Amyotrophic Lateral Sclerosis 1

Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis is a fatal neurodegenerative disease. The discovery of genes associated with amyotrophic lateral sclerosis, commencing with *SOD1* in 1993, started fairly gradually. Recent advances in genetic technology have led to the rapid identification of multiple new genes associated with the disease, and to a new understanding of oligogenic and polygenic disease risk. The overlap of genes associated with amyotrophic lateral sclerosis with those of other neurodegenerative diseases is shedding light on the phenotypic spectrum of neurodegeneration, leading to a better understanding of genotype–phenotype correlations. A deepening knowledge of the genetic architecture is allowing the characterisation of the molecular steps caused by various mutations that converge on recurrent dysregulated pathways. Of crucial relevance, mutations associated with amyotrophic lateral sclerosis are amenable to novel gene-based therapeutic options, an approach in use for other neurological illnesses. Lastly, the exposome—the summation of lifetime environmental exposures—has emerged as an influential component for amyotrophic lateral sclerosis through the gene–time–environment hypothesis. Our improved understanding of all these aspects will lead to long-awaited therapies and the identification of modifiable risks factors.

Lancet Neurol 2022

Published Online
March 22, 2022
https://doi.org/10.1016/S1474-4422(21)00414-2

See Online/Comment
https://doi.org/10.1016/S1474-4422(22)00084-9

This is the first in a Series of two papers on amyotrophic lateral sclerosis

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Introduction

Amyotrophic lateral sclerosis is a fatal neurodegenerative disease affecting motor neurons in the brain, brainstem, and spinal cord.[1] The name derives from the muscle loss (amyotrophy) and axonal loss in the lateral spinal cord columns (lateral sclerosis) characteristic of the disease. Amyotrophic lateral sclerosis presents with progressive voluntary muscles weakness, which spreads to neighbouring body segments, typically leading to death from respiratory failure within 2–4 years from diagnosis. In addition to motor neuron loss, the major neuropathological findings are intracellular cytoplasmic inclusions of eosinophilic Bunina bodies and ubiquitinated TDP-43. There is also considerable phenotypic heterogeneity in disease presentation, involving cognitive and behavioural changes in up to 60% of patients and frontotemporal dementia in about 15% of patients.

Although there are several known genetic risks for amyotrophic lateral sclerosis, about 85% of cases do not have a single genetic cause;[2] thus, the pathophysiology of the disease remains incompletely understood, which 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-pharmacological multidisciplinary care can, in some cases, improve patient outcomes, including early non-invasive ventilation use and feeding tube insertion before substantial weight loss.[1]

The scarcity of treatments has spurred intense research into the complex genetics of amyotrophic lateral sclerosis and the pathomechanisms linked to known mutations. Improved knowledge of the genetic architecture could unlock the potential of genetic therapies. Additionally, an understanding of the effect of environmental exposures, diet, and lifestyle factors—cumulatively known as the exposome—on the risk of amyotrophic lateral sclerosis is needed to identify modifiable risk factors. This Series paper will highlight the latest advances from the past 5 years pertaining to the complex genetics, pathophysiology, therapeutic development, and exposome science of amyotrophic lateral sclerosis. It is accompanied by a second, more clinically focused, paper on clinical presentation, diagnosis, and prognosis.[1]

Genetic architecture

Amyotrophic lateral sclerosis is conventionally classified as familial or sporadic (panel 1). However, this simple subdivision ignores the complex genetic architecture of the disease (figure 1A–C), which is characterised by monogenic, oligogenic, and polygenic inheritance, gene penetrance, and heritability. Mendelian familial amyotrophic lateral sclerosis accounts for 10–15% of individuals with the disease, albeit with incomplete penetrance in most kindreds.[2,3] In the remaining 85%, large genome-wide association studies (GWAS) might be able to identify rare variants and so-called private mutations—ie, mutations found in a single family that might modulate disease risk and phenotypic presentation.[4]

The proportion of patients with disease that is familial is probably under-reported,[5] because of variation in the definition of familial amyotrophic lateral sclerosis.[6] Consensus criteria for familial amyotrophic lateral sclerosis were introduced nearly a decade ago, and are based on the likelihood that two or more family members carry the same disease-causing variant. Family size is key to this definition; in families with more than 17 members, there is about a 5% chance that two members will be affected, based on the overall lifetime risk of developing

Panel 1: **Glossary of terms**

Familial amyotrophic lateral sclerosis: classically, an inherited case of amyotrophic lateral sclerosis. Clinically defined on the basis of the likelihood that two or more family members carry the same disease-causing mutations.

Sporadic amyotrophic lateral sclerosis: classically, amyotrophic lateral sclerosis occurring in a patient without evidence that the disease was inherited. Nevertheless, shares several risk genes with familial amyotrophic lateral sclerosis.

Monogenic (mendelian) inheritance: the inheritance of a trait (or disease) defined by one gene. Inheritance might be autosomal or sex-linked; dominant (only one mutant allele must be inherited) or recessive (two mutant alleles must be inherited).

Oligogenic inheritance: the 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: the inheritance of a trait (or disease) defined by the cumulative effect of many genes.

Gene penetrance: the proportion of individuals harbouring a mutant gene or gene variant that manifests a trait (or disease). High penetrance means that many individuals carrying the mutation will develop the trait (or disease); low penetrance means that few individuals will develop the trait (or disease).

Lifetime risk: the probability that a specific disease will occur in an individual or population within their lifetime.

Pathogenicity: a characteristic of a genetic variant that increases disease risk in an individual.

Heritability: measures the extent that variation in a trait (or disease) can be attributed to genetic versus environmental variation.

Gene–time–environment hypothesis of amyotrophic lateral sclerosis: posits that genetic predisposition interacts with environmental exposures over time leading to amyotrophic lateral sclerosis.

Multistep model of amyotrophic lateral sclerosis: posits that a series of steps—some genetic, some possibly environmental—leads to amyotrophic lateral sclerosis.

Genetic pleiotropy: the influence of one gene on two or more traits (or diseases).

Phenocopy: a trait (or disease) that has a similar phenotype to that associated with a specific genotype, but without harbouring that genotype.

Endophenotype: a neurobehavioural heritable trait that can be measured 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, although kindreds or ancestors might also manifest the disease.

amyotrophic lateral sclerosis (ie, one in 350).[5] Conversely, in a small family, if one parent carries a penetrant mendelian risk gene, the chance that other family members carry the allele is low, leading to an apparent sporadic case of disease.[7] Moreover, some genes for amyotrophic lateral sclerosis also cause frontotemporal dementia or other phenotypes; thus, there is an argument for including the identification of frontotemporal dementia in a kindred in the definition of familial amyotrophic lateral sclerosis, which would bring the percentage of amyotrophic lateral scletrosis cases due to familial disease closer to 20%.[5] Additionally, population studies on the family aggregation of neuropsychiatric conditions within kindreds of people with amyotrophic lateral sclerosis suggest that schizophrenia indicates familial amyotrophic lateral sclerosis, bringing the percentage closer to 30%.[5,8] Validation studies are needed to establish whether to include schizophrenia in kindreds in the familial definition of amyotrophic lateral sclerosis.

