Issues in ALS:
Why fair funding will increase innovation

Master thesis

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Layman's summary

Amyotrophic lateral sclerosis (ALS) is a devastating fatal condition, in which the nerve cells controlling muscles (motor neurons) are lost, followed by the muscles themselves. This results in paralysis and eventually death, usually within 4 years. ALS is the most common motor neuron disease, with a 1:400 risk to be affected at any time during adult life. The first diagnosis of what later would become known as ALS was given about 150 years ago, but it took until the late 20th century to start discovering the disease process and mechanics. Still, ALS is poorly understood, and only two treatments with small effects are currently available. This is likely the result of several issues on different levels, defined here as biological, technical, and structural. These problems especially restrict research progress for complex disorders such as ALS. Solving these might thus bring us closer to effective treatments.

First, the biological problems facing ALS research are the complex processes that cause it and the differences between patients. A number of complicated biological processes have been associated with ALS. To make matters worse, they are not all causative, making it difficult to distinguish the ones that can be targeted for therapy. Additionally, not all these processes are shared between patients, with the likelihood and form of ALS dependent on genes, environment, and aging. This makes it hard to find shared factors and processes that cause the disease.

Second, technical problems to research include the difficulty checking disease progress and poor design of studies. Disease risk, outlook, treatability, and the effects of treatment are measured with certain markers. However, as we do not understand the disease, making effective and reliable markers is very hard. It is also not possible to diagnose ALS before any symptoms, which causes an average delay between onset and treatment of about a year. Without reliable markers, putting patients into groups is difficult, making it hard to design studies and interpret their results. Additionally, as ALS is poorly understood, animal models are oversimplified to the point they do not reflect the disease process very well. Testing treatments in these simple models has been ineffective for finding good therapies for ALS patients.

Third, structural problems to science are formed by high competition for funding and the way we reward research. Here, competition can lead to a lack of collaboration and exploration of unconventional approaches, both of which would contribute to studying complex diseases. On the other hand, the quantity of research is often rewarded instead of its actual quality, which pushes short and simple studies. As a result, researchers cannot perform extensive complex research or develop better methods and models. Especially for complex diseases like ALS, this is a major issue, as an extensive and thorough approach is necessary. Such an approach would be designed to take the biological issues into account, would give more time to develop suitable methods and disease models, and would allow the publication of all results, not just exciting ones. Effectively, there could be a large increase in new discoveries, possibly leading to the development of better treatments.

To solve the problems posed to ALS research, a serious change is required in how research is performed and valued. Competition for funds can be limited by more general funding, where all qualified research groups get some basic funds. Additionally, research quality should be valued over quantity, considering more than just the number and score of publications. These changes would create a better environment for studying complex subjects like ALS. In turn, this would allow a deeper understanding of its cause and different forms, the improvement of markers, methods, and models, and finally the development of effective therapies.

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Abbreviations

ALS	Amyotrophic lateral sclerosis
C9ORF72	Chromosome 9 open reading frame 72
EEAT2	Excitatory amino acid transporter 2
FTD	Frontotemporal dementia
FUS	Fused in sarcoma
iPSC	Induced pluripotent stem cell
ROS	Reactive oxygen species
SOD1	Superoxygen dismutase 1
TARDBP	Transactive response DNA binding protein

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease, characterized by a progressive loss of motor neurons.[1] Motor neurons detach from their neuromuscular junction as they become dysfunctional, after which atrophy occurs in the denervated muscle fibre. During disease progression, muscle atrophy is followed by paralysis and ultimately death, usually due to respiratory failure.[1] Although lifetime risk is about 1:400, which is comparable to multiple sclerosis, survival is so short it decreases prevalence to about 4 cases per 100,000.[2]

The disease is extremely heterogeneous, and the complex underlying pathogenic processes are currently poorly understood. Onset can occur either bulbar or peripheral, resulting in different symptoms and prognoses.[3] Additionally, it may cause cognitive and behavioural changes, as severe cases may happen in combination with frontotemporal dementia (FTD).[1] Patients with a familial background (fALS) make up 10% of the total affected individuals, where genetic variants deliver a dominant contribution to pathogenesis. Up to 60% of fALS cases have variants of four major causative genes: chromosome 9 open reading frame 72 (C9ORF72), superoxygen dismutase 1 (SOD1), transactive response DNA binding protein (TARDBP), and fused in sarcoma (FUS). However, 90% of patients have a sporadic form of ALS (sALS), with up to 95% of cases having an unknown cause.[4,5] The disease is believed to be multifactorial, with genetic variants affecting predispositions to environmental factors and the aging process in a multistep model.[6] There is high variability in age of onset: while the average is 55, it ranges between 20 and 85 years. Survival also varies wildly, as it is generally between 2 and 4 years, but can range up to 10.[2]

