Published in final edited form as:

*Lancet Neurol*. 2022 May ; 21(5): 465–479. doi:10.1016/S1474-4422(21)00414-2.

# Emerging insights into the complex genetics and pathophysiology of ALS

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## Summary

ALS is a fatal neurodegenerative disease. The discovery of ALS genes, commencing with *SOD1*, started relatively gradually. Recent advances in genetic technology have led to the rapid identification of multiple new ALS genes, and a new understanding of oligogenic and polygenic disease risk. Overlap of ALS genes with other illnesses is shedding light on the phenotypic spectrum of neurodegeneration, with a better understanding of genotype-phenotype relationships. A deepening knowledge of ALS genetic architecture is elucidating the detailed molecular steps various mutations take to converge on highly shared and recurrent dysregulated pathophysiological pathways. Of critical relevance, ALS mutations are amenable to novel gene-based therapeutic options, an approach in use for other neurological illnesses. Lastly, the influence of the exposome, the summation of lifetime environmental exposures, has grown as an emergent ALS risk through the gene-time-environment hypothesis. We anticipate our improved understanding of all these aspects of ALS will lead to long-awaited therapies and the identification of modifiable risks.

### Keywords

Amyotrophic lateral sclerosis; environmental exposure; exposome; genetic architecture; gene therapy; motor neuron disease; pathophysiology

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All authors contributed to conceptualization, writing - original draft, and writing - review & editing. SAG, OH, AAC, AC, MCK, ELF contributed to funding acquisition.

## Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons in the brain, brainstem, and spinal cord.(1) The name derives from characteristic muscle loss, *amyotrophy*, and axonal loss involving the lateral spinal cord columns, *lateral sclerosis*. ALS presents with progressive voluntary muscle weakness, which spreads to neighboring body segments, typically leading to death from respiratory failure within two to four years from diagnosis. In addition to motor neuron loss, the major histopathological findings are intracellular cytoplasmic inclusions of eosinophilic Bunina bodies and ubiquitinated TAR DNA binding protein 43 (TDP-43). There is also considerable phenotypic heterogeneity in disease presentation, involving cognitive and behavioral changes in up to 60% of cases and frontotemporal dementia (FTD) in 15% of cases secondary to structural and network brain changes.

Although there are several known genetic ALS risks, the vast majority of cases, approximately 85%, lack a single common etiology;(2) thus, pathophysiology remains incompletely understood. This lack of understanding is responsible, in part, for the absence of disease-modifying therapies. Currently, there are only two approved drugs of varying efficacy, riluzole and edaravone. Non-pharmacologic multidisciplinary care may improve patient outcomes, including early non-invasive ventilation use and feeding tube insertion before significant weight loss.(1)

This lack of treatments has spurred intense research into the complex genetics of ALS and pathomechanisms linked to known mutations. A better knowledge of genetic architecture could unlock the potential of genetic therapies. Additionally, the impact of environmental exposures, diet, and lifestyle factors on ALS risk, cumulatively known as the exposome, is needed to identify modifiable risks. This review will highlight the latest ALS advances from the past five years pertaining to complex genetics, pathophysiology, therapeutic development, and exposome science. This review is accompanied by a second more clinically focused article, focused on clinical presentation, diagnosis, and prognosis.

## Genetic architecture of ALS

ALS is conventionally classified as “familial” or “sporadic” (panel 1). However, this simple subdivision ignores complex ALS genetic architecture (Figure 1A, B, C), characterized by monogenic, oligogenic, and polygenic inheritance, gene penetrance, and heritability. Mendelian familial ALS occurs in 10–15% of patients, albeit with incomplete penetrance in most kindreds.(2, 3) In the remaining 85% of patients, large genome-wide association (GWAS) studies may identify rare variants and “private” mutations, *i.e.*, found in a single family, which may modulate disease risk and phenotypic presentation.(4)

The proportion of familial disease is likely underreported,(5) due to variation in the definition of familial ALS.(6) Consensus criteria for familial ALS were introduced nearly a decade ago, based on the likelihood two or more family members carry the same disease-causing variant. Family size is critical to this definition; in families with over 17 members, there is a 5% chance two members will be affected, based on the overall lifetime risk of

developing ALS, *i.e.*, 1:350.(5) Conversely, if one parent carries a penetrant Mendelian risk gene, the chance other family members carry the allele is low in a small family, leading to an apparent "sporadic" case of disease.(7) Moreover, some ALS genes cause FTD and/or other phenotypes; thus, there is an argument for including FTD in a kindred in the familial ALS definition, which would bring the rate closer to 20%.(5) Additionally, population studies of family aggregation of neuropsychiatric conditions within ALS kindreds suggests schizophrenia indicates familial ALS disease, bringing the rate closer to 30% (see "Genetic overlap of ALS" section).(5, 8) Validation studies are needed to determine whether to include schizophrenia in kindreds in the familial definition of ALS.

## Known ALS genes

Our current knowledge of validated ALS genes derives primarily from ancestral European (Europe, USA, Canada, Australia) and Asian populations.(9) Although at least 40 ALS genes are known, four genes account for ~48% of familial and ~5% of sporadic ALS within populations of European origin.(10) These genes include *C9orf72*, *SOD1*, *TARDBP* (coding TDP-43), and *FUS*, which have lent important biological insights into ALS pathophysiology (Table 1; see "ALS genetics informs pathophysiology" section).(11) New ALS genes have been identified in the past five years, including *TBK1*, *NEK1*, *CCNF*, *C21orf2*, *ANXA11*, *TIA1*, *KIF5A*, *GLT8D1*, *LGALSL*, and *DNAJC7* (Table 1).(2, 12) These genes also add to our biological understanding of ALS, highlighting important recurrent pathways, and possible new avenues, as outlined in-depth in the "ALS genetics informs pathophysiology" section.

Importantly, ALS genes vary in pathogenicity and how susceptible they render the carrier to disease; causative genes generally lead to disease, *e.g.*, *TARDBP*, *SOD1*, *FUS*, whereas some ALS genes do not necessarily cause disease, but rather pose a risk, *e.g.*, *ANG*, *ATXN2*, *DCTN1* (Table 1). However, even causative genes are not fully penetrant, and interactions with the environment modify risk (see "Heritability in ALS" section). Thus, ALS genes exist on a continuum of higher to lower risk genes. Even the largest genomics projects may not accurately identify rare intermediate penetrance ALS variants due to the high lifetime risk and low frequency of pathogenic alleles.

Since precision treatments against specific ALS-causing mutations are gaining importance as a therapeutic paradigm (see "Gene-based ALS treatment strategies" section), distinguishing truly pathogenic versus benign variations is essential. Guidelines for interpreting the pathogenicity of variants exist, *e.g.*, American College of Medical Genetics and Genomics (ACMG) criteria,(13) and resources such as ClinGen are available.(14) Establishing the pathogenicity of recently or newly identified ALS genes will pivot on segregation analysis, neuropathological signatures, *e.g.*, aggregates, or functional investigations in preclinical model systems.(13) Large scale analyses support a reoriented view of several ALS genes and variants confined heavily to a single domain. A recent study of published data identified ~1% as pathogenic or likely pathogenic (111 mutations in 23 genes), 10% as benign or likely benign, and over 89% as of uncertain significance.(15) Of the pathogenic or likely pathogenic variants, 10% exhibited geographic heterogeneity underlining the population-specific and environmental interactions of ALS variants.(15)

## Oligogenic and polygenic models of ALS

Since Mendelian inheritance only accounts for a fraction of cases, an oligogenic model of ALS has emerged, *i.e.*, comprising a few risk genes.(16) Although oligogenic inheritance is reported in different populations, further studies are necessary. For example, a UK study of 100 consecutively recruited ALS participants found 13% harbored two pathogenic or likely pathogenic variants, which correlated with earlier disease onset by four years.(17) An Australian study solely of sporadic ALS cases (n=616) found 6·82% of participants had two or more variants, which similarly associated with earlier disease onset.(16) By contrast, an Irish study (familial n=50; sporadic n=394), did not detect an excess in apparent oligogenic inheritance, and only 1·6% of patients harbored two or more known or potential ALS variants.(18)

