP levels, but failed to confer clinical benefit in early-phase trials.^{61,62} Allele-specific ASOs aim to overcome this limitation by selectively silencing the expanded *C9orf72* allele while preserving normal gene function, demonstrating repeat-selective suppression in preclinical models.⁶³ Genome-editing strategies using CRISPR-Cas9 target the expanded G4C2 repeats at the DNA level, preventing toxic RNA and DPR generation in preclinical systems.³¹ In parallel, pathway-modifying approaches—such as autophagy enhancement and inhibition of NLRP3/NF-κB-mediated neuroinflammation—address downstream consequences of *C9orf72* dysfunction, including impaired proteostasis and microglial activation.^{36,48,54,55,98} Collectively, these strategies underscore the need for combinatorial, mechanism-driven therapeutic approaches.

Table 3. Therapeutic strategies targeting C9orf72-associated ALS and FTD

Strategy	Target	Mechanism	Clinical status	Biomarker	Key references
ASOs (BIIB078)	Repeat RNA	Reduce repeat RNA and DPR production	Phase I (failed)	poly-GP	61,62
Allele-specific ASOs	Expanded <i>C9orf72</i> allele	Selective silencing of the mutant allele while preserving normal <i>C9orf72</i>	Preclinical	poly-GP	63
CRISPR-Cas9	DNA repeat expansion	Excision or repression of expanded G4C2 repeats	Preclinical	N/A	31
Autophagy enhancers	Lysosome/autophagy pathway	Restoration of autophagic flux and proteostasis	Preclinical	p62, NfL	36,48,54
Anti-inflammatory agents	NLRP3/NF-κB signalling	Reduction of microglial activation and neuroinflammation	Preclinical	Cytokines	55

Note: Data synthesized from Refs. 27,32,44,51,52,58–60

Abbreviations: ALS: Amyotrophic lateral sclerosis; ASO: Antisense oligonucleotide; DPR: Dipeptide repeat; FTD: Frontotemporal dementia; NfL: Neurofilament light chain.

8. Clinical applications and precision medicine implementation

C9orf72 biomarkers are being rapidly translated into clinical practice for early diagnosis, patient stratification, and therapeutic monitoring, with neurofilament proteins and DPR proteins leading clinical implementation. These biomarkers enable precision medicine approaches by identifying molecular subtypes, predicting therapeutic response, and supporting clinical trial design for emerging targeted therapies.

The clinical implementation of C9orf72 biomarkers addresses critical gaps in current diagnostic approaches, which rely predominantly on clinical presentation and electrodiagnostic studies that have significant limitations for early disease detection when potential treatments could be most effective.⁹⁹ Current diagnostic delays of approximately one year after symptom onset underscore the urgent need for specific biomarkers that can support early diagnosis and therapeutic intervention.¹⁰⁰

The most clinically advanced biomarkers include neurofilament proteins and DPR proteins, which are increasingly being implemented in clinical settings as both diagnostic and therapeutic monitoring tools.²⁴

Neurofilament light chain has demonstrated exceptional clinical utility, with blood-based measurements enabling patient stratification based on clinical progression rates and distinguishing between rapid versus slow progressors.⁹² The diagnostic performance is robust, with plasma NfL effectively differentiating FTD syndromes from phenocopy cases and mimics, though it shows limitations in distinguishing slow progressors from true phenocopies.¹⁰¹

Genetic stratification represents a particularly powerful precision medicine application, with C9orf72 mutations being associated with specific biomarker profiles including elevated DPR proteins and distinct neurofilament patterns.¹⁰²

Patients with C9orf72 mutations show significantly higher plasma NfL levels compared to those with SOD1 mutations, enabling genotype-based stratification.⁷⁷ Additionally, cryptic exon-derived peptides such as UNC13A-derived peptides enable molecular subtype identification linked to TDP-43 pathology.^{90–92}

The therapeutic monitoring applications of these biomarkers are transforming clinical trial design and drug development. DPR proteins serve as excellent target engagement markers for ASO therapies, with recent advances in treatments such as tofersen for SOD1-ALS and BIIB105 for C9orf72-ALS demonstrating how biomarkers can track therapeutic efficacy in real time.⁹² Neurofilament

proteins show particular promise as prognostic biomarkers, with higher levels predicting faster disease progression rates, shorter survival, and greater cortical volume loss in FTD patients.⁷⁸