Since the first designated diagnosis of ALS almost 150 years ago, progress has been painstakingly slow. It took over a hundred years to begin dissection of the responsible molecular mechanisms and to start developing therapeutics. Currently, only two therapeutics have been approved, with another currently in phase 3 clinical trials, all of which are palliative.[1] This is the result of issues on several levels, reducing research progress. To illustrate and assess these issues, this thesis will give an overview of the historic and current ALS research progress, after which three categories of major issues slowing progress will be described: biological, technical, and structural. First, biological issues consist of the complex causation and heterogeneity inherent to the neurodegenerative disease. Second, technical issues are defined as inadequate research design, methods, and models. Third, structural issues comprise the competitive environment and quantitative focus of the scientific community and funding agencies. We argue that structural issues underlie many of the technical issues observed in complex diseases like ALS, leading to a decreased ability to solve complex biological issues. Thus, we pose that structural problems inhibit innovation, hampering the study of ALS pathology and the development of successful and significant therapeutic strategies. Finally, an effort is made to formulate solutions that may contribute to each level of issues.

1. Historic and current ALS research

The history of ALS is a long and exasperating one. Over a century of research has yielded a limited understanding of the condition, and although there are some recent notable advances, a unifying hypothesis that could bring us closer to improved therapies is sorely lacking. In the first chapter, we will describe the revolutionary methods that led to the first designated diagnosis of ALS, discuss our current understanding of the disease, and the available treatments.

1.1 Initial discovery

Diagnostic revolution

The first suspected record of ALS comes from Charles Bell in 1824, who described the now characteristic progressive muscle atrophy in a patient who maintained cognitive abilities.[7] Despite this, there was no diagnosis for the condition, and no treatment available. For most of the 19th century, complex neurological disorders were generally poorly understood and often misdiagnosed as hysteria, a term for indefinable conditions with a range of neurological and psychological symptoms.[8] Additionally, rheumatological and neurological conditions were thought to be connected because of their similar physiological effects.[9] Thus, most patients were left untreated and regarded as chronically ill, emphasizing the underdeveloped diagnostic methods of the time.

In 1862, Jaen Martin Charcot started working in a hospice with over 5000 patients, and began to improve the current diagnostic methods.[9] He later took over direct care for about 2000 patients, developing a method to evaluate detailed patient records, including pictures and notes on their symptoms and autopsies, naming it the anatomo-clinical method.[10] Combining patient data provided him with an understanding of cases on a symptomatic level, allowing him to categorize them accordingly and to differentiate diseases with similar symptoms, but disparate anatomical manifestations. Consequently, Charcot was able to give more accurate diagnoses than his contemporary colleagues.

During the years at the hospice, Charcot used his method to separate cases of acute and progressive weakness, focussing his research on the latter.[11] He refused to restrict himself to previously established diagnostic categories, rather reviewing cases independently. Accordingly, Charcot made the first major discovery related to the study of ALS in 1865. At that time, he presented a case of a young, supposedly hysteric woman, whose symptoms he described as a slowly progressive, extreme weakness, increased muscle tone and contractures, while she retained her intellect, sensory abilities, and urinary control. After her death, Charcot found the woman to have isolated lateral horn degeneration.[11] In a second patient, a child that presented weakness without contractures, he noted that degeneration was limited to the anterior horn.[12] These two patients became the reference points for future research, reinforcing his theory of a two-part organization of the motor system and their respective clinical representation.[10] Trying to rule out confounding factors, Charcot carefully selected patients for examination based on these references. Perplexingly, and despite this selection, Charcot noticed a third group, consisting of patients with atrophy, spasticity, and contractures. Autopsy of these patients revealed degeneration of both the anterior and lateral horns, providing the third essential evidence for Charcot's bipartite motor system theory.[10] The clinical representation of both parts being affected was, in fact, the first specific diagnosis of ALS. Charcot's anatomo-clinical method thus allowed him to distinguish between these three types of neurological conditions, that would unlikely have been discerned by other physicians.[10] Moreover, Charcot predicted a direct relationship between the form of neurological lesions and a patient's symptoms, and was able to accurately predict their anatomical diagnosis before autopsy, meaning a diagnosis could be given during life. While all defining features of what later became known as motor neuron disease were worked out, Charcot

only coined the term “Amyotrophic lateral sclerosis” years later, in 1874. Refusing to allow ambiguous clinical terminology, he derived the name from the anatomical observations of the involved grey matter and damaged white matter, “amyotrophy” and “lateral sclerosis”, respectively.[10]