Genes associated with amyotrophic lateral sclerosis

Our current knowledge of validated genes for amyotrophic lateral sclerosis derives primarily from ancestral European (ie, Europe, the USA, Canada, and Australia) and Asian populations.[9] Although at least 40 genes have been associated with the disease, four genes account for about 48% of familial and about 5% of sporadic cases within populations of European origin.[10] These genes are *C9orf72*, *SOD1*, *TARDBP* (coding for TDP-43), and *FUS*, and they have lent important insights into the pathophysiology of amyotrophic lateral sclerosis.[11] New genes for amyotrophic lateral sclerosis have been identified in the past 5 years, including *TBK1*, *NEK1*, *CCNF*, *C21orf2* (also known as *CFAP410*), *ANXA11*, *TIA1*, *KIF5A*, *GLT8D1*, *LGALSL*, and *DNAJC7* (table),[2,12] which have highlighted important recurrent pathways and new avenues of research.

Importantly, the genes associated with amyotrophic lateral sclerosis vary in pathogenicity and their susceptibility risk; highly penetrant mutations generally lead to disease (eg, in *TARDBP*, *SOD1*, and *FUS*), whereas some variants associated with amyotrophic lateral sclerosis do not necessarily cause the disease but rather pose a risk of developing the disease (eg, *ANG*, *ATXN2*, and *DCTN1*; table). However, even causative mutations are not fully penetrant, and interactions with the environment modify the risk of developing the disease. Thus, genetic risk represents a continuum from high

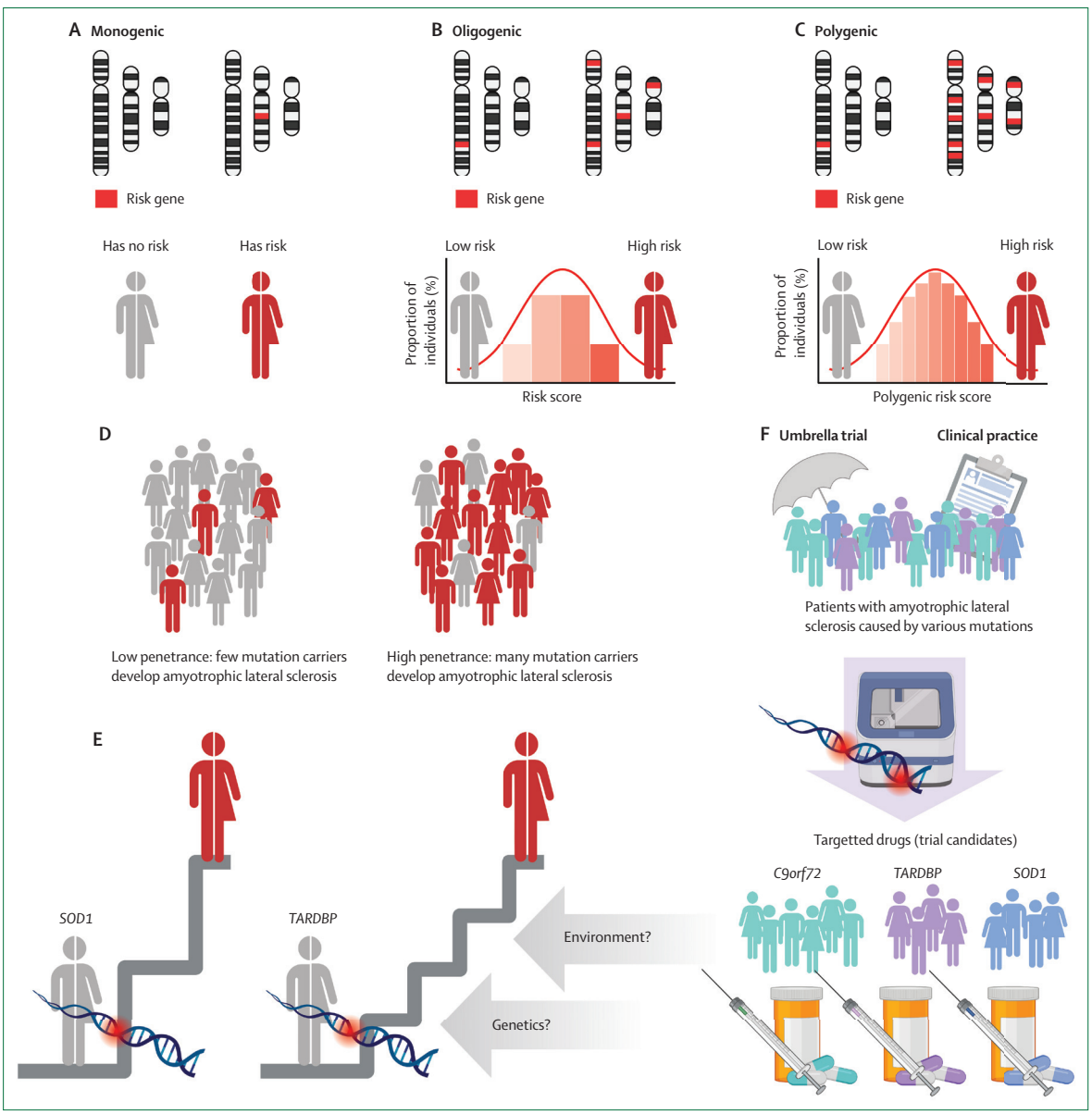

Figure 1: **The genetic architecture of amyotrophic lateral sclerosis**
The genetics of amyotrophic lateral sclerosis is characterised by (A) monogenic, (B) oligogenic, and (C) polygenic risk. Only three representative chromosomes are shown. (D) Genes for amyotrophic lateral sclerosis are not fully penetrant and the pathogenicity of some variants remains uncertain, complicating the full picture. (E) Overlaid over the genetic aspects are environmental factors, because heritability is incomplete. Thus, a multistep model for amyotrophic lateral sclerosis has emerged, which advocates that multiple steps are necessary for onset of the disease. The model posits that mutations with a larger effect require fewer steps for disease onset. Future work is needed to precisely define a step and establish when one has occurred (eg, genetic or environmental factors). (F) Several genetic therapies are under development (ie, in an umbrella trial stratified by molecular profile) and tailored precision treatments are future goals; thus, molecular profiling of patients with amyotrophic lateral sclerosis could become standard clinical practice. The figure was created in BioRender.

(rare mutations) to low (common variants). Even the largest genomics projects might not accurately identify rare intermediate-penetrance variants for amyotrophic lateral sclerosis due to the high lifetime risk and low frequency of pathogenic alleles.

Because precision treatments against specific disease-causing mutations are gaining importance as a therapeutic framework, distinguishing truly pathogenic versus benign variations is essential. Guidelines for interpreting the pathogenicity of variants exist (eg, the criteria by the American College of Medical Genetics and Genomics[13]), and resources such as ClinGen are available.[14] Establishing the pathogenicity of recently or newly identified genes for amyotrophic lateral sclerosis will pivot on segregation analysis, neuropathological signatures (eg, aggregates), or functional investigations in experimental models.[13] Large-scale analyses support a reoriented view of several genes and variants confined

For more on **ClinGen** see https://clinicalgenome.org/

For **data on genetic screening for patients with amyotrophic lateral sclerosis** see http://rshiny.tchpc.tcd.ie/users/dohertm7/journALS/App/

heavily to a single domain. A study of published data identified about 1% as pathogenic or probably pathogenic (111 mutations in 23 genes), 10% as benign or probably benign, and more than 89% as of uncertain significance. Of the pathogenic or probably pathogenic variants, 10% exhibited geographical heterogeneity underlining the population-specific and environmental interactions of variants for amyotrophic lateral sclerosis.