The implementation of precision medicine approaches is being enhanced by comprehensive biomarker panels that integrate multiple molecular readouts. Advanced liquid biopsy approaches using extracellular vesicles have achieved 94% diagnostic accuracy in presymptomatic C9orf72 carriers through TDP-43 fragment detection.^{21,94}

These minimally invasive approaches enable non-invasive monitoring and have demonstrated strong correlations with neurodegeneration markers and disease severity measures.⁹²

Clinical implementation is being facilitated by the integration of imaging and molecular biomarkers, with advanced imaging tools such as PET/MRI, sodium imaging, and quantitative susceptibility mapping being incorporated alongside biofluid biomarkers to provide multi-dimensional monitoring of disease progression.^{92,97} This integrated approach enables real-time patient stratification into molecular subtypes and supports precision trial design.

The field is rapidly advancing toward disease prevention trials, with biomarkers including blood NfL, CSF DPR proteins, CSF and blood TDP-43 cryptic peptides, and brain volumetric measures serving as key endpoints for at-risk C9orf72 populations.²¹ Network-based proteomics approaches are identifying additional candidate biomarkers and therapeutic targets through analysis of dysregulated protein co-expression modules.¹⁰³ As the field continues to evolve, the ultimate goal remains identifying biomarkers and therapeutic agents that can cure the most common form of genetically determined FTD and ALS.¹⁰⁴

9. Implications for future research

The pathophysiology of amyotrophic lateral sclerosis (ALS) remains incompletely understood.¹⁰⁵ However, advances in genetic research have revealed how mutations in key genes converge on recurrent pathological pathways, including dysfunctional autophagy, impaired RNA metabolism, mitochondrial dysfunction, cytoskeletal disruption, defective DNA repair, and oxidative stress.^{57,58,60} Commonly implicated genes include C9orf72—through hexanucleotide repeat expansions that induce proteostasis defects and oxidative stress—as well as TARDBP and FUS, whose mutations disrupt RNA processing.^{57,58} Genetic diagnostic analyses further underscore the limited heritability in most ALS cases, with rare variants frequently identified in untranslated regions (UTRs) of established

disease genes (*SOD1*, *TARDBP*, *FUS*, *VCP*, *OPTN*, *UBQLN2*)(59). These findings highlight the contribution of non-coding regulatory regions to ALS pathogenesis and reinforce the need for expanded genomic interrogation.

For *C9orf72*-associated ALS specifically, two main hypotheses—gain-of-function toxicity and loss-of-function haploinsufficiency—have been proposed to explain neuronal injury.¹⁰⁶ Yet the precise molecular mechanisms remain elusive, compounded by the absence of fully reliable animal models.¹⁰⁷ This uncertainty has left several downstream pathways underexplored, such as the interplay between DPR-induced nucleocytoplasmic transport defects and microglial neuroinflammation, or the modulatory role of repeat length variability on penetrance.⁶¹ These gaps not only obscure mechanistic clarity but also underscore the need for robust preclinical systems and biomarker discovery pipelines to inform targeted therapies.

Recent bibliometric analyses reveal a surge in *C9orf72*-focused research since 2014, catalysed by genetic breakthroughs and the Ice Bucket Challenge, with emerging hotspots in biomarker development—particularly NfL—and therapies targeting repeat expansions.⁶² Population-specific studies, such as the 13.5% prevalence of *C9orf72* expansions in Czech ALS cohorts (including 42.9% in sporadic cases overlapping with frontotemporal dementia), emphasize the diagnostic value of next-generation sequencing in early screening, particularly among bulbar-onset or FTD-linked patients.⁴⁰ These findings align with precision medicine initiatives, where expanded genomic profiling could uncover variants of uncertain significance and oligogenic interactions, enabling individualized therapeutic interventions in underrepresented populations.