Aetiological studies

For all Charcot’s extensive research of ALS, he did not provide a theory on causative disease mechanisms. He noted that one third of his cases had some exposure to a cold or damp environment, but did not attribute the aetiology to this.[10] Charcot did believe that all primary neurological diseases were hereditary, with a different penetrance and pervasiveness, allowing for alterations in neurological manifestation due to environmental factors. Despite this conviction, he did not find any familial clusters of ALS.[10] Through the years after its initial diagnosis, although the disease gained notoriety and attention from researchers, its aetiology remained mostly unknown. At the start of the 20th century, researchers could only offer the tenuous hypothesis that neuronal degeneration was caused by some intrinsic or foreign poison, influenced by an inherited or acquired disposition.[13] Their limited understanding is no surprise, as they did not yet possess the biochemical technology required to study their theories.

With the development of molecular and genetic techniques in the latter half of the 20th century, it became possible to study ALS in an unprecedented way. This led to the discovery of several correlated processes and factors, and although some were discarded, others are still studied today.[14] For instance, it was observed that some neurotransmitters and axonal transport were deregulated, protein aggregation could occur, and heavy metals were linked to the disease.[15] However, this knowledge was superficial, and an actual understanding of disease mechanisms was lacking. This is partially explained by much research being relatively primitive and non-standardized. To emphasize this, the ALS animal model predominantly used from 1956 until the development of a SOD1 model was the “wobbler” mouse. This model has symptoms similar to ALS, but was later discovered to be caused by mutations in a gene currently thought to be unrelated to ALS in humans.[5] Reproducibility was rather low, and animal models were limited in representing disease aspects, impeding clinical translation. To this extent, the early clinical trials for treating ALS consisted mostly of generalized drugs, and many trials were otherwise inadequate due to being non-randomized and non-blinded.[14] Although techniques and methods have improved tremendously, biological, methodological, and also societal problems still restrain progress towards a unifying hypothesis of ALS pathogenesis, and hamper the development of new treatment strategies. Here, we will describe the factors that are currently thought to play a role in ALS, their history and clinical translation, before discussing the problems facing ALS research and complex neurological research in general.

1.2 Current hypotheses and therapeutics

Glutamate excitotoxicity

As early as the seventies, there was evidence of a correlation between ALS and glutamate levels.[16] Since then, it has been established that glutamate levels are significantly increased in about 40% of ALS patients, and correlate with its severity.[17,18] The increase can lead to excessive stimulation and a calcium influx, which can induce severe damage or death to the affected neurons.[19] Normally, glutamate transporters are responsible for synaptic clearance of the neurotransmitter, which for motor neurons is mainly performed by astrocytic excitatory amino acid transporter 2 (EAAT2). Indeed, depletion of this protein in animal models directly causes neuronal death.[20] In mutant SOD1 animal models, expression of the transporter is reduced presymptomatically and almost completely gone at the end stage of the disease.[21] Similarly, in about 25% of patients, expression of the protein is nearly absent.[22]

Some success has been achieved in treating SOD1^{G93A} mice with ceftriaxone, a β-lactam antibiotic, stimulating EEA2 expression and delaying disease onset, progression and death.[23] In a similar study, the glutamatergic receptor antagonist memantine increased survival.[24] However, the clinical adaptation of both ceftriaxone and memantine has proven unsuccessful, as no significant improvements were observed for treated patients.[25–27] Talampanel, another glutamatergic receptor antagonist, was tested in phase 2 and 3 clinical trials. Interestingly, only the first has been published, in which the drug provides an insignificant beneficial response.[28] Finally, riluzole was shown to alter glutamatergic transmission in pre-clinical studies and went into clinical trials under this hypothesis.[29] However, the high drug levels required for this to occur are not attained in patients. Currently, the drug is shown to have a plurality of effects, although a consensus over the exact mechanism has not been reached.[30] Riluzole showed a significant but marginal effect in patients, and was thus approved as the single treatment option in Europe.[29]