Oligogenic and polygenic models

Because mendelian inheritance only accounts for a proportion of cases, an oligogenic model of amyotrophic lateral sclerosis has emerged (ie, comprising a few risk genes).[15] Although oligogenic inheritance is reported in different populations, further studies are necessary. For example, a UK study of 100 participants with amyotrophic lateral sclerosis found that 13% harboured two pathogenic or probably pathogenic variants, which was associated with earlier disease onset (by 4 years) than in participants with only one pathogenic variant.[16] An Australian multicentre study of individuals with sporadic amyotrophic lateral sclerosis (n=616) found that 7% of participants had two or more variants, which was similarly associated with earlier disease onset than that in participants with no known variants.[15] By contrast, in an Irish population-based cohort study of both familial (n=50) and sporadic (n=394) cases, only 2% of patients harboured two or more known or potential variants for amyotrophic lateral sclerosis.[17]

Polygenic risk is assessed by linkage disequilibrium score testing and mendelian randomisation, 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 that amyotrophic lateral sclerosis shared polygenic risk with several traits: positive associations with smoking and moderate physical activity, and negative associations with cognitive performance and education.[18] Mendelian randomisation additionally identified a causal link between hyperlipidaemia and risk for amyotrophic lateral sclerosis. Indeed, a multi-ethnic GWAS identified variants in *ACSL5*, which encodes an enzyme involved in fatty acid β-oxidation and lipid biosynthesis, as a risk factor for amyotrophic lateral sclerosis.[19] Mendelian randomisation also suggested a causal association between genetically determined higher leukocyte count with lower risk of amyotrophic lateral sclerosis.[20]

Heritability

Strong evidence exists of an interplay between inherited and environmental factors, including for patients that carry a highly penetrant mutation.[21] Thus, heritability—ie, the extent that variation in disease risk is attributable to genetic variation—is an important concept in amyotrophic lateral sclerosis. Heritability estimates are population-specific, reflecting the underlying genetic substructure and gene–environment interactions. Assessment of heritability has relied on twin studies (38–78%),[22] large GWAS datasets (18%),[23] and population registers (53%).[3] In the Irish amyotrophic lateral sclerosis registry, the lifetime risk for a first-degree relative of a patient with amyotrophic lateral sclerosis, without known gene mutations associated with the disease, is 0·7% (and 1·4% if the genetic status is unknown).[3] This

	Year of discovery	Inheritance pattern	Familial ALS (%)*	Sporadic ALS (%)*	Function	Associated pathophysiology
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 calcyclin; putative LLPS
C21orf2 (also known as *CFAP410*)	2016	Not established	<1%	<1%	DNA damage repair (putative); actin structure	Cytoskeletal defects
CCNF	2016	Autosomal dominant	~1–3·3%	<1%	Component of an E3 ubiquitin ligase complex; cell-cycle regulation	Proteostasis defects
DNAJC7	2019	Not established	<1%	<1%	Heat shock protein co-chaperone	Not established
GLT8D1	2019	Autosomal dominant	<1%	<1%	Glycosyltransferase; unknown cellular function, widely expressed	Not established; localised to Golgi body, 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 or trafficking defects
LGALSL	2015	Not established	<1%	<1%	Not established	Not established
NEK1	2015	Not established	~1–2%	<1%	Serine–threonine kinase; cell-cycle regulation; axonal development or guidance; axonal polarity; DNA damage repair	Putative DNA damage accumulation; protein aggregation
TBK1	2015	Autosomal dominant	~3%	<1%	Serine–threonine kinase; regulates innate immunity, autophagy, and cell-cycle	Autophagy; inflammation
TIA1	2017	Autosomal dominant	~2·2%	<1%	RNA-binding protein	Impaired RNA metabolism; LLPS

(Table continues on next page)

	Year of discovery	Inheritance pattern	Familial ALS (%)*	Sporadic ALS (%)*	Function	Associated pathophysiology
(Continued from previous page)						
ALS genes discovered before 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 repair	Impaired RNA metabolism; impaired proteostasis or autophagy; intracellular trafficking; nucleocytoplasmic transport defects; LLPS; inflammation
CHCHD10	2014	Autosomal dominant	<1%	<1%	Mitochondrial protein localised to cristae junctions in the intermembrane space	Mitochondrial and bioenergetics dysfunction
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	Not established	<1%	<1%	Histone acetyltransferase subunit of RNA polymerase II elongator complex	Ribostasis defects; cytoskeletal defects
FUS	2009	Autosomal dominant; autosomal recessive	4%	1%	RNA-binding protein; transcription regulation; splicing; RNA localisation and degradation; DNA repair	Ribostasis defects, nucleocytoplasmic transport defects, LLPS
HNRNPA1	2013	Autosomal dominant; 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 localised 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 polymerisation	Cytoskeletal or trafficking defects; impaired 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	Autosomal dominant; autosomal recessive	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-κB)	Autophagy; inflammation
TARDBP	2008	Autosomal dominant; autosomal recessive	4%	1%	RNA-binding protein; transcription regulation; splicing, RNA localisation and degradation	Ribostasis, proteostasis, and nucleo-cytoplasmic transport defects; LLPS; prion-like transmission; inflammation
TUBA4A	2014	Autosomal dominant	<1%	<1%	Microtubule protein	Cytoskeletal or trafficking defects
UBQLN2	2011	X-linked, autosomal dominant	<1%	<1%	Ubiquitin-like protein (associates with proteasome and ubiquitin ligases)	Proteostasis defects; LLPS
VAPB	2004	Autosomal dominant	<1%	<1%	Plasma and intracellular vesicle membrane protein	Proteostasis defects
VCP	2010	Autosomal dominant	1%	1%	ATPase enzyme regulating protein degradation, intracellular membrane fusion, DNA repair and replication, NF-kB activation, and cell-cycle	Proteostasis defects; inflammation

Genes are listed alphabetically. Adapted from Chia et al.[2] *Percentage of familial or sporadic ALS caused by mutations in the particular gene. ALS=amyotrophic lateral sclerosis. FTD=frontotemporal dementia. GEF=guanine nucleotide exchange factor. LLPS=liquid-to-liquid phase separation.

Table: **ALS mutations and associated pathophysiology**

lifetime risk equates to a heritability of 36·9% in the non-*C9orf72* population and 52·3% in the overall population. The missing heritability in these populations promotes a focus on epigenomics and environmental contributions. Several studies report changes to the epigenome that are linked to amyotrophic lateral sclerosis (eg, non-coding promoter and enhancer elements, and microRNAs).[24,25] Additionally, the epigenome, as an entity that is reprogrammable through environmental pressures, opens an avenue into exposome science. The gene–time–environment hypothesis of amyotrophic lateral sclerosis proposes a multistep model to account for the environmental effect on disease onset and progression.[21] In European and east Asian populations, the gene–environment interaction promotes disease in up to six steps, with fewer steps in patients harbouring known monogenic, penetrant mutations (eg, *C9orf72*, *SOD1*, *TARDBP*).[26,27] Future work is needed to precisely define a step and establish when one has occurred.[28]

Overall, on the basis of recent progress, we anticipate that genetic testing will become standard practice for profiling patients with amyotrophic lateral sclerosis 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 mutations. Ultimately, case classification will shift to using mutation status rather than the concepts of familial and sporadic disease. However, genetic testing will require establishing the optimal approach, which will have to contend with the growing number of genes for amyotrophic lateral sclerosis, dealing with polygenic risk, and deciding whether to adopt whole-genome sequencing to address intronic variants that might contribute to the disease.