Therapeutically, resolving *C9orf72*'s dual pathogenic mechanisms remains central. ASOs such as BIIB078 demonstrated adequate CNS penetration and partial DPR reduction but failed to ameliorate TDP-43 pathology or improve proteomic and clinical outcomes, highlighting the need for biomarkers reflecting disease-relevant molecular change (e.g., RNase T2 or inflammatory mediators like CCL26).⁹ Future directions include refining allele-specific CRISPR-Cas9 strategies for targeted repeat excision—which have rescued pathological phenotypes in iPSC-derived motor neurons—and combining these with RNA interference to minimize off-target microglial effects.⁶³ Preclinical evidence of viral vector-delivered editing (e.g., AAV9-Cas9) extending survival in *C9orf72* mice supports translation into early-phase trials focused on presymptomatic carriers, facilitated by novel delivery systems such as focused ultrasound and exosome-mediated

transport.¹⁰⁸ Integrative multi-target approaches—combining ASOs with small molecules like molecular tweezers to inhibit DPR aggregation and oxidative stress—represent another promising avenue.

Neuroinflammation, exacerbated by *C9orf72* haploinsufficiency in microglia, constitutes a critical yet underexplored dimension, with elevated CSF markers (e.g., chitotriosidase, MCP-1) correlating with accelerated progression and autoimmune comorbidities.¹⁰⁷ Longitudinal neuroimaging ([¹¹C]PK11195-PET for microglial activation) coupled with multi-omics profiling could elucidate the contributions of genetic modifiers such as TBK1, guiding anti-inflammatory interventions including PIKfyve inhibitors (e.g., LAM-002) that mitigate poly-GP aggregation.⁵⁷ Addressing these questions demands refined preclinical systems—particularly iPSC-derived organoids and single-cell transcriptomics—to recapitulate human repeat variability and the ALS-FTD continuum.

Future research aims to delineate the relative contributions of toxic gain- and loss-of-function mechanisms in individual patients through integrative multi-omics approaches—transcriptomic, proteomic, and epigenetic—toward constructing comprehensive models of disease.¹⁰⁹ The development of patient-derived organoids and sophisticated animal models is expected to deepen mechanistic insight and accelerate the identification of synergistic therapeutic strategies.¹¹⁰ Ultimately, bridging the gap between mechanistic understanding and clinical translation will require coordinated efforts uniting molecular neuroscience, genomics, and clinical neurology.¹¹¹ As knowledge advances, *C9orf72*-ALS stands poised at the forefront of a paradigm shift—from descriptive pathology toward precision, mechanism-driven therapeutics capable of addressing the root causes of neurodegeneration.

These strategies highlight the necessity of a systems-level understanding of *C9orf72* pathobiology to enable precision therapeutics.

10. Conclusion

The discovery of the *C9orf72* hexanucleotide repeat expansion has redefined our understanding of ALS and FTD, uniting motor and cognitive degeneration under a shared molecular umbrella. Evidence consistently points to a dual-pathogenic model, wherein toxic gain-of-function from aberrant RNA and DPR accumulation intersects with loss-of-function due to *C9orf72* haploinsufficiency, together driving cellular collapse. These mechanisms impair key homeostatic systems—autophagy, vesicular trafficking, proteostasis, and nucleocytoplasmic

transport—while amplifying neuroinflammatory cascades mediated by NF- κ B and NLRP3 activation. The resulting pathology manifests as a clinically heterogeneous yet molecularly convergent phenotype marked by TDP-43 aggregation, glial dysfunction, and elevated biomarkers such as NfL and poly-GP.

Therapeutic progress remains challenging. Although ASO therapy (BIIB078) achieved partial DPR reduction, its failure to influence TDP-43 pathology underscores the need for multi-targeted strategies that concurrently address both upstream genetic lesions and downstream inflammatory sequelae. Promising approaches—such as allele-specific ASOs, CRISPR-mediated repeat excision, small molecules restoring lysosomal flux, and immunomodulatory agents—hold potential when integrated within biomarker-guided precision frameworks.

Moving forward, interdisciplinary convergence will be pivotal. Combining single-cell transcriptomics, multi-omics profiling, patient-derived organoids, and longitudinal neuroimaging can illuminate modifier effects (e.g., TBK1, OPTN) and clarify the determinants of phenotypic variability. By aligning mechanistic discoveries with translational innovation, C9orf72-ALS stands at the frontier of mechanism-driven therapy—offering both deeper molecular insight and a tangible pathway toward clinical intervention.

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Conflict of interest

The authors declare they have no competing interests.

Author contributions

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