There is also increasing interest in polygenic risk in ALS, assessed by linkage disequilibrium score testing and Mendelian randomization, which test associations between a particular disease or clinical phenotype with genetic variants. Analysis of GWAS data from 20,806 cases versus 59,804 controls found ALS shared polygenic risk with several traits; positive associations with smoking and moderate physical activity, and negative associations with cognitive performance and education.(19) Mendelian randomization additionally identified a causal link between hyperlipidemia and ALS risk. Indeed, a multi-ethnic GWAS identified *ACSL5* as an ALS risk, an enzyme involved in fatty acid β-oxidation and lipid biosynthesis.(20) Mendelian randomization also suggested a causal relationship between genetically determined higher leukocyte count with lower ALS risk.(21)

## Heritability in ALS

ALS is a complex trait with strong evidence of an interplay between inherited and environmental factors, including for patients that carry a highly penetrant mutation.(22) Thus, heritability, the extent disease risk variation is attributable to genetic variation, is an important concept in ALS (Figure 1D). Heritability estimates are population-specific, reflecting underlying genetic substructure and gene-environment interactions. Assessing ALS heritability have relied on studies, *e.g.*, twin (38–78%),(23) large GWAS datasets (18%),(24) and population registers (53%).(3) In the Irish ALS registry, the lifetime risk for a first-degree relative of an ALS patient without known ALS gene mutations is 0·7% and 1·4% if the genetic status is unknown.(3) This equates to an ALS heritability of 36·9% in the non-*C9orf72* population and 52·3% in the overall population. This “missing heritability” promotes a focus on epigenomics and environmental contributions in ALS. Several studies report changes to the epigenome linked to ALS, *e.g.*, non-coding promoter and enhancer elements, microRNAs.(25, 26) Additionally, the epigenome, as a reprogrammable entity through environmental pressures, opens an avenue into exposome science in ALS. The gene-time-environment hypothesis of ALS proposes a “multistep” model to account for environmental impact on disease onset and progression (see “ALS exposome” section).(22) In European and East Asian populations, the gene-environment interaction promotes disease in up to 6 steps, with fewer steps in patients harboring known monogenic, penetrant mutations, *e.g.*, *C9orf72*, *SOD1*, *TARDBP*.(27, 28) Future work is needed to precisely define a “step” and determine when one has occurred.(29)

Overall, based on recent progress, we anticipate comprehensive genetic testing will become standard practice for profiling ALS patients and will identify known pathogenic mutations in up to 70% of familial and 15% of sporadic cases.(2) This practice will also lead to the discovery of novel, as yet unknown mutations in more cases. Ultimately, case classification will shift to mutation status, rather than by dichotomization of “familial” or “sporadic”. However, genetic testing for ALS will require determining the optimal approach, which will contend with the growing number of ALS genes, dealing with polygenic risk, and whether to adopt whole-genome sequencing to address intronic variants that might contribute to ALS. Further, gaining a deeper understanding of the complex genetics of ALS and factors that influence genetic variant penetrance and polygenic risk will better predict which individuals may develop ALS.

## Genetic overlap of ALS with other neurodegenerative diseases

ALS is a clinically heterogeneous condition, extending beyond corticospinal structures.(30, 31) Imaging demonstrates thalamic and amygdala involvement as well as disrupted cortical functional networks in motor and extra-motor domains.(32–34) Extra-motor domains are primarily in executive and language function, while spatial domains are relatively preserved; additionally, social, cognitive, and behavioral changes are common, which mirror the behavioral variant of FTD.(35)

Clinical ALS phenotypes are modulated by certain genetic variants;(2, 36) *SOD1* variants primarily cause motor degeneration, whereas *FUS* mutations associate with younger onset age.(2) Additionally, certain variants impact progression rate, *e.g.*, rapidly progressive $SOD1^{A5V}$ (previously known as A4V). *C9orf72* repeat expansions are most strongly linked with cognitive and behavioral changes;(37) *FUS* and *TARDBP* mutations can also present with dementia, as can some of the rarer Mendelian ALS mutations. However, most ALS patients with cognitive changes do not carry a known genetic variant. Moreover, several mutations that represent ALS risk are genetically pleiotropic and extra-motor ALS features overlap phenotypically with other neurodegenerative diseases (panel 1).(8, 38) *C9orf72* repeat expansions are the most common mutations occurring in Huntington disease (HD) “phenocopies”, patients presenting with HD without carrying the most characteristic HD-associated mutation, huntingtin (*HTT*) repeat expansions.(39) Conversely, in rare instances, patients with FTD/ALS can harbor *HTT* repeat expansions concurrent with the histopathological ALS hallmark, TDP-43 inclusions, without defining HD characteristics such as neostriatal atrophy.(40)

Although of uncertain clinical significance due to presence in single patients, mutations to ALS risk genes, *TIA1*, *TBK1*, *SQSTM1*, and *GRN* are detected in cases of dementia with Lewy bodies (DLB), a clinically heterogeneous neurodegenerative disease.(41) A 32 CAG-repeat expansion to *ATXN2* has been reported in a patient with both ALS and spinocerebellar ataxia type 2 (SCA2);(42) intermediate 32 CAG-repeats correlate with ALS,(43) but reside below the cutoff for SCA2,(44) suggesting potential overlap between the two diseases. Additionally, pathogenic mutations to *KIF5A,* known to cause hereditary spastic paraplegia (SPG10) and Charcot-Marie-Tooth disease type 2, are also described in ALS(4) and primary progressive multiple sclerosis,(45) though mutations occur in different *KIF5A*

domains in SPG10 versus ALS. Thus, the genotype-phenotype relationship among genetic mutations that cause neurodegenerative disease is highly complex. Research is needed to determine how mutations to the same gene diverge on distinct phenotypes, and, on the other hand, how mutations to different genes converge on similar phenotypes, *e.g.*, mutations to distinct gene domains, overlap in the number of disease-causing repeats. Polygenic risk(19) and environmental influence(22) are possible factors, which are highly relevant to ALS (see "Genetic architecture of ALS" and "ALS exposome" sections).

There is also evolving evidence of disease endophenotypes among ALS family members. Cohort studies describe family aggregation of neuropsychiatric disease, primarily psychosis and suicide, in kindreds of ALS probands.(46, 47) Although *C9orf72* repeat expansions account for a proportion of aggregation, they are not over-represented in typical schizophrenia.(48) Detailed family studies demonstrate non-uniform distribution of neuropsychiatric conditions, which instead cluster in up to 30% of ALS kindreds, (8) suggesting genetic pleiotropy or oligogenic inheritance. There is also evidence of overlapping polygenic risk between ALS and neuropsychiatric disease. Analysis of GWAS datasets from the ALS Project MinE and the Psychiatric Genomics Consortium found 14% polygenic overlap between ALS and schizophrenia.(49) Indeed, *GLT8D1*, a recently identified ALS risk gene, is also a schizophrenia risk gene.(50) These observations suggest that the pathogenic process underpinning some forms of ALS disrupt specific brain network patterns.(51) This may be mediated by developmental processes that render certain brain networks more vulnerable, which manifests in various family members as neuropsychiatric phenotypes or later onset neurodegeneration; however, further study is required to clarify any potential overlap of ALS with neuropsychiatric disease.

## Gene-based ALS treatment strategies

The rising number of ALS risk genes, comprising gain- and loss-of-function missense and nonsense mutations and repeat expansions, advocates gene-based approaches for treating ALS. Rapid advances have been made in gene-based therapies, which comprise several techniques, antisense oligonucleotides (ASO), RNA interference (RNAi), gene replacement therapy, and genome editing (Figure 2).(52) The optimal approach depends on the mutation and distribution/level of the encoded protein. Pathogenic gain-of-function mutations can be targeted by ASOs or RNAi but may be difficult in practice since many ALS genes are widely expressed and the wild-type protein performs essential functions. However, if the mutant protein is overexpressed, this approach could be feasible, *e.g.*, targeting mutant SOD1 protein aggregates. Loss-of-function mutations can be addressed by gene replacement therapy, which delivers a functional wild-type copy of the mutant gene. Finally, genome editing, though currently only in the preclinical stages, could potentially be leveraged to correct both gain- and loss-of-function mutations and offers the ability to specifically target the mutant allele, overcoming the weakness of ASOs and RNAi. Trial designs, such as umbrella trials, can leverage molecular phenotyping to select trial candidates (Figure 1F, E).