Genetic overlap with other neurodegenerative diseases

Amyotrophic lateral sclerosis is a clinically heterogeneous disease that extends beyond corticospinal structures.[29,30] Imaging shows thalamic and amygdala involvement as well as disrupted cortical functional networks in motor and extramotor domains (primarily involved in executive function and language),[31–33] whereas spatial domains are relatively preserved. Additionally, social, cognitive, and behavioural changes are common and mirror the behavioural variant of frontotemporal dementia.[34]

Clinical phenotypes of amyotrophic lateral sclerosis are modulated by some genetic variants;[2,35] *SOD1* variants primarily cause motor degeneration, whereas *FUS* mutations are associated with younger age of onset.[2] Additionally, some variants affect progression rate (eg, rapidly progressive SOD1^{A5V}, previously known as A4V). *C9orf72* repeat expansions are most strongly linked with cognitive and behavioural changes;[36] *FUS* and *TARDBP* mutations are also associated with dementia, as can some of the rarer mendelian mutations associated with amyotrophic lateral sclerosis. However, most affected patients with cognitive changes do not carry a known genetic variant. Moreover, several mutations that are risk factors for amyotrophic lateral sclerosis are genetically pleiotropic, and extramotor features of the disease overlap with those of other neurodegenerative diseases (panel 1).[8,37] *C9orf72* repeat expansions are the most common mutations occurring in Huntington's disease phenocopies—patients presenting with Huntington's disease without carrying the most characteristic Huntington's disease-associated mutation: *HTT* repeat expansions.[38] Conversely, in rare instances, patients with frontotemporal dementia or amyotrophic lateral sclerosis can harbour *HTT* repeat expansions concurrent with TDP-43 inclusions (the histopathological hallmark of amyotrophic lateral sclerosis), without defining Huntington's disease characteristics such as neostriatal atrophy.[39]

Although of uncertain clinical significance (because of the presence in individual patients in case reports), mutations in risk genes for amyotrophic lateral sclerosis (ie, *TIA1*, *TBK1*, *SQSTM1*, and *GRN*) are detected in patients with dementia with Lewy bodies, a clinically heterogeneous neurodegenerative disease.[40] A 32-CAG repeat expansion to *ATXN2* has been reported in a patient with both amyotrophic lateral sclerosis and spinocerebellar ataxia type 2;[41] intermediate 32-CAG repeats correlate with amyotrophic lateral sclerosis[42] but reside below the cutoff for spinocerebellar ataxia type 2,[43] suggesting a potential overlap between the two diseases. Additionally, pathogenic mutations to *KIF5A*, known to cause hereditary spastic paraplegia and Charcot-Marie-Tooth disease type 2, are also described in individuals with amyotrophic lateral sclerosis[4] and primary progressive multiple sclerosis,[44] although mutations occur in different *KIF5A* domains in those with hereditary spastic paraplegia compared with those with amyotrophic lateral sclerosis. Thus, the genotype–phenotype relationship among genetic mutations that cause neurodegenerative disease is highly complex. Research is needed to establish how the same genetic mutations diverge on distinct phenotypes and, on the other hand, how mutations to different genes converge on similar phenotypes—eg, mutations to distinct gene domains or overlap in the number of disease-causing repeats. Polygenic risk[18] and environmental influence[21] are possible factors, which are highly relevant to amyotrophic lateral sclerosis.

There is also emerging evidence of disease endophenotypes among family members of those with amyotrophic lateral sclerosis. Cohort studies describe family aggregation of neuropsychiatric disease, primarily psychosis and suicide, in kindreds of probands with amyotrophic lateral sclerosis.[45,46] Although *C9orf72* repeat expansions account for a proportion of aggregation, they are not overrepresented in individuals with typical schizophrenia.[47] Detailed family studies show a non-uniform distribution of neuropsychiatric conditions, which instead cluster in up to 30% of kindreds of patients

with amyotrophic lateral sclerosis,[8] suggesting genetic pleiotropy or oligogenic inheritance. There is also evidence of overlapping polygenic risk between amyotrophic lateral sclerosis and neuropsychiatric disease. Analysis of GWAS datasets from the Project MinE and the Psychiatric Genomics Consortium found 14% polygenic overlap between amyotrophic lateral sclerosis and schizophrenia.[48] Indeed, *GLT8D1*, a recently identified risk gene for amyotrophic lateral sclerosis, is also a schizophrenia risk gene.[49] These observations suggest that the pathogenic process underpinning some forms of amyotrophic lateral sclerosis disrupt specific brain network patterns.[50] This disruption might be mediated by developmental processes that render some 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 amyotrophic lateral sclerosis with neuropsychiatric disease.

Gene-based treatment strategies

The rising number of risk genes for amyotrophic lateral sclerosis, comprising gain-of-function and loss-of-function missense and nonsense mutations and repeat expansions, advocates for gene-based approaches for treatment. Rapid advances have been made in gene-based therapies, which comprise several techniques such as antisense oligonucleotides, RNA interference, gene replacement therapy, and genome editing (panel 2).[52] The optimal approach depends on the mutation and the distribution and amount of the encoded protein. Pathogenic gain-of-function mutations can be targeted by antisense oligonucleotides or RNA interference, but this strategy might be difficult in practice because many genes for amyotrophic lateral sclerosis are widely expressed and the wild-type protein performs essential functions. However, if the mutant protein is overexpressed, this approach could be feasible (eg, 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, although currently only in experimental stages, could potentially be leveraged to correct both gain-of-function and loss-of-function mutations and offer the ability to specifically target the mutant allele, overcoming the weakness of antisense oligonucleotides and RNA interference. Trial designs, such as umbrella trials, can leverage molecular phenotyping to select trial participants harbouring specific mutations targeted by a candidate gene therapy (figure 1F).

Antisense oligonucleotides

Antisense oligonucleotides are short, synthetic, single strands of oligonucleotides of around 20 chemically modified nucleotides with known in-vivo stability.[58] Because antisense oligonucleotides do not cross the blood–brain barrier, treating neurodegenerative disorders requires CSF delivery (eg, intrathecal or intracerebroventricular). Antisense oligonucleotides bind to target pre-mRNA or mRNA to reduce protein expression through two main mechanisms.[58] Duplex formation marks the target pre-mRNA or mRNA for degradation by endogenous ribonuclease H; alternatively, antisense oligonucleotides interfere with target pre-mRNA or mRNA translation or splicing, or both.[58] In individuals with amyotrophic lateral sclerosis, antisense oligonucleotides can potentially target *C9orf72*, *TARDBP*, *SOD1*, or *FUS* RNA foci. Several clinical trials of antisense oligonucleotides are underway in patients with amyotrophic lateral sclerosis (panel 2).[52,58] The *SOD1*-targeting tofersen (also known as BIIB067) was shown to be safe and to lower CSF SOD1 concentrations in a phase 1/2 trial, particularly in the high-dose group;[59] unfortunately, tofersen did not meet its primary endpoint in a phase 3 trial (NCT02623699). Another phase 3 trial of tofersen is also recruiting presymptomatic carriers of rapidly progressive *SOD1* mutations with blood-based biomarker evidence of disease through elevated neurofilament light chain concentrations (NCT04856982). This trial is following a framework of preventive therapy for highly penetrant *SOD1* mutation carriers. Phase 1 trials of antisense oligonucleotides designed to target *C9orf72* (BIIB078, NCT03626012; IWVE-004, NCT04931862) and *ATXN2* (BIIB105, NCT04494256) expansion repeats are also in the pipeline. Finally, a phase 1–3 trial targeting *FUS* is also ongoing (ION363, jacifusen, NCT04768972).

