A BIOINFORMATICS EXPLORATION OF DIFFERENTIAL GENE EXPRESSION IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

ABSTRACT :

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disorder characterized by the degeneration of motor neurons in the brain and spinal cord, leading to muscle weakness, paralysis, and ultimately, respiratory failure. Despite extensive research efforts, the exact etiology of ALS remains elusive, involving a complex interplay of genetic, environmental, and pathological factors. This abstract provides an overview of the current understanding of ALS pathogenesis, including protein misfolding, mitochondrial dysfunction, oxidative stress, and neuroinflammation. Diagnostic approaches, including clinical assessments, electromyography, and imaging techniques, are crucial for early detection and management of ALS. While there is currently no cure for ALS, various therapeutic strategies aim to alleviate symptoms, slow disease progression, and improve quality of life for patients. These include pharmacological interventions, such as riluzole and edaravone, as well as emerging treatments targeting genetic mutations and neuroprotective pathways. Advancements in understanding ALS pathophysiology and the development of novel therapeutic modalities offer hope for improved outcomes and ultimately, a cure for this devastating disease.

KEYWORDS :

Amyotrophic lateral sclerosis (ALS)s, Bioinformatics Tools, Neuronal Function, Inflammation, And Cellular Stress Responses

AIM AND OBJECTIVES :

Aim :

The aim of this study is to investigate the differential expression of genes associated with Amyotrophic lateral sclerosis (ALS), compared to healthy venous tissue, utilizing advanced molecular techniques. By identifying genes that are differentially expressed in Amyotrophic lateral sclerosis (ALS), we aim to gain insights into the underlying molecular mechanisms contributing to the pathogenesis of this condition.

Objectives :

1. To identify Gene Expression Profiling
2. To identify the Differentially Expressed Genes
3. To identify the Functional Annotation and Pathway Analysis
4. To identify the Validation of Candidate Genes

INTRODUCTION :

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is a devastating neurodegenerative disorder in which degeneration of the upper motor neurons in the motor cortex and lower motor neurons in the brainstem and spinal cord cause progressive denervation of voluntary muscles. ALS occurs globally, with an incidence of approximately 2 per 100,000 person-years, a prevalence of 6–9 per 100,000 persons (refs. 1,2) and a lifetime risk of approximately 1 in 350. There is evidence that the condition is increasing in incidence1,4. This may be partially accounted for by changing population demographics, and improved clinical services supporting accurate diagnosis.

What Is Amyotrophic Lateral Sclerosis (ALS)?

ALS is known to be one of the most common and aggressive neuromuscular diseases, which can hit hard following the subtle early signs that are often overlooked and lead to one of the most debilitating illnesses. Most commonly, it strikes people between 40–60 years, but also younger individuals can develop ALS. Two point six individuals/100,000 develop ALS every year, whereby men generally have a higher propensity to develop ALS than women (1). ALS slowly takes away the ability to walk, eat, or breathe from those affected. This devastating adult-onset neurodegenerative disease leads to the loss of motor neurons, the long nerve cells in the brain (upper motor neurons, UMNs) and spinal cord (lower motor neurons, LMNs), which innervate the muscles (2). These motor neurons are essential for the communication between the brain and themuscles and transmit vital instructions for mobility. When these nerve cells are dysfunctional or damaged, they gradually stop communicating with the muscles, and the brain loses its ability to control and initiate voluntary movements such as walking, chewing or talking. This results in a progressive weakness, muscle twitches (fasciculations), and atrophy of voluntary skeletalmuscles throughout the body. In the final stages of the disease, this leads to fatal paralysis and death due to respiratory failure.

The Current Views and Paradigms of ALS

Chronic diseases, such as neurodegenerative diseases (NDs), including ALS, are different from infectious diseases where, if the source of infection or epidemic can be back-tracked, the resolution to the problem generally becomes clearer, and targeted treatment can be applied. There is no global consensus on the criteria that define the start point of ALS in humans or preclinical studies involving mouse models of ALS. Nevertheless, no disease can begin without a start point. However, the same disease can have heterogenous start points that vary from person to person and follow numerous roads to the same endpoint, which is the case with ALS. This can be illustrated by

metro lines where the map is different for each line, but due to intersecting stations between different maps, the metro lines can still end at the same destination. Thus, identifying these intersections, which are the genes and pathophysiological pathways, is vital in elucidating the map that leads to ALS. Moreover, it is important to emphasize that there is a serious debate in relation to pathological and clinical and phenotypic heterogeneity and whether this means that ALS should be treated as a single entity, or if it needs stratification relying on molecular signatures that are only partially reliable given their complexity (3, 4). While recent advances in the understanding of ALS have led to new questions (5), transgenic mouse models have failed to provide rapid advances in prevention and treatment of ALS and masking the true complexity of ALS disease in monogenic models. As reviewed by Turner and Swash (5), ALS has evolved into a multisystem disorder, which may involve a final common pathway and clinical core accessible via multiple upstream etiological tributaries. Continuing clinical observations, investigating the molecular complexity of ALS, and the convergence of the resulting findings are critically important to inform the development of new therapies or prevention strategies (5). This is the main premise for this review. It can be best explained by an example of C9ORF72 mutation and the primary assaults it incurs and the connections each of these assaults maintain with multiple cellular processes in impairing them eventually leading to and converging on neurodegeneration. **Recent studies**

Recent studies have attempted to understand how the repeat expansion of GGGGCC hexanucleotide disrupts cellular physiology, and have suggested convergence on downstream, functional defects in cells, such as nucleocytoplasmic transport disruption, membrane-less organelle defects, and DNA damage, and repeat RNAs and DPRs, etc. Defects in one cellular organelle or processes usually disrupt others. Indeed, many cellular functional defects in C9ALS/FTD are connected, and all these functional connections maintained among cellular deficits cause functional impairment of these processes and lead to neurodegeneration, which is at the heart of this review (6). This study by Tang et al., has not only provided an integrated view of the disease mechanism but also revealed novel cell biology implicated in neurodegeneration through convergence of insults incurred by C90RF72 mutation in ALS patients. In our review many such hypotheses have been discussed to strongly and logically bring about the issue of convergence of various deficits leading to neurodegeneration, which is central to ALS and other neurodegenerative diseases. The main etiological factors that lie at the heart of neurodegeneration and influence degenerative processes either singly or collectively, include oxidative stress, mitochondrial damage, glutamate excitotoxicity, defective axonal transport, glial cell impairment, impaired DNA and RNA metabolism, autoimmune responses, metabolic impairment, heavy metal toxicity, viral infection, apoptosis,

proteinopathies, and proteome homeostasis (2, 3, 7).

If we take this new paradigm that ALS does not start as a multifactorial or multisystem disease, but evolves into one, we can get a more informative picture and a clear roadmap of ALS. Every early tributary and its identification, before it forms tracks, is significant at early stages. Further, recent studies have proposed ALS to be a multistep disease, and these steps may be
different across patients. An incidental analysis and identification of a reduced number of steps in patients with ALS with genetic mutations compared to those withoutmutations support the idea of ALS as a multistep disease process (8, 9). Unraveling ALS From Its Point of Origin The main question is, what triggers ALS? Which modality starts first before the disease becomes multifactorial? Is there a single thread that starts the disease and leads to a cascade effect resulting in a multifactorial disease? Is the first start point or single thread always the same, or are there diverse start points leading to the same ALS endpoint? Moreover, if there are diverse start points, are their functional correlates related? Are these etiological factors related, and what functional threads do they maintain during the disease evolution? To answer these questions, it is imperative to understand the holistic functional interaction between the various aforementioned etiological factors and the pathways they influence. For the convenience of understanding, researchers have looked at these entities singly, which has hampered the broader understanding of ALS. Various hypotheses proposed hover around single factors, failing to reconcile that these factors maintain tight and wider functional connections as the disease progresses, but are not evident at the start. We need to begin by
understanding the early onset before going into the details of later aspects of disease progression. By identifying these early symptoms and considering the underlying pathophysiological mechanisms in the design of new therapies, we may hopefully prevent the disease progression and the emergence of further manifestations and cure the damage that has already occurred. Of course, this would lie at odds with the single target idea of
ALS, but as chasing single targets as the genesis of ALS have not furthered the field in terms of therapeutic outcomes, it is time to open up and broaden our thinking. At present, it is difficult to identify the onset of the disease other than through observational study on ALS patients coinciding with the onset of focal weakness, wasting, or mental change. The main problem with this is that these onset features vary and are vague in their timing. However, we must take note of these early alterations as they may represent phenotypic changes that exist sub-clinically for many months or even years before the disease develops (10). Thus, the study of pre-symptomatic individuals may shed light on the early preponderance of highly-penetrant ALS gene mutations (11).

THE DISEASE :

What Causes ALS?