### ASOs

ASOs are short synthetic, single-stranded oligonucleotides of ~20 chemically modified nucleotides with known *in vivo* stability.(53) Since ASOs do not cross the blood-brain barrier, treating neurodegenerative disorders requires cerebrospinal fluid delivery, *e.g.*, intrathecal, intracerebroventricular. ASOs bind to target pre-mRNA or mRNA to reduce protein expression through two main mechanisms.(53) Duplex formation marks target pre-mRNA/mRNA for degradation by endogenous RNase H; alternatively, ASOs interfere with target pre-mRNA/mRNA translation and/or splicing.(53) In ALS, ASOs can potentially target C9orf72 RNA foci or TDP-43, SOD1, or FUS protein aggregates. Several clinical trials of ASOs are underway in ALS.(52, 53) The *SOD1*-targeting tofersen (BIIB067), in a phase I/II trial, demonstrated safety and lowered cerebrospinal fluid SOD1 levels, particularly in the high-dose group,(54) is now in phase III (NCT02623699). A phase III trial of BIIB067 is also recruiting presymptomatic carriers of rapidly progressive *SOD1* mutations with blood-based biomarker evidence of disease through elevated neurofilament light chain levels (NCT04856982). This trial is following a paradigm of preventive therapy for highly penetrant *SOD1* mutation carriers. Phase I trials of ASOs designed to target *C9orf72* (BIIB078, NCT03626012; IWVE-004, NCT04931862) and *ATXN2* (BIIB105, NCT04494256) expansion repeats are also in the pipeline. Finally, a phase I/III trial targeting *FUS* is also on-going (ION363, jacifusen, NCT04768972).

Details regarding methods that are currently in preclinical stages for ALS, *i.e.*, RNAi, gene replacement therapy, and genome-editing technologies, are outlined in Panel 2.

## ALS genetics informs ALS pathophysiology

Despite tremendous progress, ALS pathophysiology remains incompletely understood. However, as our knowledge of genetic architecture deepens, we are discovering the molecular steps various ALS mutations take to converge on highly shared and recurrent dysregulated nervous system pathways. The major shared pathological pathways in ALS include impaired RNA metabolism, altered proteostasis/ autophagy, cytoskeletal/ trafficking defects, mitochondrial dysfunction, and compromised DNA repair (Table 1, Figure 2).(55, 56) Among the most common ALS genes, mutant *C9orf72*, *TARDBP*, and *FUS* impair RNA metabolism; *C9orf72* repeat expansions also induce defects in protein homeostasis. Mutant *SOD1* also triggers proteostasis defects, and, additionally, mitochondrial dysfunction and oxidative stress.(55)

Repeat expansions in the *C9orf72* promoter impair gene transcription; additionally, RNA transcripts of *C9orf72* expansions aggregate into toxic RNA foci, sequestering RNA-binding proteins, which alters RNA metabolism.(55) Aberrant translation of *C9orf72* transcript expansions generates proteotoxic dipeptide repeats, *e.g.*, poly proline (P)-arginine (R) repeats [poly(PR)] and poly glycine (G)-arginine (R) repeats [poly(GR)], among others.(55) TDP-43 cytoplasmic inclusions are an almost universal ALS feature, present in ~97% of cases.(57) Although principally nuclear, TDP-43 is mislocalized to the cytoplasm in ALS, and is heavily post-translationally modified and/or truncated.(58) Mislocalized TDP-43 impairs RNA splicing, for instance, of stathmin-2 (STMN2), a protein required for microtubule stability.(59) Diminished STMN2 protein levels leads to impaired axonal

growth and motor neuron function.(59) TDP-43 inclusions are mutually exclusive with FUS, as well as SOD1, aggregates;(60) although, both TDP-43 and FUS are DNA/RNA binding proteins, which regulate transcription and RNA splicing, localization, and degradation, there is little overlap between their binding targets.(61)

Of genes discovered in the last five years, research suggests involvement in RNA metabolism (*TIA1*), proteostasis/ autophagy (*CCNF*, *NEK1*, *TBK1*), and cytoskeletal/ trafficking (*ANXA11*, *C21orf2*, *KIF5A*).(12, 55) Interestingly, and potentially novel, the *DNAJC7*-, *GLT8D1*-, and *LGALSL*-mediated mechanisms of neurodegeneration are uncertain. *DNAJC7* is a heat-shock protein co-chaperone, which could possibly be linked to proteostasis/ autophagy.(12) It is hypothesized *GLT8D1*, a glycosyltransferase, may impair ganglioside biosynthesis and O-linked β-N-acetylglucosamine modification.(62) The cellular role of *LGALSL*, galectin-like, is completely unknown; however, galectins are glycosylating enzymes, which may suggest a potential link between *LGALSL* and *GLT8D1* in ALS. Therefore, the discovery of novel ALS genes may unlock as yet unknown research avenues and pathological processes.

## Nucleocytoplasmic transport defects in ALS

Nucleocytoplasmic transport (NCT) is a highly regulated process, which conveys RNA and protein cargo between the nucleus and cytoplasm.(63) NCT is mediated by large, multi-subunit nuclear pore complexes comprised of nucleoporins, which act in concert with cytoplasmic importins (import protein cargo from cytoplasm to nucleoplasm) and nuclear exportins (export protein cargo from cytoplasm to nucleoplasm).(63) NCT protein cargo transport directionality is governed by small GTPase Ras-related nuclear (Ran) proteins by binding to importins and exportins. Recent studies report both morphological and functional defects in NCT in ALS animal and cell models, also present in tissue from sporadic and familial ALS patients.(63) Specially, NCT and nuclear envelope morphology are impaired by *C9orf72* repeat expansions,(64, 65) insoluble TDP-43 aggregates,(66) and mutant *FUS*. (67) In patients, abnormal immunoreactivity against nucleoporins, importins, and Ran is detected in motor cortex and spinal motor neurons from *TARDBP* mutant and sporadic ALS patients, even independent of *C9orf72* repeat expansions.(66–68) Impaired NCT may represent a universal pathology in neurodegenerative diseases, since it is also present in Alzheimer's disease(69) and HD.(70)

## C9orf72 dipeptide repeat proteins and neurotoxicity

Research is also uncovering the mechanism of toxicity of *C9orf72* repeat expansion-derived dipeptide repeats, which, in addition to impairing NCT, alter chromatin structure.(71) Poly(PR) expression in mouse produces neuronal loss and gliosis, resulting in motor and memory defects.(71) Poly(PR) binds to DNA and localizes with heterochromatin, disrupting the condensed state, leading to aberrant histone methylation and altered gene expression. (71) Furthermore, poly(PR) produces nuclear lamina invaginations and impairs NCT.(71) Poly(PR) also co-localizes with heterochromatin in cortex from *C9orf72* ALS patients. (71) These dipeptide repeats can trigger TDP-43 proteinopathy, forging a link between *C9orf72* repeat expansions and TDP-43 pathology.(72, 73) Poly(GR) and Poly(GA) induce cytoplasmic TDP-43 inclusions;(72, 73) additionally, Poly(GR) sequesters NCT proteins.

(72) Encouragingly, an ASO targeting C9orf72 GGGGCC repeats reduces poly(GR) burden, TDP-43 pathology, and neurodegeneration.(72) Poly(GR) aggregates co-localize with TDP-43 inclusions in disease related brain tissue from ALS patients, suggesting pathological involvement.(74) Importantly, studies are not fully concordant, possibly due to differing model systems; thus, this research direction requires further investigation.

## Liquid-to-liquid phase separation

In addition to impaired NCT, emerging interest is focused on liquid-to-liquid phase separation (LLPS) in ALS.(75) LLPS occurs when a homogenous fluid separates into two liquid phases, forming a dynamic, organelle-like structure lacking a membrane.(75) LLPS is related to several pathophysiological processes in ALS, including NCT, RNA metabolism, DNA repair, protein aggregation, and axonal transport.(75) Stress granules are the most widely studied LLPS and form under cellular duress; normally, however, stress granules are dynamic and reversible once the cellular stress subsides. In ALS, however, stress granule dynamics are impaired, leading to persistent granules of several RNA and protein aggregates, as well as TDP-43 and FUS, which possess so-called low-complexity domains that predispose to aggregation.(75) Arginine-rich *C9orf72* repeat expansion-derived dipeptide repeats undergo LLPS and induce stress granule assembly, impairing dynamics.(76) A recent study demonstrates how LLPS of elevated cytoplasmic TDP-43 levels occurs, even independent of stress granules, recruiting nucleoporins, importins, and Rans.(77) Although *TARDBP*, *FUS*, and *C9orf72* are the major ALS genes related to LLPS, multiple less common ALS risk genes are also involved, *hnRNPA1*, *hnRNPA2B1*, *TIA1*, and *UBQLN2*.(75) Thus, LLPS is an exciting research direction in ALS shared by several risk genes and also intertwined with well-established pathophysiological mechanisms.