Pathophysiology

Despite tremendous progress, the pathophysiology of amyotrophic lateral sclerosis remains incompletely understood. However, as our knowledge of the genetic architecture deepens, we are discovering the molecular steps that various mutations take to converge on recurrent dysregulated nervous system pathways. The major shared pathological pathways in individuals with amyotrophic lateral sclerosis include impaired RNA metabolism, altered proteostasis or autophagy, cytoskeletal or trafficking defects, mitochondrial dysfunction, and compromised DNA repair (table; figure 2).[60,61] Among the most common genes for amyotrophic lateral sclerosis, mutant *C9orf72*, *TARDBP*, and *FUS* impair RNA metabolism; *C9orf72* repeat expansions, *TARDBP*, and *SOD1* also induce defects in protein homoeostasis. Mutant *SOD1* also triggers mitochondrial dysfunction and oxidative stress.[60]

Repeat expansions in *C9orf72* lead to mutant protein and haploinsufficiency from the wild-type allele; additionally, RNA transcripts of *C9orf72* expansions aggregate into toxic RNA foci, sequestering RNA-binding proteins and altering RNA metabolism.[60] Aberrant translation of *C9orf72* transcript expansions generates proteotoxic dipeptide repeats—eg, poly proline (P)–arginine (R) repeats (poly[PR]) and poly glycine (G)–arginine (R) repeats (poly[GR]).[60] TDP-43 cytoplasmic inclusions are an almost universal feature of amyotrophic lateral sclerosis, present in about 97% of cases.[62] Although

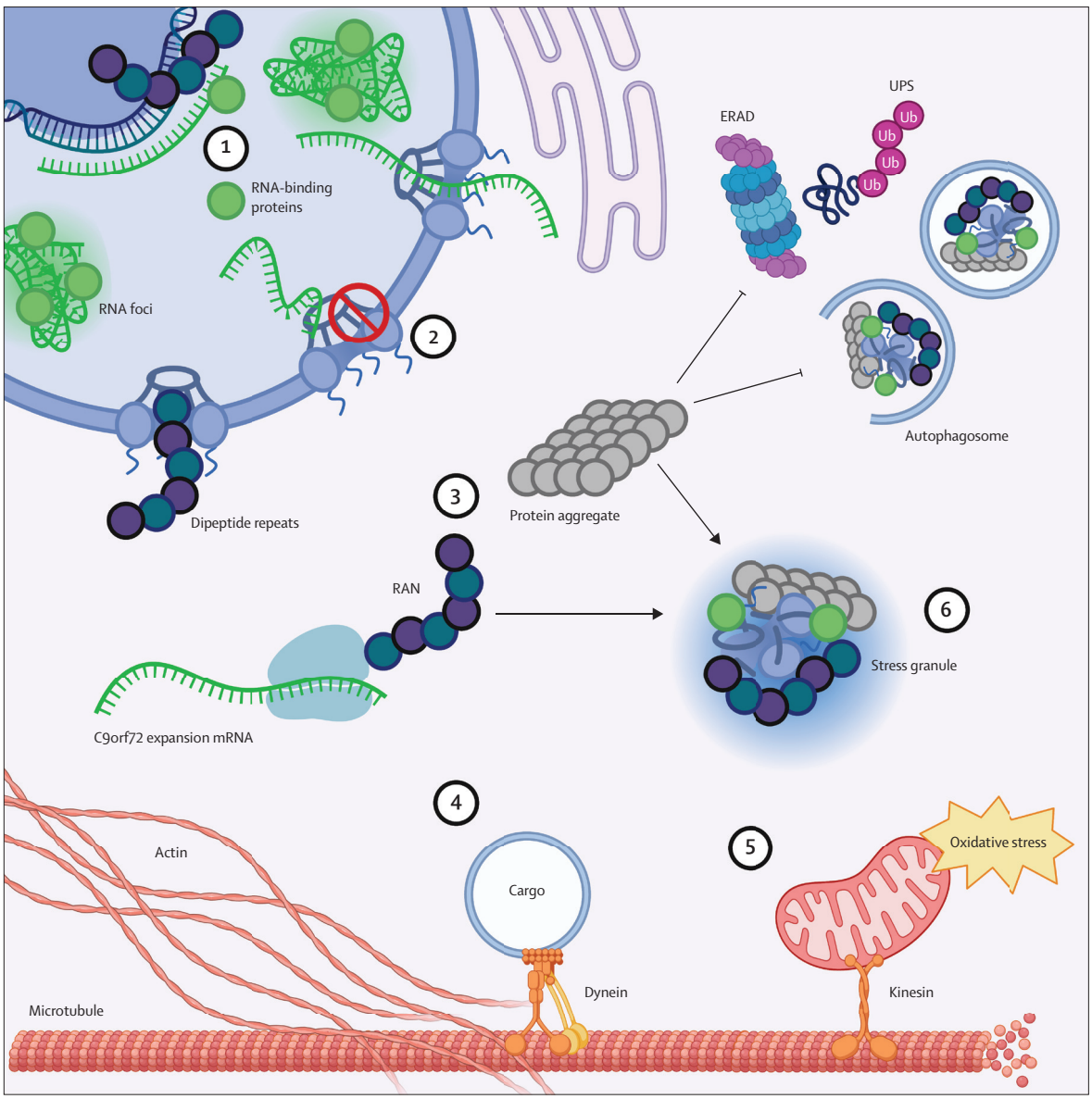

Figure 2: **The pathophysiology of amyotrophic lateral sclerosis**
Pathological pathways centre on impaired RNA metabolism, altered proteostasis or autophagy, cytoskeletal or trafficking defects, mitochondrial dysfunction, and compromised DNA repair. Numbering from top left downwards: (1) Mutant RNA-binding proteins (eg, FUS and TDP-43) disrupt RNA transcription and splicing. C9orf72 repeat expansion RNAs aggregate into RNA foci, sequestering RNA-binding proteins and impairing RNA metabolism. Additionally, haplo-insufficiency from the single remaining wild-type *C9orf72* allele leads to loss-of-function of native C9orf72 protein function, related to multiple mechanisms such as trafficking, autophagy, and DNA repair. (2) Mutant *C9orf72*, *FUS*, and *TARDBP* functionally impair nucleocytoplasmic transport and induce nuclear envelope morphology defects and cytoplasmic inclusions of nucleocytoplasmic transport components (eg, nucleoporins, importins, and RANs). (3) Repeat-associated non-AUG translation of *C9orf72* repeat expansions yields dipeptide repeats, which are toxic through several pathways, including protein aggregation, chromatin alterations, and DNA damage; impaired nucleocytoplasmic transport; and component sequestration. Additional cytoplasmic protein aggregation (eg, TDP-43 and SOD1) induces proteostasis and autophagy defects. Protein aggregates block the ERAD response and UPS, preventing aggregate clearance. Mutations to ubiquitination proteins (eg, CCNF and UBQLN2) additionally dysregulate the UPS. Protein aggregates and RNA-binding proteins also accumulate into stress granules, which become persistent in individuals with amyotrophic lateral sclerosis. Mutations to vesicle-forming proteins (eg, OPTN, VAPB, and VCP) disrupt vesicular transport and distribution, leading to dysfunctional autophagy and proteostasis. (4) Mutations to the tubulin transport machinery (eg, DCTN1, KIF5A, and TUBA4A) and actin (eg, PFN1) induce cytoskeletal or trafficking defects, which impair distribution of vital organelles throughout cells (eg, mitochondria and cargo-laden vesicles). (5) Protein aggregates (eg, TDP-43 and SOD1) and mutations to mitochondrial protein components (eg, CHCHD10) trigger mitochondrial and bioenergetics dysfunction and raise oxidative stress. (6) Liquid-to-liquid phase separation of aggregation-prone proteins (eg, FUS and TDP-43) drives formation of stress granules. This figure was created in BioRender. ERAD=endoplasmic reticulum-associated protein degradation. RAN=GTPase Ras-related nuclear protein. UPS=ubiquitin proteasome system.