ALS is no longer viewed as one disease or a disease with a single unified cause. It is now considered a clinicopathological syndrome, caused by a complex convergence of environmental influences coupled with genetic susceptibility and age-related loss of cellular homeostasis, leading to neurodegeneration (12). In 1824, the surgeon and philosopher Sir Charles Bell first described a rare condition, the clinical features of which matched those of ALS (13). It was not until 1874, when the neurologist Jean-Martin Charcot gave the disease its modern name and described it as a neurological problem of a mysterious nature (14). A though ALS had already been discovered in the 19th century, the causes and the basic underlying mechanisms of ALS remain obscure (15). Currently, no durable treatments exist that can prevent, reverse, or even alter the course of ALS, with recent drug candidates (Riluzole and Edaravone) (16, 17) providing extremely modest increases in life expectancy of those affected. Even if we already knew the causes of ALS, the sheer complexity and heterogeneity of the disease challenge the development of optimal treatments. ALS patients seem to be distinct from each other, similar to the variability we see in cancer patients that may have molecularly and genetically distinct forms of the disease. Thus, targeted therapies need to be developed and tailored to the affected individuals. It would be inaccurate to say that nothing has been learned

about ALS since it was first described, but unfortunately, it is true that even after 2 centuries, the start points in ALS pathology remain a mystery. The knowledge we have acquired through research has not yet yielded dividends in terms of clinical breakthroughs that can make a difference in the quality of life of ALS patients (18). This could be largely attributed to the

heterogeneity in the start points that initiate disease in different individuals, implying that a combination of diverse factors (endogenous and/or environmental) participate in progressive

motor neuron stress. This progressive stress culminates in the activation of aforementioned pathways that incur insults on several neurological compartments encompassing the central and peripheral nervous system. Thus, it is logical to suppose that the interactions between genetic and environmental risk factors lead to degeneration of the neuromuscular junctions (NMJ), which is a hallmark of ALS onset and pathogenesis. To explain this further, the neuromuscular junction assembly and their plasticity is tightly regulated, through a cross-talk between motor nerve endings, muscle fibers and glial cells, at all stages of life starting from embryonic, postnatal to adult life.

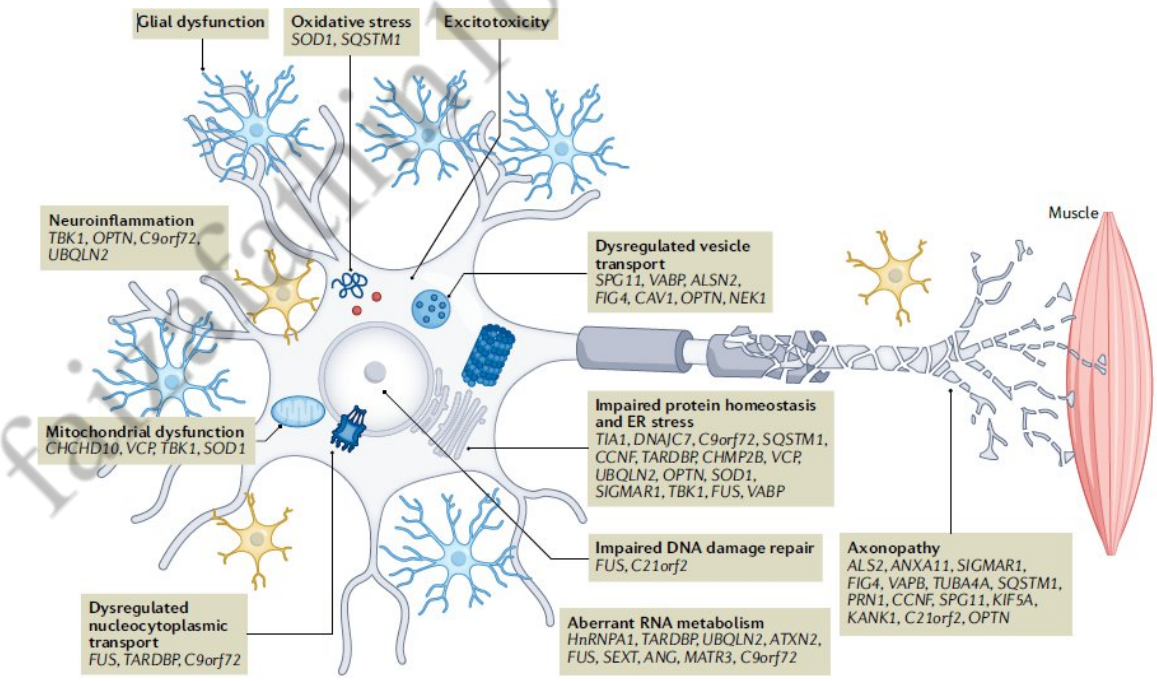
Any alteration in their ability to communicate is possibly responsible for its destruction in pathological states. Thus, the neuromuscular junction dismantling plays an important role in the onset of ALS. Further, the insults on the neuromuscular system overtime results in musculature

weakness and atrophy leading to gradual paralysis, and death from respiratory failure, which typically happens in ALS within 2–3 years from the ALS disease onset (19). Early Signs and Pathogenic Process in Motor **Neurons and Disease Progression**

Early Onset of ALS – What and Where to Look?

"Hit hard, hit early" applies to any disease, including ALS. However, we have a poor understanding of the early signs that are not disease-specific, generally do not exceed a certain threshold, and are often ignored. Numerous NDs that afflict humans start years or decades subliminally before any symptoms are noticed, and ALS is no exception (20). According to Eisen et al., ALS may even start at birth, because early programming of metabolic abnormalities that shape a disease can take decades before a full blown disease phenotype is expressed or any structural change is visible (10).

ALS, being a non-cell-autonomous disease, further complicates the issue of diffuse, non-specific, easy to ignore symptoms. Over the course of the disease, the degeneration and death of motor neurons leading to skeletal muscle denervation will progress, likely aided by inflammatory signaling by glial cells and other cells of the peripheral immune system. Degenerative nerve diseases are known to affect many activities of the human body, including talking, breathing, chewing, swallowing, balance, movement, and heart function. Although the causes for these degenerations in NDs (Amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, Lewy body Spinal muscular atrophy, Huntington's disease, and Friedreich's ataxia disease) are not known, it is believed that toxins, chemicals, and viruses can



trigger this process, which becomes progressive over time (21). Elucidation of interactions between cellular degeneration and system-level degeneration will provide the much-needed disease map in

its earliest symptomatic phases, and based on this understanding will lead to the improved development of relevant biomarkers for disease progression and subsequently to targeted treatments, in addition to addressing the biological basis of disease heterogeneity at the clinical and molecular levels. Early Symptomology and Indications There are several common early clinical symptoms, all of which are underpinned into muscles, neuronal dysfunction or neuronal degeneration resulting in the loss of voluntary movement in almost all NDs, including ALS:

• **Loss of Coordination** is one of the first signs of ALS, starting slowly and increasing in frequency over months or years before becomingmingled with other symptoms. It is attributed to the damage to the nerve paths connecting the brain to the spinal cord (18).

• **Muscular weakness** occurs as a result of motor neuron loss and a lack of signaling to muscle fibers (18).

• **Vocal pitch changes** are often seen in ALS patients and are attributed to Laryngeal dysfunction. Laryngeal dysfunction occurs due to the loss of neurons, which affect the bulbar nerves (18).

• **Slurred speech or Dysarthria** is different from Laryngeal dysfunction and is caused by the loss of motor function. Speech is slurry, and fluctuations in the volume are uncontrollable because of lack of coordination in speech muscles, and, as a result, the movement of the muscles around the mouth may be exaggerated (18).

• **Cramps and muscle twitching:** are an early warning sign of ALS that is often ignored, and it is a consequence of nerveendings pressing against the muscles. The fasciculation, which is one of the most prominent features of ALS, is itself evidence of the increased excitability at the level of LMNs (22).

• **Uncontrollable laughing and crying** (**pseudobulbar affect**) occur when nerve degradation leads to improperly inhibited emotions (18).

• **Breathing difficulties** (such as increased breathlessness, shortness of breath, breathing discomfort) are the first and foremost reason for short survival. Death is often attributed to a loss of control of the respiratorymuscles. This diaphragmatic dysfunction is caused by marked loss of motor units leading to weak inspiratory strength, respiratory fatigue, hypercapnia, and hypoxemia. Breathing problems do not occur immediately but affectmost ALS patients, eventually resulting in death (23).

• **Problems swallowing (Dysphagia)** is a common symptom seen at late stages of ALS and is caused by the lack of muscular control that is guided by the nerve cells that control muscle movements. Predominantly, the ALS patients with bulbar involvement demonstrate severe swallowing difficulties, but dysphagia is also seen in non-bulbar involvement ALS patients.

Bilateral degeneration of the UMNs in the primary motor areas also impairs other motor areas, leading to a substantial reduction of “swallowing related” cortical activation .

• **Weakness of the neck muscles** is seen in almost all ALS patients at some stage, and this inability of the muscles to support head results into “dropped head syndrome,” and at the extreme, the patient will be unable to look straight ahead. This is attributed to the deterioration of cervical paraspinal extensor muscles at the back of the neck . While the subliminal signs serve as an early warning system in predicting the disease, the transition from pre-symptomatic to symptomatic phases of the neurodegenerative process implies possible intervention of pathogenic mechanisms which must be different from the starting events . Thus, to design a therapeutic intervention, a critical understanding of these transition states in necessary so they can be stopped in tracks rather than progressing to the next stage. This is what is critically lacking in defining effective treatments for ALS. Neurodegeneration Is the Core of ALS As with other NDs, the symptomatology described above shows that neurodegeneration is the core event that causes all symptoms of ALS, in addition to many NDs as well. The neurodegenerative process in ALS is characterized by the loss of motor neurons (i.e., the nerve cells that regulate voluntary muscle movement). This loss leads tomuscle weakness andmuscle wasting throughout the body because of the degeneration of both the UMNs and LMNs.