## Cell-to-cell prion-like transmission

The low-complexity domains from TDP-43 and FUS contain prion-like motifs.(75) Self-propagating prion spread of amyloid-β and tau is a well-studied phenomenon in Alzheimer's disease. Cell-to-cell transmission of aggregation prone proteins is a developing focus in ALS, including of wild-type and mutant SOD1,(78) dipeptide repeats,(79, 80) and TDP-43.(81)

## Inflammatory pathways in ALS

Dysregulated inflammatory pathways are a recurrent thread in ALS.(82) Central and peripheral inflammation are present in *C9orf72*, *SOD1*, and *TARDBP* animal models and in familial ALS patients.(82) This pathophysiology is characterized by immune cell infiltration into the central nervous system, dysregulated peripheral immune cell counts, induction of an activated immune phenotype, and altered cytokine production (Figure 3A, B).(82) Cytotoxic CD8 T cells infiltrate the central nervous system of mutant $SOD1^{G93A}$ mice and selectively destroy motor neurons; genetic ablation of this immune cell population slows motor neurodegeneration.(83) Furthermore, mutant $SOD1^{G93A}$ CD8 T cells express elevated levels of interferon gamma (IFNγ), a cytokine linked to ALS progression.(83) ALS patients with loss of normal C9orf72 activity secondary to abnormal *C9orf72* repeat expansions lose the ability to regulate interferon production via the innate immune system (cGAS/STING pathway), leading to type I interferon-mediated systemic and central nervous

system inflammation.(84) Similar elevated interferon production is associated with TDP-43 pathology in cell and animal models of ALS.(85) Blocking innate immunity signaling in mutant *TARDBP* mice normalizes interferon levels and both slows disease progression and lengthens survival.(85) Simultaneous with the increase in cytotoxic immune cells, ALS is characterized by lower levels of immune-regulatory and anti-inflammatory Tregs(82) and CD4 T cells.(86) In addition, less frequent ALS mutations induce inflammation, including *OPTN*, *SQSTM1*, *TBK1*, and *VCP*.(82) Thus, inflammation may modulate ALS progression and survival. In sporadic patients lacking any know genetic etiology, the mechanism of immune dysregulation in ALS remains uncertain, although it is a characteristic feature.(86, 87) Similar to ALS with a determined genetic cause, patients with sporadic ALS have altered peripheral immunity, induction of an activated immune phenotype, and changes in peripheral cytokine levels.(82)

### Pathophysiology summary

Overall, emergent research directions in ALS pathophysiology constitute NCT, LLPS, cell-to-cell and transmission. These pathways are interrelated, and also feed into other pathological aspects, *e.g.*, abnormal ribostasis, proteostasis, and trafficking, mitochondrial dysfunction, DNA repair defects and inflammation. Thus, future work is needed to generate a holistic view of pathophysiology in ALS (see “Conclusion” section).

## ALS exposome

Although burgeoning genetic discoveries have deepened our understanding of ALS etiology and mechanisms, most are “sporadic” cases lacking a known genetic cause. Moreover, incomplete heritability of known mutations suggests environmental factors are involved.(22) This has led to the gene-time-environment hypothesis, which suggests that genetic predisposition interacts with environmental exposures over time leading to ALS.(22) Thus, the role of an individual’s cumulative lifelong exposure, the exposome, on ALS risk represents a developing research direction to better understand etiology and identify modifiable risks to prevent disease. Furthermore, the multi-step model also supports environmental effects in ALS, since a series of steps are required for disease onset,(88) even in individuals with known and penetrant mutations.(27)

Several studies have investigated the ALS exposome, which is broad, and encompasses exogenous toxicant exposures, *e.g.*, environmental pollutants,(89) medical events, *e.g.*, brain trauma,(89) and lifestyle factors, *e.g.*, intense physical activity,(90) military service.(89) Here, we focus on exogenous environmental exposures that increase risk and/or accelerate disease progression (Appendix Table). A 2017 meta-analysis highlights some commonly studied ALS-environment links (odds ratio [OR]>1), encompassing lead exposure, heavy metals, pesticides, agricultural chemicals, and solvents.(89) Studies in the past five years add to the growing literature of ALS environmental risk factors, as outlined in Appendix Table. The table is not exhaustive, but rather provides an overview of existing and emerging research directions in diverse geographic locations and genetically distinct populations.

Importantly, not all ALS exposome studies are concordant (Appendix), which may arise from population size or characteristics (*e.g.*, location, genetics), exposure duration,

adjustment parameters, and methodology (*e.g.*, historical estimates versus analyte measurements). Thus, despite a significant body of work and identified ALS-environmental links, large prospective cohort studies are needed.(91) These will require detailed registries of patient medical information linked to personal-level data and occupational and residential history with banked biosamples. Studies should evaluate how the exposome modifies disease progression and outcomes,(92) as well as onset risk. Furthermore, environmental, residential, and occupational risks may not be geographically uniform, necessitating large prospective cohorts across diverse regions, possibly globally. Additionally, geographically distinct populations may also be genetically distinct, which could modify their exposure risk. Although gene-environment interaction studies have been conducted for single gene candidates,(90) multi-Omics studies will be needed that bridge genetics,(93) *i.e.*, mono-, oligo- and polygenic risk, with the exposome, to truly comprehend ALS risk and progression.

## Conclusion

Much progress has been made towards a more comprehensive picture of ALS, aided by a new understanding of complex genetics and the discovery of novel disease mechanisms. The advent of genetic therapies has realized preclinical and early clinical trials of candidate ALS genetic therapies. Our growing body of knowledge advocates a shift in clinical practice, trial design, and emerging research questions in ALS. Regarding clinical practice, we anticipate genetic testing will become routine, profiling ALS patients by mutation or genetic/polygenic risk, rather than the previous dichotomization of “familial” or “sporadic”. Genetic profiling should also be leveraged to transform how we conduct forthcoming ALS clinical trials, especially for candidate genetic therapies, by stratifying trial participants by mutation status. This will also ultimately impact management, as we shift gears to a more tailored precision approach for treating ALS patients. For preventive therapies, improved predictive algorithms will identify most-at-risk individuals, as our understanding of penetrance and oligo/polygenic risk crystallizes. We expect this will tie in with environmental factors; multi-Omics platforms could generate an integrated perspective on gene-exposome architecture rather than on individual genetic or exposome contributions. Machine learning and big data may play a role in these ambitious goals,(94) for instance, in prioritizing ALS genes,(95) particularly in view of ALS complexity. Emerging questions will continue to refine our picture of ALS. Given the phenotypic spectrum of ALS with other neurological diseases and the genetic overlap among various conditions, should we switch to a molecular classification? Could we integrate that with an exposome classification? These questions are not unique to ALS, since most neurodegenerative diseases are sporadic. To meet the challenges of this complex disease, future ALS studies will rely on large multi-center cohorts and integrated multi-Omics platforms, necessitating international collaborative projects. Findings from these collaborative patient-based projects will drive our improved understanding of ALS pathogenesis, and lead to needed and long-awaited therapies.

## Search strategy and selection criteria

We searched PubMed for English language articles with the terms: “amyotrophic lateral sclerosis,” “ALS,” “motor neuron disease,” “MND,” “GWAS,” “genetic,” “risk,”

"oligogenic," "polygenic," "C9orf72," "SOD1," "TARDBP," "FUS," "TBK1," "NEK1," "CCNF," "C21orf2," "ANXA11," "TIA1," "KIF5A," "GLT8D1," "LGALSL," "DNAJC7," "genotype-phenotype," "Alzheimer's disease," "Huntington's disease", "Parkinson's disease," "pathophysiology," "mechanism," "nucleocytoplasmic transport," "liquid-to-liquid phase separation," "RNA splicing," "cell-to-cell transmission," "prion," "immune system," "gene therapy", "antisense oligonucleotide, "RNAi," "AAV9," "CRISPR," "exposure," "environment," "pollutant," "toxin," "metals," "traffic." The search focused on articles published from 2016 to 2021, though seminal older articles were also considered. We also included articles from the authors' personal reference lists. Selected articles were based on relevance to this review. Additionally, we searched clinicaltrials.gov for "amyotrophic lateral sclerosis" with "gene therapy", "antisense oligonucleotide, "RNAi," "small interfering RNA," "short hairpin RNA," "AAV9," "CRISPR."