Mitochondrial dysfunction

Some degree of mitochondrial dysfunction is commonly observed in neurodegenerative conditions.[31] Interestingly, abnormalities are not seen in every ALS mouse model: while SOD1^{G37R} mice show mitochondrial swelling and vacuolization at presymptomatic stages, SOD1^{G85R} do not.[32,33] Despite this, a number of mutant SOD1-dependent mechanisms were proposed, including impaired energy metabolism, disrupted calcium homeostasis and protein build-up.[31] Additionally, dysregulated transport of mitochondria is hypothesized to contribute to degeneration, but not fully understood. Treating SOD1^{G93A} mice with creatine had a beneficial effect on disease onset and survival, giving rise to several clinical trials which all were unsuccessful.[34,35]

Axonal disorganization

Intracellular transport over the axon is vital for maintaining neuron structure and function, and is often perturbed in neurodegenerative diseases.[5] Some forty years ago, ALS patients were revealed to have aberrant proximal accumulation of neurofilaments, mitochondria, lysosomes and other vesicles.[36] Normally, neurofilaments are responsible for determining axon shape and promote growth, as the most abundant cytoskeletal protein in motor neurons.[21] Indeed, in mouse models with overexpressed or mutant neurofilament, a selective motor neuron dysfunction and subsequent death is observed.[37,38] The causal mechanism is poorly understood, but hypothesized to include insufficient delivery and excessive retrograde transport of mitochondria and trophic factors.[21] In ALS mouse models specifically, a more complex dynamic is seen: in SOD1^{G37R} mice, neurofilament overexpression ameliorated degeneration of motor neurons and increased disease survival. This is thought to be the result of protection against the effects of mutant SOD1, possibly through a buffer effect of the proximally accumulated neurofilaments against pathological calcium levels, or initial enhancement of axonal transport.[39,40] Perhaps the poorly understood mechanics and balance between beneficial and deleterious effects have prevented clinical development of successful axon-targeting drugs. Additionally, the few drugs that were developed proved ineffective, as the compounds were unable to cross the blood-brain barrier.[41]

Free radicals

Reactive oxygen species (ROS) have been associated with neurodegenerative conditions for a long time, and are suggested to be one of the primary initiating factors in ALS.[5] The majority of ROS are produced by cellular respiration in mitochondria. When such free radicals are not neutralized by antioxidants, they can cause significant damage to DNA and proteins, inducing oxidative stress. As SOD1 normally has a function in removing ROS, it is possible that mutations can reduce enzymatic function, thereby causing oxidative stress.[42] However, it is hypothesized that mutant SOD1 rather plays an indirect role through interacting with mitochondria, and its enzymatic activity is usually not

affected.[43] Oxidative stress and RNA dysregulation are often seen simultaneously in patients, but it remains uncertain which process precedes and possibly causes the other, as evidence for both theories exists.[44,45] One the one side, oxidative stress might cause mislocalization and aggregation of TARDBP and FUS, which regulate RNA. On the other side, perturbed RNA regulation might affect mitochondrial free radical generation through associated proteins. A possible mechanism for this is a modified interaction of TARDBP and FUS with the mitochondria.[5]

Modelling of this phenomenon has proved difficult, as mouse models yield inconsistent results: while increased ROS are associated with disease progression in $SOD1^{G93A}$ mice, this was not seen in $SOD1^{G37R}$ mice.[46,47] Besides creatine (mentioned before), two other antioxidative drugs are found to have beneficial effects in mice: coenzyme Q10 and edaverone.[48] Conversely, coenzyme Q10 does not show any amelioration in human trials.[49,50] Edaverone, however, showed marginal improvements in three major phase 3 clinical trials. The first of these trials demonstrated no significant differences between groups of late-stage patients, but slight beneficial effects in moderately and severely affected individuals.[51] This prompted a second trial focusing on severely affected patients, which did not exhibit any beneficial effect.[52] The third study rather aimed at moderately affected patients, and narrowed inclusion criteria even more. Here, functional rating and quality of life were significantly improved in treated patients compared to controls, leading to approval of the drug in Japan.[53] As all clinical trials so far have been performed on the Japanese population, it is uncertain whether the same effects will be demonstrated in western populations, as there is a significant disparity between patient populations of eastern and western descent.[54]