In support of these symptoms, it is important to mention that the evidence emerging from autopsies demonstrating ALS being a cerebral pathology is associated with regions beyond the primary motor cortex. Cognitive impairment, occasional psychosis, and subtle dysexecutive neuropsychological symptoms were recognized in earlier studies, followed by more recent positron emission tomography supporting strong cerebral involvement in ALS . Furthermore, ubiquitinated inclusions of the protein transactive response DNA binding protein 43 kDa (TDP-43) are found in both ALS and frontotemporal dementia (FTD) . Notably, overt FTD manifests early in about 10–15% of patients with ALS (31), and seems to be strongly associated with a G4- C2-hexanucleotide repeat expansion in chromosome 9 open reading frame 72 (C9orf 72) . Together this link between ALS and FTD not only extends ALS as a motor system disease into the frontotemporal lobes but may also connect with early symptomatology related to balance, expression of thought, planning, personality, and speech, in addition to motor symptoms. The clinically distinctive presentations of ALS might be viewed as a failure of several evolutionarily-interlinked functions, namely upper limb functions, in particular hand functions, linked to the development of bipedalism (involving changes in corticomotoneuronal connections), impaired vocalization, swallowing, and breathing (involving the brainstem functional complex); and FTD involving selective impairments of cognitive functions linked to socialization . This leads to the suggestion of the presence of discrete systems whose separation defines the ultimate expression of

the neurodegenerative process. Thus, at early stages when these symptoms appear, selective vulnerability (a long-held view) of neurons can give clues on what next to come, which has given birth to the idea of ALS being a multisystem disease primarily dictated by the properties intrinsic to the motor system and the individual neuron . Indeed, early comparisons of healthy and neurodegenerative functional connectome of the brains provide valuable insight into the evolution of symptoms and neurodegeneration . Key Tenets of Neurodegeneration

Neurodegeneration is a central tenet of ALS, and a striking estimation is that one-third of large motor neurons must be lost before there is any visible atrophy . Both UMNs and LMNs are involved in ALS, and the progressive degeneration of the motor neurons eventually leads to death in ALS patients . Motor neurons are unique and vital cells. They are the longest cells in the body with high energy demands and are responsible for integrating signals from the brain and the sensory systems to control voluntary and involuntary movements. To maintain a seamless communication between brain and body, axon structure and dynamics are vital to drive retrograde and anterograde transport of material along the length of motor neurons. The cargoes of the transport proteins influence many functional and developmental pathways in motor neurons such as the axon guidance pathway, metabolism, energetics (ATP synthesis) and mitochondria, apoptosis, excessive glutamate release, and neuroinflammation. A disruption in any of these processes is implicated in neurodegeneration. Notably, given their extreme size and energy demands, motor neurons are prone to be directly impacted by dire consequences from malfunctions in any of these pathways . Furthermore, the functionality and survival of motor neurons usually are intrinsically supported by different types of glial cells. Consequently, glial cells also play an integral role in neurodegenerative processes .

During disease progression, neuronal degeneration spreads to other brain regions, and the pattern of this degenerative process is often domino-like. It has been suggested that the ripple effect is based on the disruptive effects of neuronal dysfunction and death on both pre- and postsynaptic neurons . Neurotransmission is a highly energy-dependent process and thus, synapses are the most vulnerable regions of neurons. The differences among synapses in their structure, metabolism, and signalling mechanisms might act as determinants of neuronal vulnerability. The biochemical, structural, and genetic changes that occur in the cellular milieu in which neurons reside (including glial cells (astrocytes, oligodendrocytes, Schwann cells, microglia) and vascular cells), considerably influence the fate of neurons in NDs. Thus, it is pivotal to investigate the intrinsic and extrinsic factors that contribute to the vulnerability of neurons need and to delineate their role in disease pathology. As neurodegeneration is similarly central to other NDs (such as Parkinson’s disease, Huntington’s disease, and Alzheimer’s disease), this process can be best understood when

visualized in parallel with other NDs. Although in each disease, specific neuron subclasses in specific brain areas are affected, neurodegeneration, and the resulting loss of neurons is a unifying feature of all NDs.

Subliminal Signs of Neurodegeneration :

During the Emergence of a Disease Process Mild and vague symptoms that repeatedly occur over time are rarely investigated further by patients who are affected by them. However, analogous to the observation that unconsciously perceived subliminal language is able to exert long-lasting effects on neuronal signals and durably affect neural architecture, the subtle prodromal symptomsmay induce comparable long-lasting effects. Consequently, a greater understanding of what these symptoms implicate, and the pathophysiological mechanisms that underpin them may yield valuable insights into the early molecular events that trigger the onset of the disease and its progression and lead to improved diagnosis earlier in the disease course.

Structural and functional MRI studies highlighted that early neurodegeneration is likely compensated by increased activation of the remaining neurons in the same brain region and in a wider sense also by activation of additional parts of cerebral networks resulting in equal behavioral performance between affected and non-affected subjects . This explains, at least in part, how early symptoms go unnoticed or unreported for some time. However, the compensation is overridden at some point during the disease . This overriding process is crucial to understanding the disease as this holds the balance between manifestation and transitory non-manifestation of the disease, and is critical when the disease has paused due to unknown reasons while remaining subliminally active. There may be a molecular dynamic operating below the threshold that has a direct bearing on early clinical symptoms. It is conceivable that clinically-discernable and concerning symptoms only manifest when a molecular event exceeds a certain threshold. A common question is “what is cause and what is consequence?”. This questionmay be too simplistic to fully untangle themultifactorial nature of this disease. The consequences of a particular mutation or group of mutations or molecular events may become a driving cause in itself where the initial cause or event dies off or ceases.

That is, the secondary events become a key player, almost like a relay where the baton gets handed over, and the race progresses forward. Indeed, one molecular process may begin where the other process or processes stop.

As this concept of functional compensation appears to emerge as a common compensatory mechanism in different sporadic and inherited NDs, early detection of the onset of neurodegeneration remains crucial as it may allow for early interventions preventing disease progression. Although neuropathology is considered the diagnostic gold standard, it is invasive and

requires biopsies that can be obtained usually at autopsy. As early neurodegenerative signs are heterogeneous with respect to phenotype and underlying pathophysiological mechanisms, we need a new generation of effective non-invasive diagnostic methods detecting the diverse nuances of early signs of neurodegeneration. Such methods could facilitate the early diagnosis and, thus, the selection of appropriate available pharmacological interventions, as well as the development of new, more effective targeted therapies.

DNA damage and repair :

Postmitotic cells such as neurons are highly susceptible to DNA damage and if unrepaired, cell death ensues. DNA damage has been demonstrated in pathological studies showing elevated oxidized deoxyguanosine (OdG) in CNS tissue and biofluids from patients with ALS. Recent evidence has been reported of elevated apurinic/apyrimidinic DNA sites (loss of purine/pyrimidine base from the DNA strand) and activation of the DNA damage response (DDR) in ALS Several genes mutated in ALS lead to elevated DNA damage or have roles in DNA repair. *C9orf72*-related ALS shows elevated staining for γH2AX, a phosphorylated histone that acts as an important regulator of the DDR, in spinal motor neurons. iPSC-derived motor neurons from patients with *C9orf72*-related ALS show a similar elevation in γH2AX which is partially rescued by inhibition of oxidative stress. *C9orf72* hexanucleotide expansions are prone to formation of G-quadruplex DNA structures which promote the formation of R-loops. These DNA–RNA hybrid structures are substrates for DNA strand breaks, and have been detected in spinal cord tissue from patients with *C9orf72*-related ALS and mouse models in association with defective ataxia telangiectasia mutated (ATM)-mediated DNA repair signalling. Recently TDP-43 has been shown to play a role in DDR signalling via association with DDR proteins. Loss of nuclear TDP-43 was also shown to correlate with strand breaks and DDR response in spinal cord tissue from patients with sALS. Further in vitro assessment in cell models of DNA damage showed that TDP-43 participates in nonhomologous end-joining-mediated repair. *TARDBP* mutations prevent this activity and lead to DNA damage, and DNA damage itself can cause TDP-43 mislocalization. Loss of TDP-43 also increases R-loop formationand genome instability and the *TARDBP* mutation causes the same abnormalities . *FUS* and *NEK1* mutations have also been implicatedin dysregulation of DNA repair. The emerging picture is of DNA damage and impaired repair being a common pathological feature. This is supported by multiple lines of evidence implicating loss of DDR or DNA repair functions of ALS-related genes. A causal link for DNA damage in motor neuron loss is not yet supported by the available evidence, but therapeutic opportunities to enhance repair or mitigate damage (for example, by amelioration of oxidative stress) are worth further investigation. Dysregulated RNA metabolism and nucleocytoplasmic transport (NCT) Disrupted RNA metabolism appears to play a key role in the pathophysiology of ALS. Both TDP-43 and FUS are

RNA-binding proteins (RBPs) with major roles described in multiple aspects of RNA biology including splicing, transcription, stability, export and microRNA biogenesis. Both proteins have binding sites for over 5,000 genes and regulate expression levels of hundreds of mRNAs. The most profound mutation-associated changes in expression occur in genes with very long introns and roles in synaptic function. *C9orf72* mutations lead to the accumulation of RNA foci which sequester a range of RBPs including TDP-43 and RNA export factors. This sequestration has inevitable downstream effects on RNA metabolism including dysregulated splicing. Targeting the repeat expansion in *C9orf72* with antisense oligonucleotides (ASOs) was shown to prevent the formation of RNA foci and downstream effects and is under clinical investigation. The sequestration of RBPs to *C9orf72* RNA foci also licenses them for nuclear export, allowing the pathological transcripts to escape to the cytoplasmic ribosomal machinery, resulting in the production of toxic DPRs