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

SAG and ELF receive funding from the National ALS Registry/CDC/ATSDR (1R01TS000289; R01TS000327); National ALS Registry/CDC/ATSDR CDCP-DHHS-US (CDC/ATSDR 200–2013-56856); NIEHS K23ES027221; NIEHS R01ES030049; NINDS R01NS127188; NeuroNetwork for Emerging Therapies, the NeuroNetwork Therapeutic Discovery Fund, the Peter R. Clark Fund for ALS Research, the Sinai Medical Staff Foundation, Scott L. Pranger, University of Michigan. OH receives funding from Science Foundation Ireland (13/RC2015, 16/RC/3948), Thierry Latran Foundation, Health Research Board (Ireland). AAC is an NIHR Senior Investigator (NIHR202421). This is an EU Joint Programme - Neurodegenerative Disease Research (JPND) project. The project is supported through the following funding organisations under the aegis of JPND - www.jpnd.eu (United Kingdom, Medical Research Council (MR/L501529/1; MR/R024804/1) and Economic and Social Research Council (ES/L008238/1)) and through the Motor Neurone Disease Association. This study represents independent research part funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. AC received funding from the Italian Ministry of Health (Ministero della Salute, Ricerca Sanitaria Finalizzata, grant RF-2016–02362405); the Progetti di Rilevante Interesse Nazionale program of the Ministry of Education, University and Research (grant 2017SNW5MB); the European Commission's Health Seventh Framework Programme (FP7/2007–2013 under grant agreement 259867); and the Joint Programme–Neurodegenerative Disease Research (Strength, ALS-Care and Brain-Mend projects), granted by Italian Ministry of Education, University and Research. This study was performed under the Department of Excellence grant of the Italian Ministry of Education, University and Research to the "Rita Levi Montalcini" Department of Neuroscience, University of Torino, Italy. MCK receives funding from the National Health and Medical Research Council of Australia Program Grant (APP1132524), Partnership Project (APP1153439) and Practitioner Fellowship (APP1156093) schemes.

Declaration of interests

SAG declared consulting fees from Biogen and ITF Pharma, a patent "Methods for treating amyotrophic lateral sclerosis", and participation on a data safety monitoring board for Watermark. OH declared grants from Science foundation Ireland and Health Research Board, consulting fees from Novartis, Cytokinetics, Denali Pharma, Stitching Foundation, and La Caixa, payment or honoraria from Biogen, participation on a data safety monitoring board for Accelsiors and steering committee for Cytokinetics, and Editor-in-Chief for ALS and FTD Journal. AAC declared consulting fees from Mitsubishi Tanabe Pharma, Biogen Idec, Cytokinetics, Wave Pharmaceuticals, Apellis, Amylyx, Novartis, and Eli Lilly. AC declared grants from Biogen to his institution, payments or honoraria from Biogen and Amylyx, and participation on a data safety monitoring board for Ely Lilly and ABScience and advisory board for Mitsubishi Tanabe, Roche, Denali Pharma, Cytokinetics, Biogen, and Amylyx. MGS declared no conflicts of interest. MCK declared support from National Health and Medical Research Council of Australia, honorary role as President of the Brain Foundation, and Editor-in-Chief of the Journal of Neurology, Neurosurgery and Psychiatry. ELF declared a patent "Methods for treating amyotrophic lateral sclerosis".

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**Panel 1.**

## Glossary of terms

**Familial ALS:**

Classically, an inherited ALS. Clinically defined based on the likelihood two or more family members carry the same disease-causing variant.

**Sporadic ALS:**

Classically, ALS occurring in a patient without evidence it was inherited. Nevertheless, shares several ALS risk genes with familial ALS.

**Monogenic (Mendelian) inheritance:**

Inheritance of a trait (or disease) defined by one gene. Inheritance may be autosomal or sex-linked dominant (only one mutant allele must be inherited) or recessive (two mutant alleles must be inherited).

**Oligogenic inheritance:**

Inheritance of a trait (or disease) defined by a few genes. This term is frequently used as an intermediate between monogenic and polygenic inheritance.

**Polygenic inheritance:**

Inheritance of a trait (or disease) defined by the cumulative effective of many genes.

**Gene penetrance:**

The proportion of individuals harboring a mutant gene or gene variant that manifest a trait (or disease). High penetrance means many individuals will develop the trait (or disease); low penetrance means few individuals will develop the trait (or disease).

**Lifetime risk:**

Probability a specific disease will occur in an individual or population within their lifetime.

**Pathogenicity:**

A genetic variant that increases disease risk in an individual.

**Heritability:**

Measures the extent variation in a trait (or disease) can be attributed to genetic versus variation in environmental factors.

**Complex trait:**

A trait (or disease) dictated by polygenic inheritance and environmental interaction.

**Gene-time-environment hypothesis of ALS:**

Posits that genetic predisposition interacts with environmental exposures over time leading to ALS.

**Multi-step model of ALS:**

Posits that a series of “steps”, some genetic, some possibly environmental, which lead to ALS.

**Genetic pleiotropy:**

A gene that influences two or more traits (or diseases).

**Phenocopy:**

A trait (or disease) that “copies” the phenotype associated with a specific genotype, but without harboring that genotype.

**Endophenotype:**

A neurobehavioral heritable trait that can be measured in both affected and unaffected individuals to assess genetic susceptibility for psychiatric illnesses

**Proband:**

An individual in a family with a heritable trait (or disease); generally, the proband is the first individual to seek medical attention for a genetic disease, though kindreds and/or ancestors may also (have) manifest(ed) the disease.

**Panel 2.**

## Gene-based treatment strategies

### RNAi

**Comprises two approaches:**

Small interfering RNA (siRNA) and short hairpin RNA (shRNA).(96)

**siRNAs:**

Are generally duplexes of two strands of approximately 20 modified nucleotide base pairs long, internalized into cells.(96)

**siRNA mechanism:**

The strand of the siRNA complementary to the gene target binds to Dicer protein and recruits argonaute proteins and target mRNA, generating a RNA-induced silencing complex (RISC). RISC cleaves the target gene mRNA, leading to gene knockdown.(96)

**shRNAs:**

Hairpin structures of either natural or modified nucleotide bases, which can be delivered by viral vectors.(96)

**siRNA mechanism:**

After internalization into cells, shRNAs are first cut by Dicer to remove the hairpin, and then follows the same pathway as siRNAs through RISC.(96)

**Clinical applications:**

RNAi is FDA-approved to treat hereditary transthyretin amyloidosis.(96)

**State of RNAi in ALS:**

Strategies are being tested in preclinical ALS models(52) but have not yet entered ALS clinical trials.

### Gene replacement therapy

**Mechanism:**

This approach leverages specific viruses to provide patients harboring loss-of-function mutations a functional copy of a gene.(52) Viruses can cross the brain-blood barrier and may consequently be administered intravenously, which is a significant advantage. Currently, two vectors are employed, *i.e.*, lentivirus and adeno-associated virus (AAV), which deliver the replacement gene by mRNA or cDNA, respectively.

**Clinical applications:**

Onasemnogene abeparvovec, an AAV9-mediated gene replacement therapy for *SMN1*, is FDA-approved. A phase I open-label, dose escalation clinical trial assessed a single intravenous injection of onasemnogene abeparvovec in *SMN1* pediatric participants (n= 15; NCT02122952).(97) Onasemnogene abeparvovec was safe and significantly improved motor function and survival (100% vs 8%) versus historical cohorts. The extremely promising results warranted Fast Track, Breakthrough Therapy, and Priority

Review designation at the FDA, culminating in approval for treating patients less than two years of age and demonstrating the feasibility of this approach for treating neuromuscular disease.

### State of gene replacement therapy in ALS:

The most common ALS mutations, *C9orf72*, *SOD1*, *TARDBP*, and *FUS*, are toxic gain-of-function, and therefore not amenable to gene replacement therapy. However, gene delivery of neurotrophic factors is being investigated in preclinical models.(52) Moreover, less frequent but penetrant loss-of-function ALS mutations may become viable candidates as research advances.