Panel 2: Gene-based treatment strategies for amyotrophic lateral sclerosis

RNA interference

Comprises two approaches: small interfering RNA (siRNA) and short hairpin RNA (shRNA).[51] siRNAs are generally duplexes of two strands of about 20 modified nucleotide base pairs long, that can be internalised into cells.[51] The strand of the siRNA complementary to the gene target binds to endoribonuclease Dicer protein and recruits argonaute proteins and target mRNA, generating an RNA-induced silencing complex (RISC). RISC cleaves the target gene mRNA, leading to gene knockdown.[51] shRNAs are hairpin structures of either natural or modified nucleotide bases, which can be delivered by viral vectors.[51] After internalisation into cells, shRNAs are first cleaved by endoribonuclease Dicer protein to remove the hairpin, and then follow the same pathway as siRNAs through RISC.[51]

RNA interference is approved by the US Food and Drug Administration (FDA) to treat hereditary transthyretin amyloidosis.[51] Strategies are being tested in experimental models of amyotrophic lateral sclerosis,[52] but have not yet entered clinical trials.

Gene replacement therapy

This approach uses viruses as vectors to provide patients harbouring loss-of-function mutations a functional copy of a gene.[52] Viruses can cross the brain–blood barrier and might consequently be administered intravenously, which is a considerable advantage. Currently, two vectors are employed, lentivirus, which delivers the replacement gene by mRNA, and adeno-associated virus (AAV), which delivers the replacement gene by cDNA.

Onasemnogene abeparvovec, an AAV9-mediated gene replacement therapy for *SMN1*, is approved by the US FDA. A phase 1, open-label, dose-escalation, clinical trial assessed a single intravenous injection of onasemnogene abeparvovec in children with the *SMN1* mutation (n=15; NCT02122952).[53] The treatment was safe and significantly improved motor function and survival (100% *vs* 8%) compared with historical cohorts. The extremely promising results warranted Fast Track, Breakthrough Therapy, and Priority Review designation by the FDA, culminating in approval for treating patients younger than 2 years and showing the feasibility of this approach for treating neuromuscular disease.

The most common mutations for amyotrophic lateral sclerosis (ie, *C9orf72*, *SOD1*, *TARDBP*, and *FUS*) result in toxic gain-of-function, and are therefore not amenable to gene replacement therapy. However, gene delivery of neurotrophic factors is being investigated in experimental models.[52] Moreover, less frequent but penetrant loss-of-function mutations might become viable candidates as research advances.

Genome-editing technologies

These technologies aim 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.[54] The CRISPR RNA guide targets the locus of interest by simple base pairing, which means that a guide can be designed to target any gene of interest.[54] Gene editing can modify chromosomal DNA, but that can have unintended consequences, such as unwanted deletions or chromosomal rearrangements.[55] CRISPR can do more targeted changes than other technologies can (eg, single-base editing),[54] which do not require a double-stranded DNA break. Additionally, CRISPR technology can modulate transcription and edit RNA, expanding its potential applications.[54]

There are no clinical applications of such technologies to date, but they are being tested in experimental models of amyotrophic lateral sclerosis against *SOD1* mutations and *C9orf72* repeat expansions.[52,56,57]

principally nuclear, TDP-43 is mislocalised to the cytoplasm in patients with amyotrophic lateral sclerosis, and is heavily post-translationally modified or truncated, or both.[63] Mislocalised TDP-43 impairs RNA splicing, for instance, of stathmin-2, a protein required for microtubule stability.[64] Diminished stathmin-2 concentrations lead to impaired axonal growth and motor neuron function.[64] Patients with amyotrophic lateral sclerosis and TDP-43 inclusions do not have FUS and SOD1 aggregates;[65] although both TDP-43 and FUS are RNA-binding proteins, which regulate transcription and RNA splicing, localisation, and degradation, there is little overlap between their binding targets.[66]

Of genes discovered in the past 5 years, research suggests involvement in RNA metabolism (*TIA1*), proteostasis or autophagy (*CCNF*, *NEK1*, *TBK1*), and cytoskeletal or trafficking defects (*ANXA11*, *C21orf2*, *KIF5A*).[12,60] The *DNAJC7*-mediated, *GLT8D1*-mediated, and *LGALSL*-mediated mechanisms of neurodegeneration are uncertain. DNAJC7 is a heat shock protein co-chaperone, which could possibly be linked to proteostasis or autophagy.[12] It is hypothesised that GLT8D1, a glycosyltransferase, might impair ganglioside biosynthesis and O-linked β-N-acetylglucosamine modification.[67] The cellular role of galectin-related protein (encoded by *LGALSL*) is completely unknown; however, galectins are galactoside-binding proteins. Therefore, the discovery of novel genes for amyotrophic lateral sclerosis might unlock as yet unknown research avenues and pathological processes.

Nucleocytoplasmic transport defects

Nucleocytoplasmic transport is a highly regulated process, which conveys RNA and protein cargo between the nucleus and cytoplasm.[68] This process is mediated by large, multi-subunit nuclear pore complexes consisting of nucleoporins, which act in concert with cytoplasmic importins (importing protein cargo from cytoplasm to nucleoplasm) and nuclear exportins (exporting protein

cargo from cytoplasm to nucleoplasm).[68] Transport directionality for protein cargo is governed by small GTP-binding nuclear Ran proteins by binding to importins and exportins. Studies report both morphological and functional defects in nucleocytoplasmic transport in animal and cell models of amyotrophic lateral sclerosis, also present in tissue from patients with sporadic or familial disease.[68] Specifically, nucleocytoplasmic transport and nuclear envelope morphology are impaired by *C9orf72* repeat expansions,[69,70] insoluble TDP-43 aggregates,[71] and mutant *FUS*.[72] In patients with amyotrophic lateral sclerosis with mutant *TARDBP* or sporadic disease, abnormal immunoreactivity against nucleoporins, importins, and GTP-binding nuclear Ran proteins is detected in motor cortex and spinal motor neurons, even independent of *C9orf72* repeat expansions.[71–73] Impaired nucleocytoplasmic transport might represent a universal pathology in neurodegenerative diseases, because it is also present in patients with Alzheimer's disease[74] and in those with Huntington's disease.[75]

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 nucleocytoplasmic transport, alter chromatin structure.[76] Poly(PR) expression in mouse models produces neuronal loss and gliosis, resulting in motor and memory defects.[76] Poly(PR) binds to DNA and localises with heterochromatin, disrupting the condensed state, leading to aberrant histone methylation and altered gene expression.[76] Furthermore, poly(PR) produces nuclear lamina invaginations and impairs nucleocytoplasmic transport.[76] Poly(PR) also co-localises with heterochromatin in cortex from affected patients with the *C9orf72* repeat expansion.[76] These dipeptide repeats can trigger TDP-43 proteinopathy, forging a link between *C9orf72* repeat expansions and TDP-43 pathology.[77,78] Poly(GR) and Poly(GA) induce cytoplasmic TDP-43 inclusions;[77,78] additionally, poly(GR) sequesters nucleocytoplasmic transport proteins.[77] Encouragingly, an antisense oligonucleotide targeting *C9orf72* GGGGCC repeats reduces poly(GR) burden, TDP-43 pathology, and neurodegeneration.[77] Poly(GR) aggregates co-localise with TDP-43 inclusions in brain tissue from patients with amyotrophic lateral sclerosis, suggesting pathological involvement.[79] Importantly, studies are not fully concordant, possibly due to differing model systems; thus, these findings require further investigation.