. This has become a potential target for therapeutic intervention by inhibition of the nuclear export adaptor SRSF1. Deficiencies in NCT have been identified in multiple ALS model systems. DPRs resulting from repeat-associated non-AUG (RAN) translation of *C9orf72* repeats promote SG formation , and an emerging model is one in which aberrant recruitment of NCT factors to SGs disrupts NCT, leading to neurodegeneration. Inhibitors of the integrated stress response (ISR) such as ISRIB, a stabilizer of eIF2B functions were able to protect against these effects and related compounds are in clinical studies in ALS

REVIEW OF LITERATURE

Amyotrophic lateral sclerosis (ALS) is a neuro degenerative disorder affecting primarily the motor system, but in which extra-motor manifestations are increasingly recognized. The loss of upper and lower motor neurons in the motor cortex, the brain stem nuclei and the anterior horn of the spinal cord gives rise to progressive muscle weakness and wasting. ALS often has a focal onset but subsequently spreads to different body regions, where failure of respiratory muscles typically limits survival to 2-5 years after disease onset. In up to 50% of cases, there are extra-motor manifestations such as changes in behaviour, executive dysfunction and language problems. In 10%-15% of patients, these problems are severe enough to meet the clinical criteria of frontotemporal dementia (FTD). In 10% of ALS patients, the family history suggests an autosomal dominant inheritance pattern. The remaining 90% have no affected family members and are classified as sporadic ALS. The causes of ALS appear to be heterogeneous and are only partially understood. To date, more than 20 genes have been associated with ALS. The most common genetic cause is a hexanucleotide repeat expansion in the C9orf72 gene, responsible for 30%-50% of familial ALS and 7% of sporadic ALS. These expansions are also a frequent cause of frontotemporal dementia, emphasizing the molecular overlap between ALS and FTD. To this day there is no cure or effective treatment for ALS and the cornerstone of treatment remains multidisciplinary care, including nutritional and respiratory support and symptom management. In this review, different aspects of ALS are discussed, including epidemiology, aetiology, pathogenesis, clinical features, differential diagnosis, investigations, treatment and future prospects.

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ALS. These expansions are also a frequent cause of frontotemporal dementia, emphasizing the molecular overlap between ALS and FTD. To this day there is no cure or effective treatment for ALS and the cornerstone of treatment remains multidisciplinary care, including nutritional and respiratory support and symptom management. In this review, different aspects of ALS are discussed, including epidemiology, aetiology, pathogenesis, clinical features, differential diagnosis, investigations, treatment and future prospects.

Amyotrophic lateral sclerosis (ALS) is a devastating disease caused by degeneration of motor neurons. As with all major neurodegenerative disorders, development of disease-modifying therapies has proven challenging for multiple reasons. Nevertheless, ALS is one of the few neurodegenerative diseases for which disease-modifying therapies are approved. Significant discoveries and advances have been made in ALS preclinical models, genetics, pathology, biomarkers, imaging and clinical readouts over the last 10–15 years. At the same time, novel therapeutic paradigms are being applied in areas of high unmet medical need, including neurodegenerative disorders. These developments have evolved our knowledge base, allowing identification of targeted candidate therapies for ALS with diverse mechanisms of action. In this Review, we discuss how this advanced knowledge, aligned with new approaches, can enable effective translation of therapeutic agents from preclinical studies through to clinical benefit for patients with ALS. We anticipate that this approach in ALS will also positively impact the field of drug discovery for neurodegenerative disorders more broadly.

Amyotrophic Lateral Sclerosis (ALS) is a prototypical neurodegenerative disease characterized by progressive degeneration of motor neurons both in the brain and spinal cord. The constantly evolving nature of ALS represents a fundamental dimension of individual differences that underlie this disorder, yet it involves multiple levels of functional entities that alternate in different directions and finally converge functionally to define ALS disease progression. ALS may start from a single entity and gradually becomes multifactorial. However, the functional convergence of these diverse entities in eventually defining ALS progression is poorly understood. Various hypotheses have been proposed without any consensus between the for-and-against schools of thought. The present review aims to capture explanatory hierarchy both in terms of hypotheses and mechanisms to provide better insights on how they functionally connect. We can then integrate them within a common functional frame of reference for a better understanding of ALS and defining future treatments and possible therapeutic strategies. Here, we provide a philosophical understanding of how early leads are crucial to understanding the endpoints in ALS, because invariably, all early symptomatic leads are underpinned by neurodegeneration at the cellular, molecular and genomic levels. Consol-

idation of these ideas could be applied to other neurodegenerative diseases (NDs) and guide further critical thinking to unveil their roadmap of destination ALS.

Amyotrophic lateral sclerosis (ALS) is an idiopathic, fatal neurodegenerative disease of the human motor system. In this Seminar, we summarise current concepts about the origin of the disease, what predisposes patients to develop the disorder, and discuss why all cases of ALS are not the same. In the 150 years since Charcot originally described ALS, painfully slow progress has been made towards answering these questions. We focus on what is known about ALS and where research is heading—from the small steps of extending longevity, improving therapies, undertaking clinical trials, and compiling population registries to the overarching goals of establishing the measures that guard against onset and finding the triggers for this neurodegenerative disorder.

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is characterized by the degeneration of both upper and lower motor neurons, which leads to muscle weakness and eventual paralysis. Until recently, ALS was classified primarily within the neuromuscular domain, although new imaging and neuropathological data have indicated the involvement of the non-motor neuraxis in disease pathology. In most patients, the mechanisms underlying the development of ALS are poorly understood, although a subset of patients have familial disease and harbour mutations in genes that have various roles in neuronal function. Two possible disease-modifying therapies that can slow disease progression are available for ALS, but patient management is largely mediated by symptomatic therapies, such as the use of muscle relaxants for spasticity and speech therapy for dysarthria.

Amyotrophic lateral sclerosis (ALS) is a neuro degenerative disease characterized by progressive degeneration of upper and lower motoneurons, leading to muscle weakness and paralysis, and finally death. Considerable recent advances have been made in basic research and preclinical therapeutic attempts using experimental models, leading to increasing clinical and translational research in the context of this disease. In this review we aim to summarize the most relevant findings from a variety of aspects about ALS, including evaluation methods, animal models, pathophysiology, and clinical findings, with particular emphasis in understanding the role of every contributing mechanism to the disease for elucidating the causes underlying degeneration of motoneurons and the development of new therapeutic strategies.

SOFTWARE AND METHODOLOGY

SOFTWARE USED:

1. Data Acquisition:

Publicly available transcriptomic datasets of Amyotrophic lateral sclerosis (ALS) tissues and healthy venous tissues will be obtained from repositories such as the Gene Expression Omnibus (GEO) or the Sequence Read Archive (SRA). Relevant metadata, including sample characteristics and experimental conditions, will be extracted for downstream analysis.

2. Data Preprocessing:

Raw sequencing reads will undergo quality control assessment using tools such as FastQC to identify and remove low-quality reads, adapter sequences, and other artifacts. Subsequently, reads will be aligned to the reference genome (e.g., GRCh38) using splice-aware aligners like HISAT2 or STAR to generate aligned read files (BAM format).

3. Quantification of Gene Expression:

Aligned reads will be quantified to measure gene expression levels using tools like featureCounts or HTSeq. This step involves counting the number of reads that align to each gene, which serves as the basis for differential expression analysis.

4. Differential Expression Analysis:

Differential expression analysis will be performed using bioinformatics tools such as DESeq2, edgeR, or limma-voom. These tools employ statistical models to identify genes that are significantly differentially expressed between Amyotrophic lateral sclerosis (ALS) tissues and healthy venous tissues, considering factors such as sample variance and experimental design.

5. Visualization of Results:

Visualization tools such as heatmaps, volcano plots, and gene set enrichment plots will be utilized to visualize the results of the differential expression analysis and pathway analysis, aiding in the interpretation of the data and identification of key biological processes and pathways associated with Amyotrophic lateral sclerosis (ALS)s.

6. Validation of Results:

Selected candidate genes identified from the differential expression analysis will be validated using independent datasets or experimental validation techniques such as quantitative PCR (qPCR) in an independent cohort of Amyotrophic lateral sclerosis (ALS) patients and healthy controls to confirm their differential expression.

7. Interpretation of Results:

The results of the bioinformatics analysis will be interpreted to gain insights into the molecular mechanisms underlying Amyotrophic lateral sclerosis (ALS) pathogenesis, identifying potential biomarkers and therapeutic targets for further investigation.

METHODS :

Isolation of human PBMCs from ALS patients and healthy controls :

Isolation of human PBMCs :

PBMCs were prepared by centrifugation. Peripheral blood was layered (density = 1.077) and centrifuged at 950 g for 30 min. After isolation on a Ficoll-Histopaque layer (Sigma Aldrich, Italy), cell viability was assayed by a trypan blue exclusion test and cells were then used for RNA extraction.

To identify differential expression genes (DEGs) using IDEP (Integrated Differential Expression and Pathway analysis), you would typically follow these steps:

1. Data Input:

- Prepare your gene expression data in a suitable format (e.g., tab-delimited text file, Excel file).
- Ensure that your data includes gene expression values for multiple samples under different experimental conditions or treatments.

2. Upload Data to IDEP:

- Navigate to the IDEP web interface (http://bioinformatics.sdstate.edu/idep/).
- Click on the "Start Analysis" button to access the analysis tools.
- Select the "Differential Expression Analysis" option.