**Genome-editing technologies**

### Mechanism:

Aims to correct a disease-causing genetic mutation in a patient; several technologies exist, but RNA-guided CRISPR-Cas9 is prominent due to its numerous advantages.(98) The CRISPR RNA guide targets the locus of interest by simple base pairing, which means a guide can be designed to target any gene of interest.(98) CRISPR can cut chromosome DNA to modify it, thought this can have unintended consequences, such as unwanted deletions or chromosomal rearrangements.(99) CRISPR can perform more targeted changes, *e.g.*, single-base editing,(98) which does not require a double-stranded DNA break. Additionally, CRISPR technology can modulate transcription and edit RNA, expanding its potential applications.(98)

### Clinical applications:

None to date.

### State of genome-editing technology in ALS:

Is currently being tested in preclinical ALS models against *SOD1* mutations and *C9orf72* repeat expansions.(52, 100, 101)

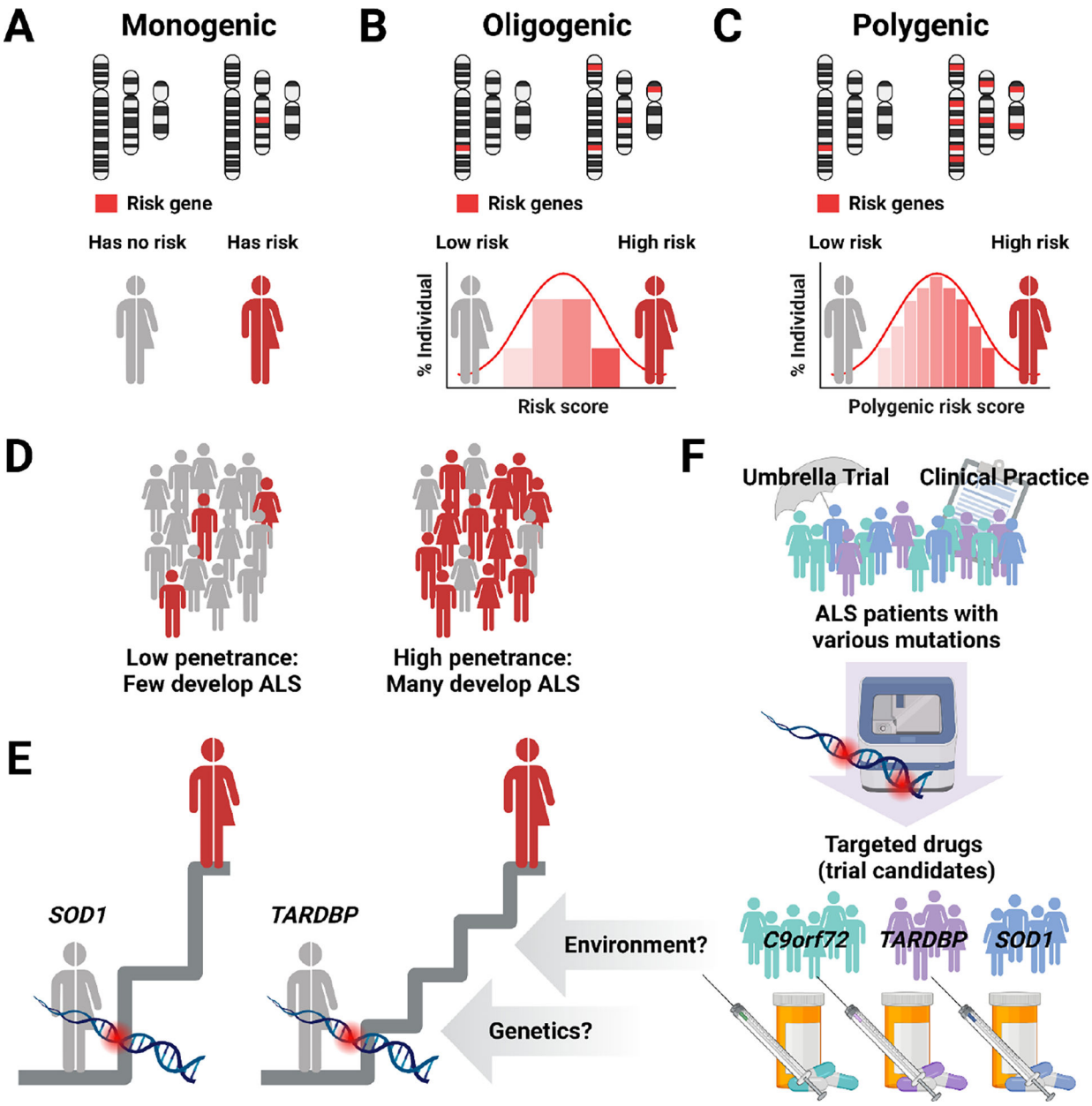


**Figure 1. ALS genetic architecture.**

ALS genetics is characterized by (**A**) monogenic, (**B**) oligogenic, and (**C**) polygenic risk. Only three representative chromosomes shown. (**D**) ALS genes are not fully penetrant and the pathogenicity of certain variants remains uncertain, complicating the full picture. Left: For a population of gene carriers, low penetrance variants lead to a low frequency of ALS onset (red figures). Right: For a population of gene carriers, high penetrance variants lead to a high frequency of ALS onset (red figures). (**E**) Overlaid over the genetic aspects are environmental factors, since heritability is incomplete. Thus, a multistep ALS model has emerged, which advocates that multiple “steps” are necessary for ALS onset. Left: Larger-effect mutations, *e.g.*, mutant *SOD1*, require fewer steps for ALS onset (red figure). Right: Smaller-effect mutations, *e.g.*, mutant *TARDBP*, require more steps for ALS onset

(red figure). Future work is needed to precisely define a “step” and determine when one has occurred, *e.g.*, genetic or environmental factors. (**F**) Several genetic therapies are in the clinical trial pipeline (umbrella trial, stratified by molecular profile) and tailored precision treatments are future goals; thus, molecular profiling of ALS patients could become standard clinical practice. SNP, single nucleotide variant. Created with BioRender.com.