Liquid-to-liquid phase separation

In addition to impaired nucleocytoplasmic transport, there is emerging interest in liquid-to-liquid phase separation (LLPS) in amyotrophic lateral sclerosis.[80] LLPS occurs when a homogeneous fluid separates into two liquid phases, forming a dynamic, organelle-like structure lacking a membrane.[80] This process is related to several pathophysiological processes in amyotrophic lateral sclerosis, including nucleocytoplasmic transport, RNA metabolism, DNA repair, protein aggregation, and axonal transport.[80] Stress granules are the most widely studied example of LLPS and form under cellular duress; normally, however, stress granules are dynamic and reversible once the cellular stress subsides. Yet, in amyotrophic lateral sclerosis, 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.[80] Arginine-rich *C9orf72* repeat expansion-derived dipeptide repeats undergo LLPS and induce stress granule assembly, impairing dynamics.[81] An in-vitro study on various cell types shows how LLPS occurs during increased cytoplasmic TDP-43 concentrations, even independent of stress granules, recruiting nucleoporins, importins, and GTP-binding nuclear Ran proteins.[82] Although *TARDBP*, *FUS*, and *C9orf72* are the major LLPS-related genes for amyotrophic lateral sclerosis, multiple, less common risk genes are also involved, such as *HNRNPA1*, *HNRNPA2B1*, *TIA1*, and *UBQLN2*.[80] Thus, LLPS is an exciting research direction, because it is shared by several risk genes and is 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.[80] Self-propagating 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 amyotrophic lateral sclerosis research, including of wild-type and mutant SOD1,[83] dipeptide repeats,[84,85] and TDP-43.[86]

Inflammatory pathways

Dysregulated inflammatory pathways are a recurrent thread in patients with amyotrophic lateral sclerosis.[87] Central and peripheral inflammation are present in mutant *C9orf72*, *SOD1*, and *TARDBP* animal models and in patients with familial amyotrophic lateral sclerosis.[87] This pathophysiology is characterised by immune cell infiltration into the CNS, dysregulated peripheral immune cell counts, induction of an activated immune phenotype, and altered cytokine production (figure 3).[87] Cytotoxic CD8 T cells infiltrate the CNS of mutant *Sod1*^{G93A} mice and selectively destroy motor neurons; genetic ablation of this immune cell population slows motor neurodegeneration.[88] Furthermore, mutant *SOD1*^{G93A} CD8 T cells express increased concentrations of interferon γ, a cytokine linked to amyotrophic lateral sclerosis progression.[88] Patients with amyotrophic lateral sclerosis and loss of C9orf72 activity secondary to *C9orf72* repeat expansions lose the ability to regulate interferon production via the innate immune system (cGAS–STING pathway), leading to type 1 interferon-mediated systemic and CNS inflammation.[89]

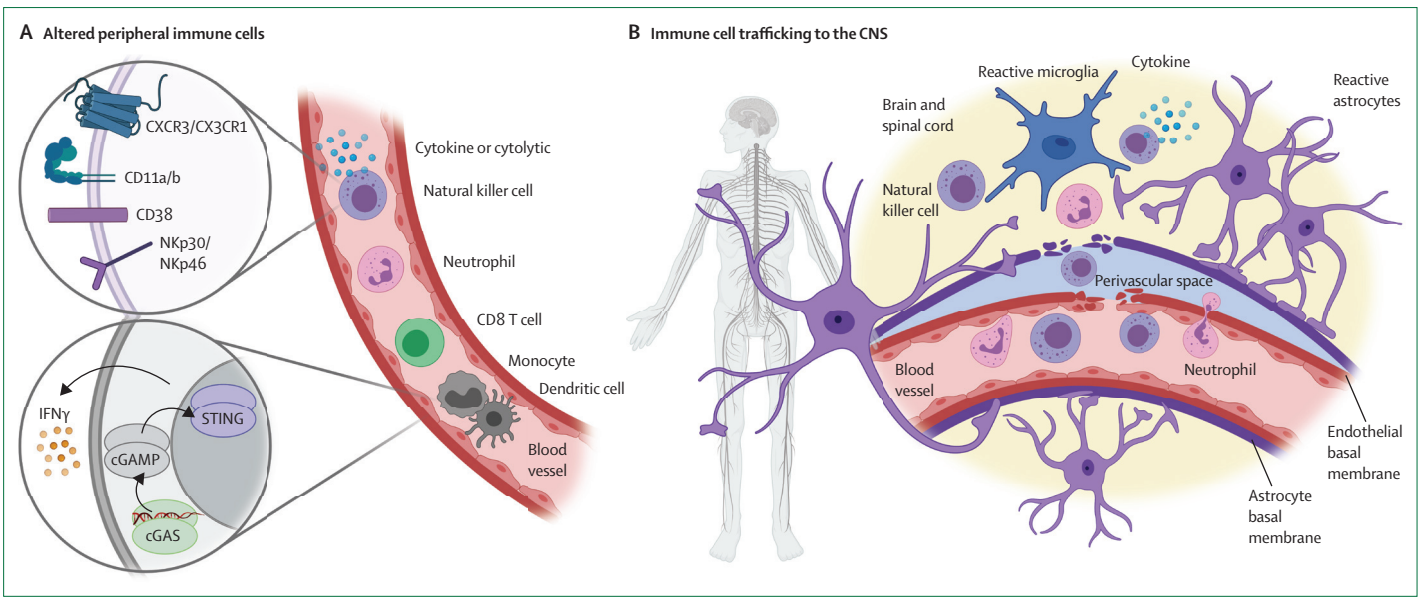

Figure 3: **Inflammatory pathways in amyotrophic lateral sclerosis**
(A) Various peripheral immune cell populations in blood have differential levels of expression in patients with amyotrophic lateral sclerosis, including innate (eg, neutrophils and natural killer cells) and adaptive (CD8 T cells) cells. In patients with amyotrophic lateral sclerosis, circulating natural killer cells over-express surface markers of cytotoxic function (eg, CD38, NKG2D, NKp30, and NKp46) and trafficking (eg, CD11a, CD11b, CXCR3, and CX3CR1). Circulating monocytes and dendritic cells expressing mutant TARDBP and C9orf72 repeat expansions increase IFNγ production. (B) Peripheral immune cells traffic to the CNS in patients with amyotrophic lateral sclerosis (eg, neutrophils and natural killer cells). This figure was created in BioRender. cGAMP= cyclic guanosine monophospate–adenosine monophosphate. cGAS=cGAMP synthase. IFNγ=interferon γ. STING=stimulator of interferon genes protein.