3. Input Data Format:

- Follow the instructions to upload your gene expression data file.
- Specify the experimental design (e.g., control vs. treatment groups) and any relevant experimental factors or covariates.

4. Preprocessing:

- IDEP performs data preprocessing steps, including normalization and filtering, to ensure the quality and reliability of the input data.
- Normalization methods such as TMM (Trimmed Mean of M values) or RPKM (Reads Per Kilobase Million) are commonly applied to correct for biases and differences in sequencing depth.

5. Differential Expression Analysis:

- IDEP employs statistical methods (e.g., DESeq2, edgeR) to identify genes that are differentially expressed between experimental conditions.
- Differential expression analysis assesses the statistical significance of expression differences using appropriate statistical tests (e.g., negative binomial test, t-test).
- The results typically include a list of DEGs along with associated statistical metrics (e.g., fold change, p-value, adjusted p-value).

6. Visualization:

- IDEP provides interactive visualization tools to explore and interpret the results of the differential expression analysis.
- Volcano plots, heatmaps, and expression profiles are commonly used to visualize DEGs and their expression patterns across samples and conditions.

7. Pathway Analysis:

- Optionally, IDEP offers integrated pathway analysis tools to identify biological pathways and processes that are significantly enriched with DEGs.
- Pathway enrichment analysis helps to elucidate the functional implications of gene expression changes and identify potential targets for further investigation.

8. Interpretation:

- Interpret the results of the analysis in the context of your research questions and hypotheses.

- Validate the identified DEGs using additional experimental techniques (e.g., qRT-PCR, Western blotting) if necessary.

9. Report Generation:

Generate summary reports, tables, and figures to document the results of the analysis for publication or further analysis.

By following these steps, you can effectively use IDEP to identify and analyze differential expression genes in your gene expression data.

RNA extraction :

Samples were homogenized and total RNA was isolated by Trizol® reagent (Life Science Technologies, Italy) following the manufacturer's protocol. RNAs were quantified using a Nanodrop ND-100 Spectrophotometer (Nanodrop Technologies, Wilmington, USA) and a 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit, Waldbronn, Germany); RNAs with a 260:280 ratio of ≥1.5 and an RNA integrity number of ≥8 were deep sequenced (Supplementary Figure 1).

RNA-seq library preparation, sequencing and analysis :

Sequencing libraries were prepared with the Illumina TruSeq Stranded RNA Library Prep, version 2, Protocol D, using 500-ng total RNA (Illumina,USA). The qualities of the libraries were assessed by 2100 Bioanalyzer with a DNA1000 assay. Libraries were quantified by qPCR using the KAPA Library Quantification kit for Illumina sequencing platforms (KAPA Biosystems); RNA processing has been carried out using Illumina NextSeq 500 Sequencing, using 8 samples for each run mixing samples and controls in each flowcell to avoid not manageable batch-effects. FastQ files were generated via llumina bcl fastq2.

Quality validation and read mapping :

Quality of individual sequences were evaluated using FastQC software (see Code Availability 1) after adapter trimming with cutadapt software. Per base sequence quality plots, showing the Phred quality score distribution among all sample reads . Gene and transcript intensities were computed using STAR/RSEM software using the "--*strandness forward*" option . Human genome reference used for the alignment was GRCh38 in a rapidly evolving field like the one of non coding RNA analysis it is indeed fundamental to use up-to-date reference versions containing all the available information about annotated coding and non coding RNAs.

Mapping results are summarized in Percentage of uniquely mapped reads is 21.8% on average, with standard deviation 10.35%. Remaining reads belong to ribosomal RNA because of the non perfect ribosomal RNA depletion. Since 10 to 25 M reads are suggested for RNA-Seq experiments this dataset is suitable for differential expression analysis, since about 60% of samples (21/36) have at least 10 M reads. Furthermore, biological replicates are the most effective strategy to improve statistical power in such experiments. For this reason, we decided to share our dataset with the scientific community, to have the possibility of integrating our data with similar data obtained in different laboratories.

Expressed transcripts per sample were evaluated imposing a minimum threshold of 5 counts per gene to consider it as expressed. shows the amount of coding and non coding transcripts detected in each sample. With the exception of 4 samples which have a low number of detected transcripts (probably due to a lower number of available reads), 7000 to 14000 expressed coding genes and 500 to 3000 non coding genes were detected per sample. This is in accordance with the currently available knowledge about non coding transcripts that result to be globally less ex-pressed than coding ones within the cell.

RESULT :

The table given below represents the result of PCA (Principal Component Analysis) of the given samples from the website IDEP 2.0,

PCA result :

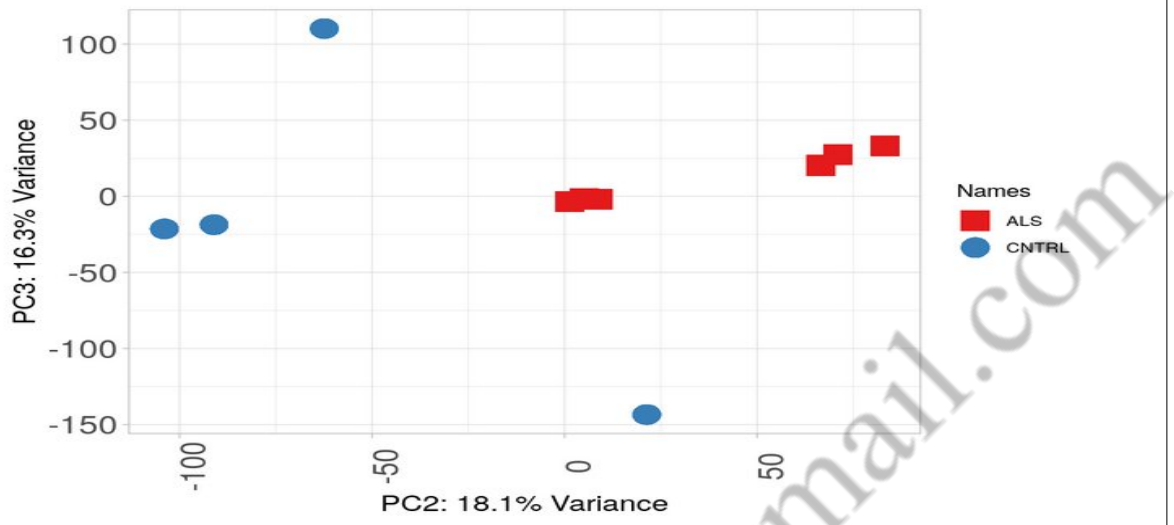
	PC1	PC2	PC3	PC4	PC5	Names
ALS1	-42.3260323314891	1.39651092697851	-3.56405047354511	-66.7507403624897	51.696874564064	ALS
ALS2	-32.9088984370693	66.5350034568339	20.3220065450026	26.8263727892167	-25.0675759439897	ALS
ALS3	-21.6560583641391	70.9823272403322	27.2970137587069	27.1004607420255	31.9046019892579	ALS
ALS4	-37.8308189471935	83.2112329349417	33.0644398411761	55.0007166965846	-5.28667052421283	ALS
ALS5	-33.9075900385675	5.09973990300215	-1.65821826355693	-70.5216198060151	-28.7890525550179	ALS
ALS6	-36.9417977880599	8.75754189547091	-2.0730004818388	-63.7166443034338	-27.6125145865538	ALS
CNTRL1	-88.6939494762574	-103.910536534057	-21.4379527240874	43.9597555856965	-31.2691018507772	CNTRL
CNTRL2	-83.9889274536923	-91.0230767604145	-18.6772501991106	32.7282895177179	34.4349578992951	CNTRL
CNTRL3	169.216332436817	21.3488632530537	-143.448671390227	14.7088294776708	0.525703559824239	CNTRL
CNTRL4	209.037740399651	-62.3976063161416	110.175683387481	0.66457966302645	-0.537222551889747	CNTRL

The follow

PCA plot

PCA plots

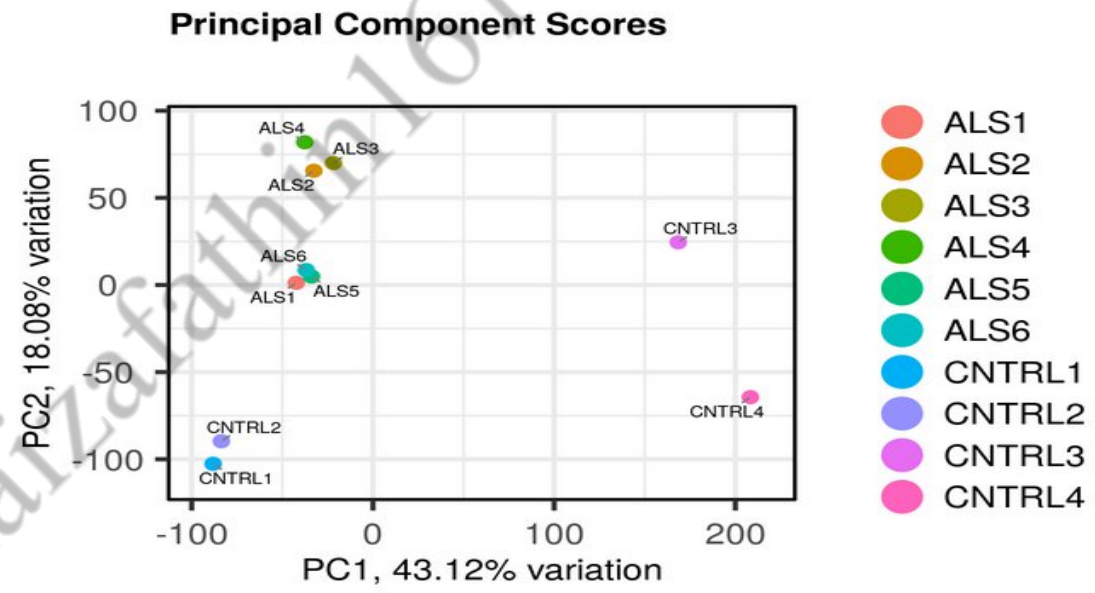
Principal Component Analysis



Biplot :