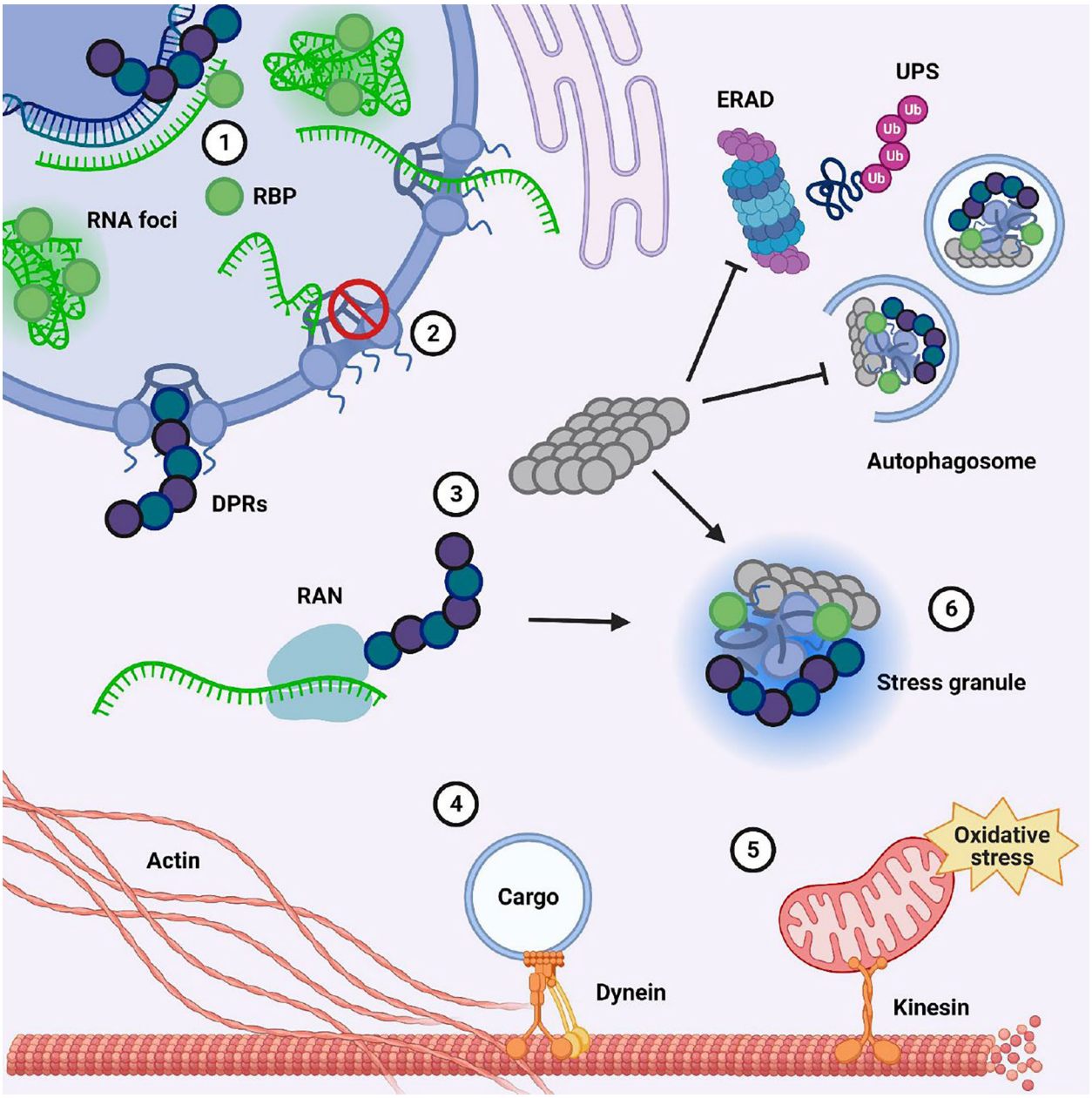


**Figure 2. ALS pathophysiology.**

Shared ALS pathological pathways center on impaired RNA metabolism, altered proteostasis/ autophagy, cytoskeletal/ trafficking defects, mitochondrial dysfunction, and compromised DNA repair. Numbering from top left downwards: (**1**) Mutant RNA-binding proteins (RBPs), *e.g.*, FUS, TDP-43, disrupt RNA transcription and splicing. C9orf72 repeat expansion RNAs aggregate into RNA foci, sequestering RBPs and impairing RNA metabolism. Additionally, haploinsufficiency from the single remaining normal *C9orf72* allele leads to loss-of-function of native C9orf72 protein function, related to multiple aspects, trafficking, autophagy, DNA repair. (**2**) Mutant *C9orf72*, *FUS*, and *TARDBP* functionally impair nucleocytoplasmic transport (NCT) and induce nuclear envelope morphology defects and cytoplasmic inclusions of NCT components, *e.g.*, nucleoporins,

importins, and Ran (small GTPase Ras-related nuclear proteins). (**3**) Repeat-associated non-AUG translation of C9orf72 repeat expansions yields dipeptide repeats (DPRs), which are toxic through several pathways, including protein aggregates, chromatin alterations and DNA damage, impaired NCT and component sequestration. Additional cytoplasmic protein aggregation (*e.g.*, TDP-43, SOD1) induces proteostasis and autophagy defects. Protein aggregates block the endoplasmic reticulum-associated protein degradation (ERAD) response and ubiquitin proteasome system (UPS), preventing aggregate clearance. Mutations to ubiquitination proteins (*e.g.*, CCNF, UBQLN2) additionally dysregulate the UPS. Protein aggregates and RBPs also accumulate into stress granules, which become persistent in ALS. Mutations to vesicle-forming proteins (*e.g.*, OPTN, VAPB, VCP) disrupt vesicular transport and distribution, leading to dysfunctional autophagy and proteostasis. (**4**) Mutations to the tubulin transport machinery (*e.g.*, DCTN1, KIF5A, TUBA4A) and actin (*e.g.*, PFN1) induce cytoskeletal/ trafficking defects, which impairs distribution of vital organelles throughout cells (*e.g.*, mitochondria, cardo-laden vesicles). (**5**) Protein aggregates (*e.g.*, TDP-43, SOD1) and mutations to mitochondrial protein components (*e.g.*, CHCHD10) trigger mitochondrial and bioenergetics dysfunction and raise oxidative stress. (**6**) liquid-to-liquid phase separation of aggregation prone proteins (*e.g.*, FUS, TDP-43) drives formation of stress granules. Created, in part, with BioRender.com.

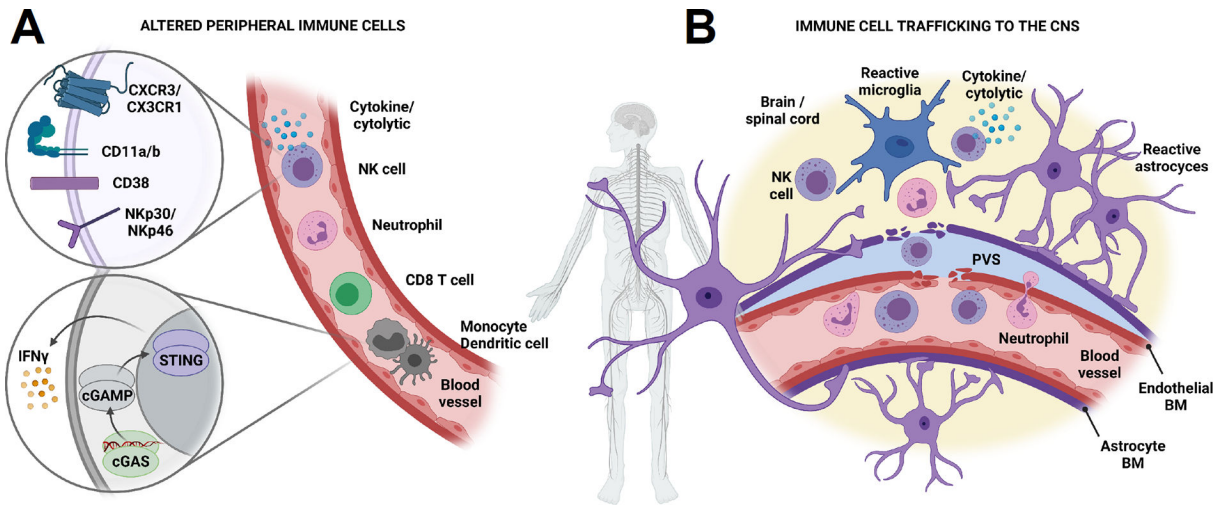


**Figure 3. ALS inflammatory pathways.**

This pathophysiology in ALS is characterized by dysregulated peripheral immune cell counts, immune cell infiltration (trafficking) into the central nervous system, induction of an activated immune phenotype, and altered cytokine production. (**A**) Various peripheral immune cell populations in blood have differential levels in ALS, *e.g.*, innate (neutrophils, natural killer (NK) cells) and adaptive (CD8 T cells). Circulating NK cells in ALS increase expression of surface markers of cytotoxic function (CD38, NKG2D, NKp30, NKp46) and trafficking (CD11a, CD11b, CXCR3, CX3CR1). Circulating monocytes and dendritic cells expressing mutant *TARDBP* and *C9orf72* repeat expansions increase interferon gamma (IFNγ) production. (**B**) Peripheral immune cells traffic to the central nervous system (CNS) in ALS, e.g., neutrophils, NK cells. BM, basal membrane; CD11a, cluster of differentiation 11a; CD11b, cluster of differentiation 11b; CD38, cluster of differentiation 38; CXCR3, C-X-C motif chemokine receptor 3; CX3CR1, C-X3-C motif chemokine receptor 1; NKG2D, killer cell lectin like receptor K1 (*KLRK1*); NKp30, natural cytotoxicity triggering receptor 3 (NCR3); NKp46, Natural cytotoxicity triggering receptor 1 (NCR1); PVS, perivascular space. Created, in part, with BioRender.com.