Similar increased interferon production is associated with TDP-43 pathology in cell and animal models of amyotrophic lateral sclerosis.[90] Blocking innate immunity signalling in mutant *Tardbp* mice normalises interferon concentrations, slows disease progression, and lengthens survival.[90] Simultaneous with the increase in cytotoxic immune cells, amyotrophic lateral sclerosis is characterised by decreased concentrations of immune-regulatory and anti-inflammatory Tregs[87] and CD4 T cells.[91] Additionally, less frequent mutations for amyotrophic lateral sclerosis induce inflammation, including those in *OPTN*, *SQSTM1*, *TBK1*, and *VCP*.[87] Thus, inflammation might modulate the progression of amyotrophic lateral sclerosis and survival. In patients with sporadic disease lacking any known genetic causes, the mechanism of immune dysregulation remains uncertain, although it is a characteristic feature.[91,92] Similar to amyotrophic lateral sclerosis with a determined genetic cause, patients with sporadic disease have altered peripheral immunity, induction of an activated immune phenotype, and changes in peripheral cytokine concentrations.[87]

Overall, the emerging research directions in the pathophysiology of amyotrophic lateral sclerosis are nucleocytoplasmic transport, LLPS, and cell-to-cell transmission. These pathways are interrelated and feed into other pathological aspects, such as abnormal ribostasis, proteostasis, and trafficking; mitochondrial dysfunction; DNA repair defects; and inflammation. Future work is needed to generate a holistic view of the pathophysiology of amyotrophic lateral sclerosis.

The exposome and amyotrophic lateral sclerosis

Although burgeoning genetic discoveries have deepened our understanding of the aetiology of amyotrophic lateral sclerosis, most cases are sporadic and do not have a known genetic cause. Moreover, incomplete heritability of known mutations suggests that environmental factors are involved.[21] This consideration has led to the gene–time–environment hypothesis, which suggests that genetic predisposition interacts with environmental exposures over time leading to the development of amyotrophic lateral sclerosis.[21] Thus, the role of an individual's cumulative lifelong exposure (the exposome) on the risk of amyotrophic lateral sclerosis represents a developing research direction to better understand the aetiology and identify modifiable risk factors to prevent disease. Furthermore, the multistep model also supports environmental effects in amyotrophic lateral sclerosis, because a series of steps are required for disease onset,[93] even in individuals with penetrant mutations.[26]

Several studies have investigated the exposome related to amyotrophic lateral sclerosis, which is broad and encompasses exogenous toxicant exposures (eg, environmental pollutants[94]), medical events (eg, brain trauma[94]), and lifestyle factors (eg, intense physical activity[95] and military service[94]). Some exogenous environmental exposures can increase the risk of disease or accelerate disease progression (appendix pp 3–6). A 2017 meta-analysis highlighted some commonly studied links between amyotrophic lateral sclerosis and the

See Online for appendix

environment (odds ratio>1), encompassing lead exposure, heavy metals, pesticides, agricultural chemicals, and solvents.[94] Studies in the past 5 years add to the growing literature of environmental risk factors for amyotrophic lateral sclerosis (appendix pp 3–6).

Importantly, not all exposome studies are concordant (appendix p 2), which might arise from different population sizes or characteristics (eg, location or genetics), exposure duration, adjustment parameters, and methodology (eg, historical estimates *vs* analyte measurements). Thus, despite a considerable body of evidence and identified links between amyotrophic lateral sclerosis and the environment, large prospective cohort studies are needed.[96] These studies will require detailed registries of patient medical information linked to personal data and occupational and residential history with banked biosamples. Studies should evaluate how the exposome modifies disease progression and outcomes,[97] as well as onset risk. Furthermore, environmental risks might not be geographically uniform, necessitating large prospective cohorts across diverse regions, possibly globally. Additionally, geographically distinct populations might also be genetically distinct, which could modify their exposure risk. Although gene–environment interaction studies have been done for single-gene candidates,[95] multiomics studies will be needed that bridge genetics[98] (ie, monogenic, oligogenic, and polygenic risk) with the exposome, to truly comprehend amyotrophic lateral sclerosis risk and progression.

Conclusions and future directions

Much progress has been made towards a more comprehensive picture of amyotrophic lateral sclerosis, aided by a new understanding of the complex genetics behind the disease and the discovery of novel disease mechanisms. The advent of genetic therapies has realised experimental and early clinical trials of genetic therapies. Our growing body of knowledge advocates for a shift in clinical practice, trial design, and emerging research questions. Regarding clinical practice, we anticipate genetic testing will become routine, with the profiling of patients by mutation or genetic or polygenic risk, rather than the previous dichotomisation of familial or sporadic. Genetic profiling should also be leveraged to transform how forthcoming clinical trials are conducted, especially for genetic therapies, by stratifying trial participants by mutation status. This stratification will also ultimately impact management, as we shift gears to a more tailored precision approach. For preventive therapies, improved predictive algorithms will identify individuals most at risk, as the understanding of penetrance and oligogenic or polygenic risk crystallises. This development will tie in with environmental factors; multiomics platforms could generate an integrated perspective on gene–exposome architecture rather than on individual genetic or exposome contributions.

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," and "traffic." The search focused on articles published from Jan 1, 2016, to Oct 15, 2021, although well known and seminal older articles were also considered. We also included articles from the authors' personal reference lists. Articles were selected on the basis of relevance to this Review. Additionally, we searched the ClinicalTrials.gov registry using "amyotrophic lateral sclerosis" with "gene therapy", "antisense oligonucleotide, "RNAi," "small interfering RNA," "short hairpin RNA," "AAV9," and "CRISPR."

Machine learning and big data might play a part in these ambitious goals;[99] for instance, in prioritising genes for amyotrophic lateral sclerosis,[100] particularly in view of the disease's complexity. Emerging questions will continue to refine our picture of amyotrophic lateral sclerosis. Given the phenotypic spectrum of the disease and its overlap with other neurological diseases, and the genetic overlap among various conditions, should we switch to a molecular classification? Could we integrate such a classification with an exposome classification? These questions are not unique to amyotrophic lateral sclerosis, because most neurodegenerative diseases are sporadic. To meet the challenges of this complex disease, future studies will rely on large multicentre cohorts and integrated multiomics platforms, necessitating international collaborative projects. Findings from these collaborative projects will improve our understanding of disease pathogenesis and lead to much needed and long-awaited therapies.

Contributors

All authors contributed to conceptualisation, the writing of the original draft, and review and editing of later drafts.

Declaration of interests

SAG declares 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 declares 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 is Editor-in-Chief for the journal *Amyotrophic Lateral Sclerosis and Frontotemporal Dementia*. AA-C declares consulting fees from Mitsubishi Tanabe Pharma, Biogen Idec, Cytokinetics, Wave Pharmaceuticals, Apellis, Amylyx, Novartis, and Eli Lilly. AC declares

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. MCK has an honorary role as President of the Brain Foundation and as Editor-in-Chief of the *Journal of Neurology, Neurosurgery and Psychiatry*. ELF declares a patent "Methods for treating amyotrophic lateral sclerosis". MGS declares no competing interests.

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 and R01NS120926; 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, and the Health Research Board (Ireland). AA-C is a Senior Investigator for the National Institute for Health Research (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: Medical Research Council (MR/L501529/1; MR/R024804/1), Economic and Social Research Council (ES/L008238/1), and the Motor Neurone Disease Association. This study represents independent research partly funded by the 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); the Joint Programme–Neurodegenerative Disease Research (Strength, ALS-Care and Brain-Mend projects), granted by Italian Ministry of Education, University and Research; and the Department of Excellence grant of the Italian Ministry of Education, University and Research to the Rita Levi Montalcini Department of Neuroscience, University of Turin, Turin, 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. Funding from Horizon 2020, the ALS Association, and My Name'5 Doddie Foundation are also acknowledged.

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