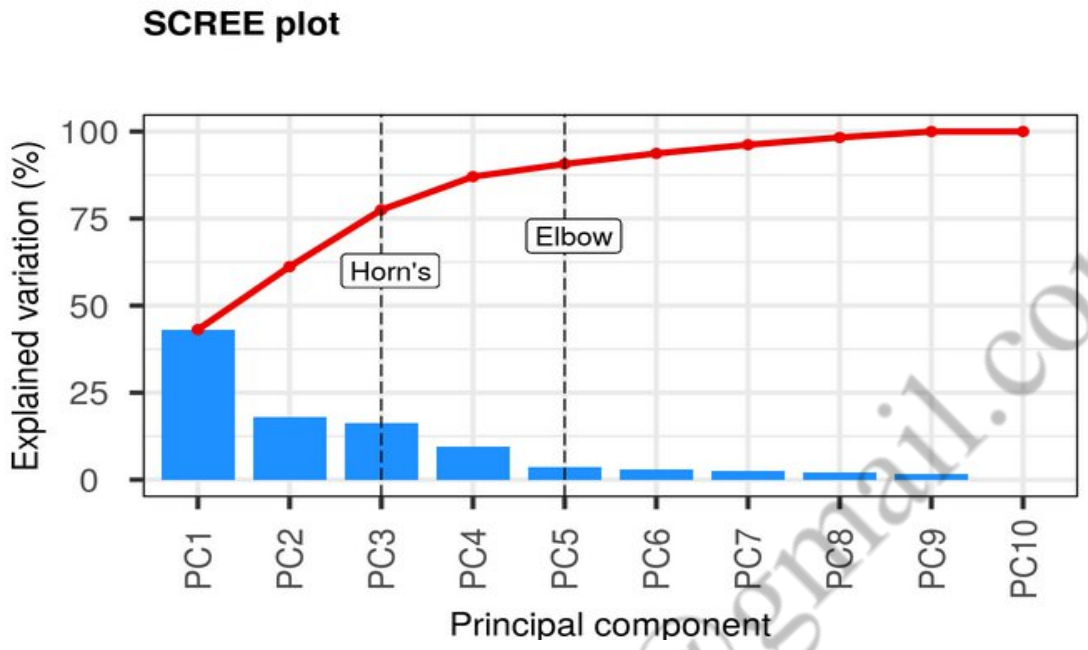
PCA tools tab

Biplot



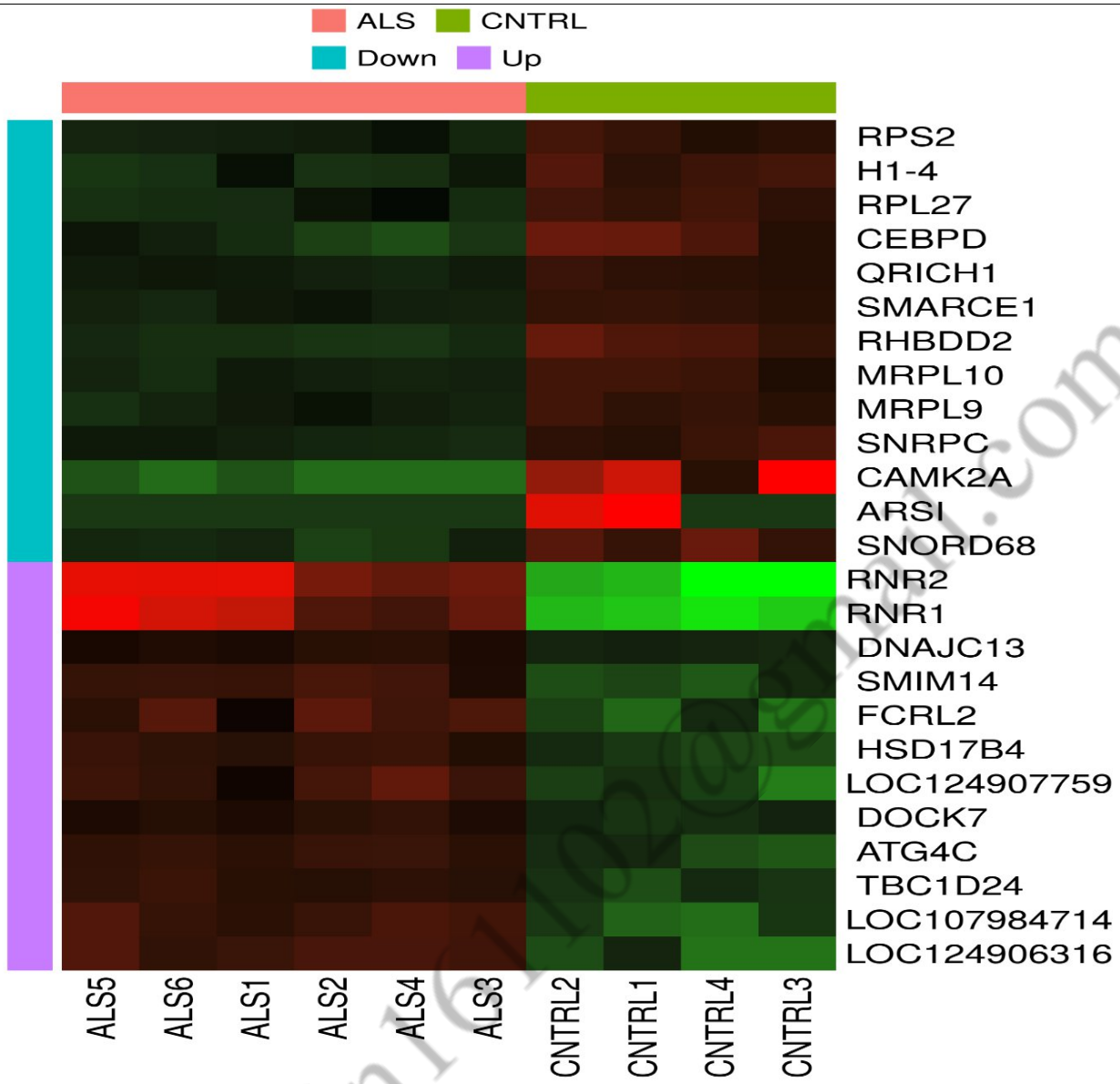
Scree plot:

Scree Plot

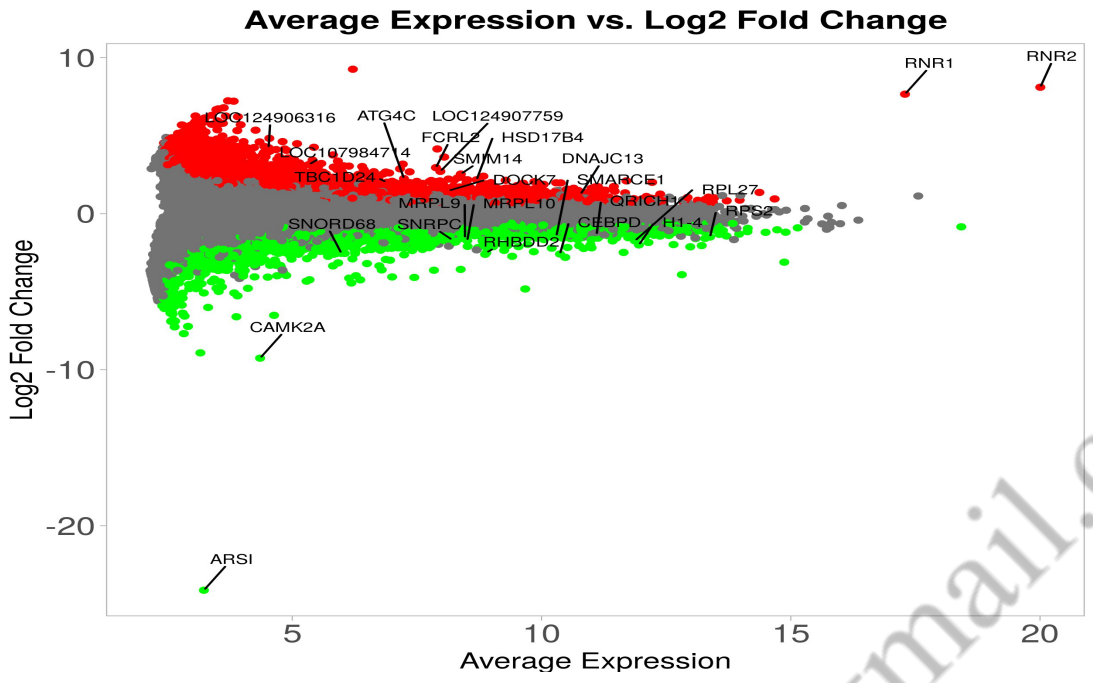


The following plots were taken form the IDEP 2.0 an integrated web application for differential expression and pathway analysis. By loading the gene counts csv file in the IDEP 2.0, we can derive these plots from the website. Such as HeatMap plot, MA plot, Scatter plot and Volcano plot for the differentialy expressed genes.