**Table 1.**

**Summary of most recently (since 2015; top) and all other (bottom) identified ALS mutations and associated pathophysiology.**

Genes listed alphabetically within top and bottom portions. Adapted, with modifications, from Chia et al. Lancet Neurology, 2018.(2)

| Gene | Year of discovery | Genetic effect | Familial ALS (%) | Sporadic ALS (%) | Function | Associated ALS pathophysiology |
|---|---|---|---|---|---|---|
| **Top: Alphabetical summary of ALS genes discovered since 2015** | | | | | | |
| ***ANXA11*** | 2017 | Autosomal dominant | ~1 | ~1·7 | Calcium-dependent phospholipid-binding protein, vesicle trafficking | Annexin A11 inclusions, impaired binding to calcylin, putative LLPS |
| ***C21orf2*** | 2016 | ND | <1 | <1 | Putative, DNA damage repair, actin structure | Cytoskeletal organization |
| ***CCNF*** | 2016 | Autosomal dominant | ~1–3·3 | <1 | Component of an E3 ubiquitin-protein ligase complex, cell-cycle regulation | Proteostasis defects |
| ***DNAJC7*** | 2019 | ND | <1 | <1 | Heat-shock protein co-chaperone | ND |
| ***GLT8D1*** | 2019 | Autosomal dominant | <1 | <1 | Glycosyltransferase, unknown cellular function, widely expressed | ND, localized to Golgi, suggested role in impaired ganglioside synthesis and addition of O-linked β-N-acetylglucosamine |
| ***KIF5A*** | 2018 | Autosomal dominant | ~0·5–3 | <1 | Kinesin microtubule motor protein | Cytoskeletal/trafficking defects |
| ***LGALSL*** | 2015 | ND | <1 | <1 | ND | ND |
| ***NEK1*** | 2015 | ND | ~1–2 | <1 | Serine/threonine kinase, cell-cycle regulation, axonal development/guidance, axonal polarity, DNA damage repair | Putative DNA damage accumulation, protein aggregation |
| ***TBK1*** | 2015 | Autosomal dominant *de novo* | ~3 | <1 | Serine/threonine kinase, regulates innate immunity, autophagy, cell-cycle | Autophagy, inflammation |
| ***TIA1*** | 2017 | Autosomal dominant | ~2·2 | <1 | RNA-binding protein | Impaired RNA metabolism, LLPS |
| **Bottom: Alphabetical summary of ALS genes discovered prior to 2015** | | | | | | |
| ***ALS2*** | 2001 | Autosomal recessive | <1 | <1 | GEF | Vesicular trafficking defects |
| ***ANG*** | 2006 | Risk factor | <1 | <1 | Ribonuclease | Angiogenesis |
| ***ATXN2*** | 2010 | Autosomal dominant Risk factor | <1 | <1 | RNA-binding protein | Ribostasis defects, putative LLPS |
| ***C9orf72*** | 2011 | Autosomal dominant | 40 | 7 | Putative GEF, endosome trafficking and autophagy regulation, DNA damage | Impaired RNA metabolism, impaired proteostasis/autophagy, intracellular trafficking, NCP defects, LLPS, inflammation |
| ***CHCHD10*** | 2014 | Autosomal dominant | <1 | <1 | Mitochondrial protein localized to cristae junctions in the intermembrane space | Mitochondrial and bioenergetics dysfunction |

| Gene | Year of discovery | Genetic effect | Familial ALS (%) | Sporadic ALS (%) | Function | Associated ALS pathophysiology |
|---|---|---|---|---|---|---|
| ***CHMP2B*** | 2006 | Autosomal dominant | <1 | <1 | ESCRT-III complex component | Impaired proteostasis, vesicular trafficking defects |
| ***DCTN1*** | 2003 | Autosomal dominant Risk factor | <1 | <1 | Dynactin microtubule motor protein subunit | Axon trafficking defects |
| ***ELP3*** | 2009 | ND | <1 | <1 | Histone acetyltransferase subunit of RNA polymerase II elongator complex | Ribostasis defects, cytoskeletal defects |
| ***FUS*** | 2009 | Autosomal dominant Autosomal recessive *de novo* | 4 | 1 | RNA-binding protein, transcription regulation, splicing, RNA localization/ degradation, DNA damage | Ribostasis defects, NCP defects, LLPS |
| ***HNRNPA1*** | 2013 | Autosomal dominant *de novo* Risk factor | <1 | <1 | RNA-binding protein | Ribostasis defects, LLPS |
| ***HNRNPA2B1*** | 2013 | Autosomal dominant Risk factor | <1 | <1 | RNA-binding protein | Ribostasis defects, LLPS |
| ***MATR3*** | 2014 | Autosomal dominant | <1 | <1 | RNA-binding protein localized to nuclear matrix | Ribostasis defects |
| ***NEFH*** | 1994 | Autosomal dominant Risk factor | <1 | <1 | Neurofilament protein | Axon trafficking defects |
| ***OPTN*** | 2010 | Autosomal dominant Autosomal recessive | <1 | <1 | Coiled-coil containing protein regulating membrane trafficking, vesicle trafficking, and transcription activation | Autophagy, inflammation |
| ***PFN1*** | 2012 | Autosomal dominant | <1 | <1 | Actin-binding protein regulating actin polymerization | Cytoskeletal/trafficking defects, axon growth |
| ***SETX*** | 1998 | Autosomal dominant | <1 | <1 | Helicase | Ribostasis defects |
| ***SPG11*** | 2010 | Autosomal recessive | <1 | <1 | Putative transmembrane protein phosphorylated upon DNA damage | DNA damage |
| ***SOD1*** | 1993, first ever discovered ALS mutation | Autosomal dominant Autosomal recessive *de novo* | 12 | 1–2 | Superoxide anion detoxifying enzyme | Proteostasis defects, oxidative stress, prion-like transmission, inflammation |
| ***SQSTM1*** | 2011 | Autosomal dominant | ~1 | <1 | Ubiquitin-binding autophagy adaptor protein, regulates NF-kB | Autophagy, inflammation |
| ***TARDBP*** | 2008 | Autosomal dominant Autosomal recessive *de novo* | 4 | 1 | RNA-binding protein, transcription regulation, splicing, RNA localization/ degradation | Ribostasis defects, NCP defects, LLPS, prion-like transmission, inflammation |
| ***TUBA4A*** | 2014 | Autosomal dominant | <1 | <1 | Microtubule protein | Cytoskeletal/trafficking defects |
| ***UBQLN2*** | 2011 | X-linked autosomal dominant | <1 | <1 | Ubiquitin-like protein, associates with proteasome and ubiquitin ligases | Proteostasis defects, LLPS |

| Gene | Year of discovery | Genetic effect | Familial ALS (%) | Sporadic ALS (%) | Function | Associated ALS pathophysiology |
|---|---|---|---|---|---|---|
| ***VAPB*** | 2004 | Autosomal dominant | <1 | <1 | Plasma and intracellular vesicle membrane protein | Proteostasis defects |
| ***VCP*** | 2010 | Autosomal dominant *de novo* | 1 | 1 | ATPase enzyme regulating protein degradation, intracellular membrane fusion, DNA repair/replication, NF-kB activation, cell-cycle | Proteostasis defects, inflammation |

*ALS2*, alsin Rho guanine nucleotide exchange factor ALS2; *ANG*, angiogenin; *ANXA11*, annexin A11; *ATXN2*, ataxin 2; *C9orf72*, chromosome 9 open reading frame 72; *C21orf2*, chromosome 21 open reading frame 2; *CCNF*, cyclin F; *CHCHD10*, coiled-coil-helix-coiled-coil-helix domain containing 10; *CHMP2B*, charged multivesicular body protein 2B; *DCTN1*, dynactin subunit 1; *DNAJC7*, DnaJ homolog subfamily C member 7; *ELP3*, elongator acetyltransferase complex subunit 3; ESCRT-III complex, Endosomal Sorting Complex Required for Transport III; FTD, frontotemporal dementia; *FUS*, Fused in Sarcoma; GEF, guanine nucleotide exchange factor; *GLT8D1*, glycosyltransferase 8 domain containing 1; *HNRNPA1*, heterogeneous nuclear ribonucleoprotein A1; *HNRNPA2B1*, heterogeneous nuclear ribonucleoprotein A2/B1; *KIF5A*, kinesin family member 5A; *LGALSL*, galectin-like; LLPS, liquid-to-liquid phase separation; *MATR3*, matrin 3; ND, not determined; NCP, nucleocytoplasmic transport; *NEFH*, neurofilament heavy chain; *NEK1*, NIMA (never in mitosis gene a)-related kinase 1; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; *OPTN*, optineurin; *PFN1*, profilin 1; *SPG11*, SPG11 vesicle trafficking associated, spatacsin; *SETX*, senataxin; *SOD1*, superoxide dismutase 1; *SQSTM1*, sequestosome 1; *TARDBP*, TAR DNA binding protein; *TBK1*, TANK-binding kinase 1; *TIA1*, TIA-1 cytotoxic granule-associated RNA binding protein; *TUBA4A*, tubulin alpha 4a; *UBQLN2*, ubiquilin 2; *VAPB*, vesicle-associated membrane protein-associated protein B and C; *VCP*, valosin-containing protein.