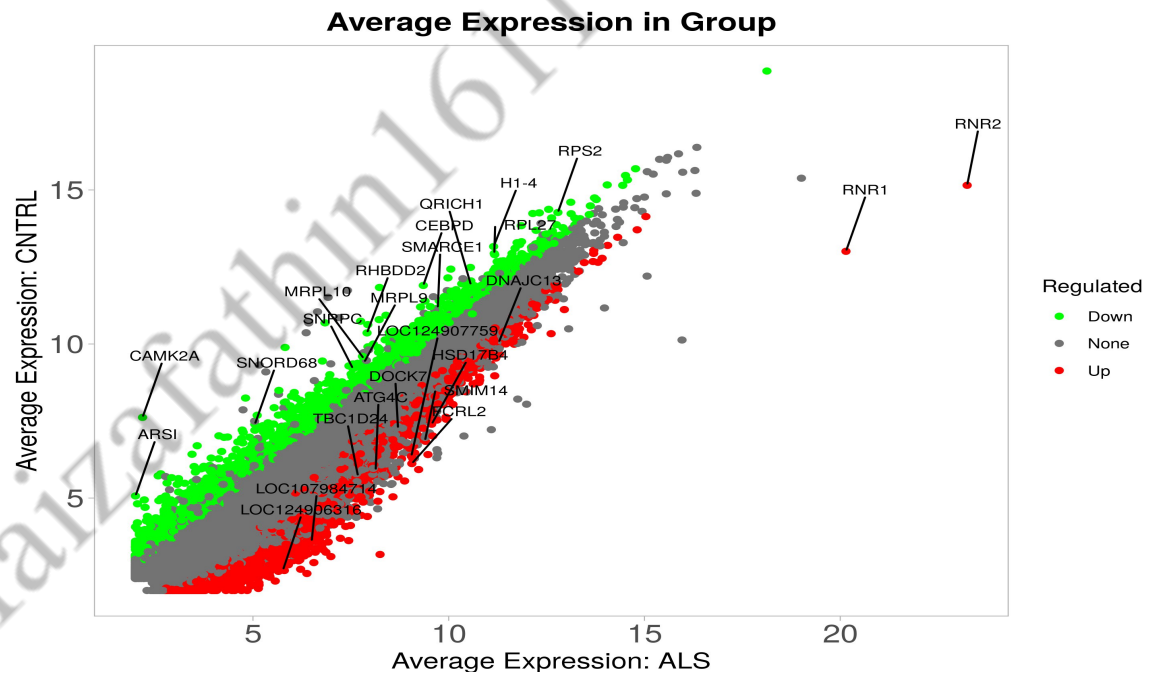
a) HeatMap Plot :



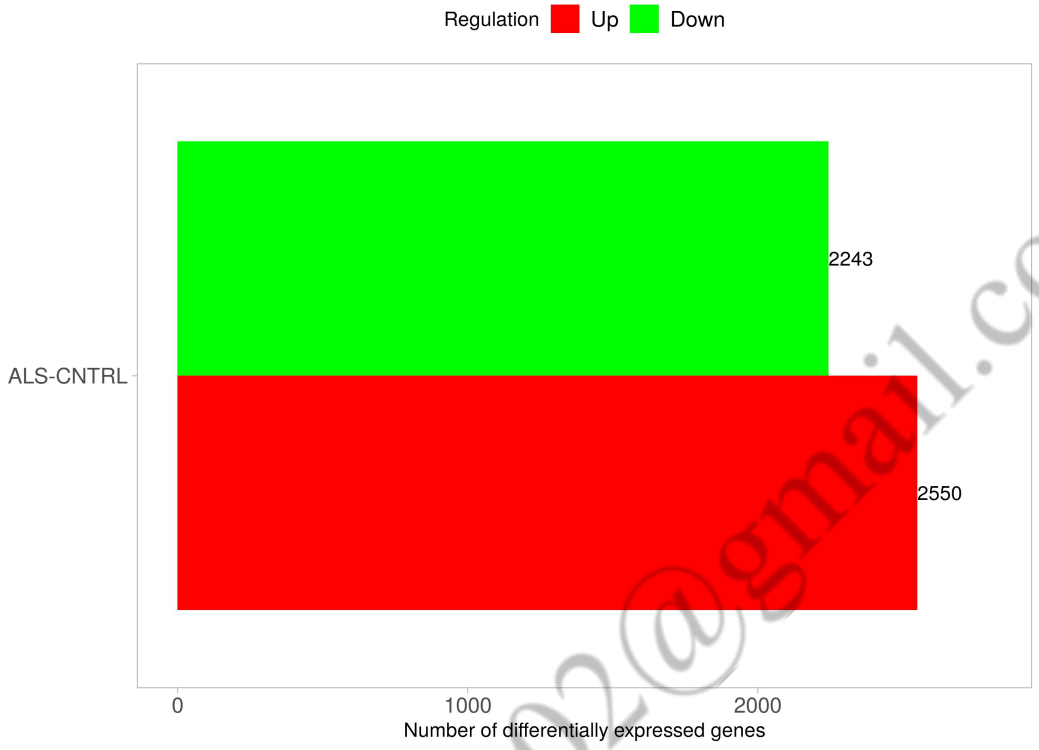
b) ma plot :



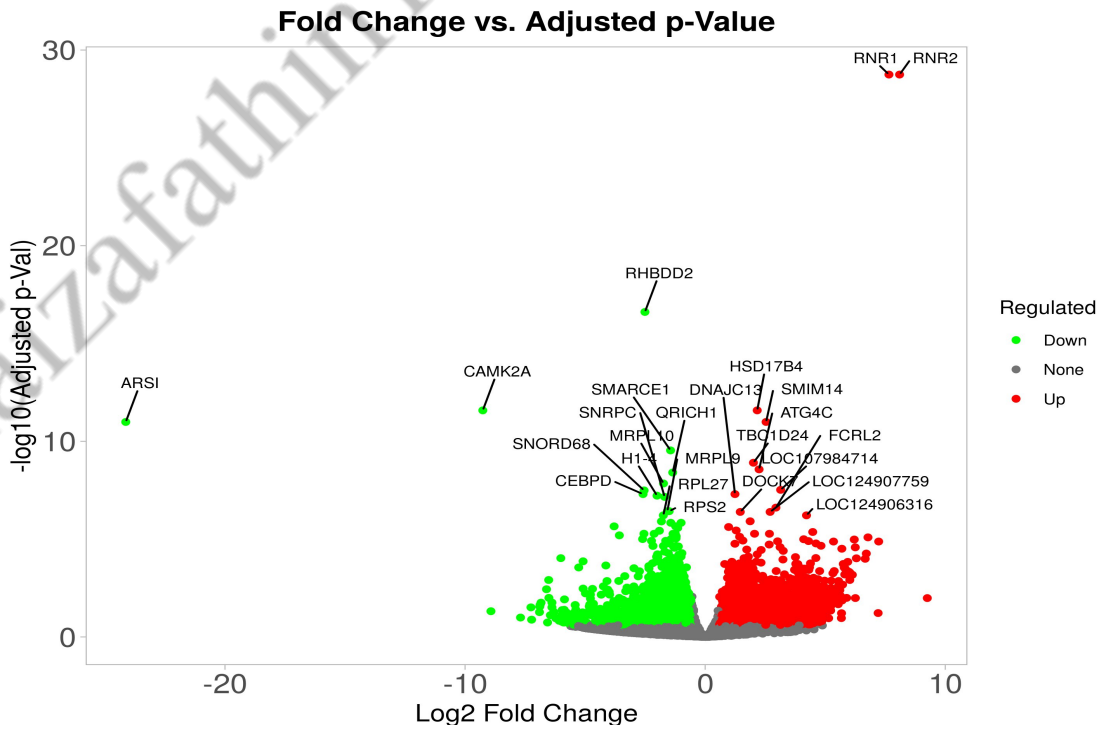
c) scatter plot :



d) sig gene plot :



e) volcano plot :



CONCLUSION :

In conclusion, our differential expression analysis utilizing bioinformatics tools has provided valuable insights into the molecular landscape of Amyotrophic lateral sclerosis (ALS)s. Through comprehensive data preprocessing, differential expression analysis, and functional annotation, we have identified a panel of genes that are significantly dysregulated in Amyotrophic lateral sclerosis (ALS) tissues compared to healthy venous tissues. These differentially expressed genes are implicated in various biological processes and pathways, shedding light on the underlying molecular mechanisms contributing to Amyotrophic lateral sclerosis (ALS) pathogenesis. Furthermore, our findings offer potential biomarkers for Amyotrophic lateral sclerosis (ALS) diagnosis and prognosis, as well as novel therapeutic targets for intervention. The robust statistical analysis and stringent false discovery rate correction employed in our study enhance the reliability and reproducibility of the results, ensuring the validity of the identified gene signatures. However, it is important to acknowledge the limitations of our study, including the inherent heterogeneity of Amyotrophic lateral sclerosis (ALS) samples, potential confounding factors, and the reliance on publicly available transcriptomic datasets. Future research efforts should aim to validate our findings using independent cohorts and experimental validation techniques to further corroborate the identified gene signatures and elucidate their functional significance

REFERENCES

1. Longinetti, E. & Fang, F. Epidemiology of amyotrophic lateral sclerosis: an update of recent literature. *Curr. Opin. Neurol.* 32, 771–776 (2019).

2. Brown, C. A., Lally, C., Kupelian, V. & Flanders, W. D. Estimated prevalence and incidence of amyotrophic lateral sclerosis and SOD1 and C9orf72 genetic variants. *Neuroepidemiology* 55, 342–353 (2021).

3. Ryan, M., Heverin, M., McLaughlin, R. L. & Hardiman, O. Lifetime risk and heritability of amyotrophic lateral sclerosis. *JAMA Neurol.* 76, 1367–1374 (2019).

4. GBD 2016 Motor Neuron Disease Collaborators. Global, regional, and national burden of motor neuron diseases 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 17, 1083–1097 (2018).

5. Masrori, P. & Van Damme, P. Amyotrophic lateral sclerosis: a clinical review. *Eur. J. Neurol.* 27, 1918–1929 (2020).

6. Elamin, M. et al. Cognitive changes predict functional decline in ALS: a population-based longitudinal study. *Neurology* 80, 1590–1597 (2013).

7. Phukan, J. et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J. Neurol. Neurosurg. Psychiatry* 83, 102–108 (2012).

8. Neumann, M. et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130–133 (2006).

9. Ling, S. C., Polymenidou, M. & Cleveland, D. W. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 79, 416–438 (2013).

10. Tan, R. H., Ke, Y. D., Ittner, L. M. & Halliday, G. M. ALS/FTLD: experimental models and reality. *Acta Neuropathol.* 133, 177–196 (2017).

11. Hardiman, O. et al. Amyotrophic lateral sclerosis. *Nat. Rev. Dis. Prim.* 3, 17085 (2017).

12. Mackenzie, I. R. et al. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with *SOD1* mutations. *Ann. Neurol.* 61, 427–434 (2007). This paper highlights the important molecular features of SOD1-ALS, which is not TDP-43

proteinopathy and may partially explain why treatments evaluated in *SOD1*-transgenic mouse models have not generally translated well to the clinic.

13. Vance, C. et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 323, 1208–1211 (2009).

14. Kato, S. et al. Copper chaperone for superoxide dismutase co-aggregates with superoxide dismutase 1 (SOD1) in neuronal Lewy body-like hyaline inclusions: an immunohistochemical study on familial amyotrophic lateral sclerosis with SOD1 gene mutation. *Acta Neuropathol.* 102, 233–238 (2001).

15. Ramos-Campoy, O. et al. Systematic screening of ubiquitin/p62 aggregates in cerebellar cortex expands the neuropathological phenotype of the C9orf72 expansion mutation. *J. Neuropathol. Exp. Neurol.* 77, 703–709 (2018).

16. Turner, M. R. & Talbot, K. Mimics and chameleons in motor neurone disease. *Pract. Neurol.* 13, 153–164 (2013).

17. Brooks, B. R., Miller, R. G., Swash, M. & Munsat, T. L. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Mot. Neuron Disord.* 1, 293–299 (2000).

18. de Carvalho, M. et al. Electrodiagnostic criteria for diagnosis of ALS. *Clin. Neurophysiol.* 119, 497–503 (2008).

19. Shefner, J. M. et al. A proposal for new diagnostic criteria for ALS. *Clin. Neurophysiol.* 131, 1975–1978 (2020).

20. Petrov, D., Mansfield, C., Moussy, A. & Hermine, O. ALS clinical trials review: 20 years of failure. Are we any closer to registering a new treatment? *Front. Aging Neurosci.* 9, 68 (2017).

21. Lacomblez, L. et al. A confirmatory dose-ranging study of riluzole in ALS. ALS/Riluzole Study Group-II. *Neurology* 47, S242–S250 (1996).

22. Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P. & Meininger, V. Dose-ranging study

of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet* 347, 1425–1431 (1996).

23. Bensimon, G., Lacomblez, L. & Meininger, V. A controlled trial of riluzole in amyotrophic lateral sclerosis. ALS/Riluzole Study Group. *N. Engl. J. Med.* 330, 585–591 (1994).

24. Kretschmer, B. D., Kratzer, U. & Schmidt, W. J. Riluzole, a glutamate release inhibitor, and motor behavior. *Naunyn Schmiedebergs Arch. Pharmacol.* 358, 181–190 (1998).

25. Wang, S. J., Wang, K. Y. & Wang, W. C. Mechanisms underlying the riluzole inhibition of glutamate release from rat cerebral cortex nerve terminals (synaptosomes). *Neuroscience* 125, 191–201 (2004).

26. Andrews, J. A. et al. Real-world evidence of riluzole effectiveness in treating amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 21, 509–518 (2020).

27. The Writing Group on behalf of the Edaravone (MCI-186) ALS 19 Study Group Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* 16, 505–512 (2017).

28. Sawada, H. Clinical efficacy of edaravone for the treatment of amyotrophic lateral sclerosis. *Expert. Opin. Pharmacother.* 18, 735–738 (2017).

29. Bourke, S. C. et al. Effects of non-invasive ventilation on survival and quality of life in patients with amyotrophic lateral sclerosis: a randomised controlled trial. *Lancet Neurol.* 5, 140–147 (2006).

30. Lechtzin, N. et al. Early use of non-invasive ventilation prolongs survival in subjects with ALS. *Amyotroph. Lateral Scler.* 8, 185–188 (2007).

31. Rosen, D. R. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362, 59-62 (1993).

32. Ranganathan, R. et al. Multifaceted genes in amyotrophic lateral sclerosisfrontotemporal dementia. *Front. Neurosci.* 14, 684 (2020).

33. van Rheenen, W. et al. Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology. *Nat. Genet.* 53, 1636–1648 (2021).

34. van Rheenen, W. et al. Genome-wide association analyses identify new risk variants and the genetic architecture of amyotrophic lateral sclerosis. *Nat. Genet.* 48, 1043–1048 (2016).

35. Shepheard, S. R. et al. Value of systematic genetic screening of patients with amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 92, 510–518 (2021).

36. Zhang, S. et al. Genome-wide identification of the genetic basis of amyotrophic lateral sclerosis. *Neuron* 110, 992–1008.e11 (2022).

37. Al-Chalabi, A. et al. Analysis of amyotrophic lateral sclerosis as a multistep process:a population-based modelling study. *Lancet Neurol.* 13, 1108–1113 (2014).

38. Julian, T. H. et al. A review of Mendelian randomization in amyotrophic lateral sclerosis. *Brain* 145, 832–842 (2021).

39. Julian, T. H. et al. Physical exercise is a risk factor for amyotrophic lateral sclerosis: convergent evidence from Mendelian randomisation, transcriptomics and risk genotypes. *EBioMedicine* 68, 103397 (2021).

40. Bandres-Ciga, S. et al. Shared polygenic risk and causal inferences in amyotrophic lateral sclerosis. *Ann. Neurol.* 85, 470–481 (2019).

41. McKay, K. A. et al. Military service and related risk factors for amyotrophic lateral sclerosis. *Acta Neurol. Scand.* 143, 39–50 (2021).

42. Ingre, C., Roos, P. M., Piehl, F., Kamel, F. & Fang, F. Risk factors for amyotrophic lateral sclerosis. *Clin. Epidemiol.* 7, 181–193 (2015).

43. Lacorte, E. et al. Physical activity, and physical activity related to sports, leisure and occupational activity as risk factors for ALS: a systematic review. *Neurosci. Biobehav. Rev.* 66, 61–79 (2016).

44. Armon, C. Smoking may be considered an established risk factor for sporadic ALS. *Neurology* 73, 1693–1698 (2009).

45. Barber, S. C. & Shaw, P. J. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. *Free. Radic. Biol. Med.* 48, 629–641 (2010).

46. Ferraiuolo, L., Kirby, J., Grierson, A. J., Sendtner, M. & Shaw, P. J. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nat. Rev. Neurol.* 7, 616–630 (2011).

47. D'Amico, E., Factor-Litvak, P., Santella, R. M. & Mitsumoto, H. Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. *Free Radic. Biol. Med.* 65, 509–527 (2013).

48. Mitsumoto, H. et al. Oxidative stress biomarkers in sporadic ALS. *Amyotroph. Lateral Scler.* 9, 177–183 (2008).

49. Kim, K. Glutathione in the nervous system as a potential therapeutic target to control the development and progression of amyotrophic lateral sclerosis. *Antioxidants* 10, 1011 (2021).

50. Cuadrado, A. et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. *Nat. Rev. Drug Discov.* 18, 295–317 (2019).

51. Jimenez-Villegas, J. et al. NRF2 as a therapeutic opportunity to impact in the molecular roadmap of ALS. *Free Radic. Biol. Med.* 173, 125–141 (2021).

52. Cohen, T. J. et al. An acetylation switch controls TDP-43 function and aggregation propensity. *Nat. Commun.* 6, 5845 (2015).

53. Colombrita, C. et al. TDP-43 is recruited to stress granules in conditions of oxidative insult. *J. Neurochem.* 111, 1051–1061 (2009).

54. Goh, C. W. et al. Chronic oxidative stress promotes GADD34-mediated phosphorylation of the TAR DNA-binding protein TDP-43, a modification linked to neurodegeneration. *J. Biol. Chem.* 293, 163–176 (2018).

55. Zuo, X. et al. TDP-43 aggregation induced by oxidative stress causes global mitochondrial imbalance in ALS. *Nat. Struct. Mol. Biol.* 28, 132–142 (2021).