Protein aggregation

Many neurodegenerative diseases display an accumulation or aggregation of proteins, which is also seen in ALS.[5] The most prevalent mutated genes in ALS (SOD1, C9ORF72, TARDBP, and FUS) are all associated with proteins found in aggregates. This said, the emergence, role, and effects of the aggregates are still not fully understood. It is thought that the intracellular aggregates may contribute to motor neuron degeneration by sequestering vital cellular components, impairing chaperone function, and diminishing autophagy and ubiquitin-mediated proteolysis.[55–57] Both the autophagy and ubiquitin-proteasome system pathways are of great importance for protein homeostasis in healthy neurons, and may be critical for maintaining physiological protein levels.[5] Lithium carbonate and pioglitazone are neuroprotective compounds thought to function through degrading aggregates, and have shown some beneficial effects in animal models.[35] While a trial for pioglitazone failed, a pilot study of lithium carbonate did prove beneficial.[58] This sparked three additional clinical trials, which all failed to demonstrate the previously seen effects.[59–61] This is thought to be the result of inappropriate patient selection for the initial trial, confounding its results.[35]

Glial toxicity

Defects in astroglia were detected as early as the nineties, when it was discovered that a large portion of ALS patients has a dysfunctional astroglia-specific glutamate transporters.[22] Now, it is known that the neuroinflammation accompanying neuronal degeneration is associated with microglia and astrocyte activation, excessive release of inflammatory cytokines and subsequent T-cell infiltration.[62] This is also thought to be the case in ALS. Microglia respond to neurological damage, releasing cytokines and chemokines in an effort to restore it, whereas astrocytes normally assist neurons by providing trophic support and pruning synapses. However, microglial overactivation and cytokine overproduction can lead to consequent overactivation of astrocytes, resulting in neuronal death[63]. In SOD1 mouse models, perturbations in astrocyte functions are shown to correlate with disease progression, but the mechanism behind this is not yet understood.[21] Notably, glia have been

associated with both beneficial and deleterious effects, dependent on the stage of the disease, underscoring the complexity of immunological mechanisms in neurodegeneration.[64]

A host of therapeutics were found to have beneficial effects in SOD1 mice and were used in clinical trials. Unfortunately, most of these proposed therapeutics have proven unsuccessful.[35] One notable exception is masitinib, which was shown to prevent neuroinflammation in SOD1^{G93A} rats.[65] Unexpectedly, the treatment markedly improved survival even after onset of paralysis. This has recently prompted a clinical trial with masitinib as an add-on to riluzole therapy, where the drug was shown to have a significant beneficial effect for moderately affected patients.[66] However, this does not apply for fast-progressing patients. Despite the limited effects, these results are encouraging, and a subsequent confirmatory clinical trial has been initiated.[66] While the trial subjects represented five times more ALS patients than comprised in the edaverone trials, inclusion criteria were still stringent, and it will be of interest to test therapeutic efficacy in an extended group of patients.

2. Problems facing ALS research

Regarding the progress of ALS research described here, we can observe a number of issues. Some of these are rather apparent, and inherent to the disease. These are biological problems like the complex causality and heterogeneity of ALS. Other issues are technical in nature, seen in the approach of research. Here, gains could be made to reproducibility, translation of animal models to a clinical setting, and trial design. Lastly, and perhaps overlooked in the context of ALS, there are implicit structural issues concerning the publication of results and acquisition of funding. In the following chapter we will discuss these issues, attempting to offer solutions that will contribute to future research and a more innovative development of therapeutics.

2.1 Biological issues

Complex causality

As discussed in the previous chapter, it is clear that the pathogenic process of ALS is extremely complex. Consequently, it has proven an arduous process pinpointing the disparate causative factors leading to the aforementioned abnormalities in neural functions (impaired neurotransmitter homeostasis, mitochondrial dysfunction, axonal disorganization, oxidative stress, proteostatic imbalance and glial cell toxicity).[5] It has long been understood that the disease is multifactorial, with the likelihood of acquiring the disease derived from a combination of genetic variants, environmental exposures and the normal aging process.[67] Recently, it has been hypothesized that these factors cause varying levels of dispositions, and interact in a multistep process to ultimately initiate the pathogenic process.[6]

Several environmental exposures have been reported to be associated with ALS. The strongest correlation was seen for chronic exposure to lead.[68] Indeed, lead toxicity-induced neuropathy is generally characterized by symptoms similar to ALS, and might accelerate pathogenesis in individuals with other predispositions, such as genetic ones.[68] This would also fit into the multistep hypothesis of the disease.[6] Other heavy metals, like mercury and aluminium, have been correlated to ALS as well, albeit only supported by suggestive evidence.[68] To a lesser extent, head trauma has been implicated in pathogenesis.[68] Although it is argued to be biologically plausible, there is no universal definition, consistent methodology, nor adequate statistical analysis in the various studies of head trauma in patients.[68] Moreover, head trauma and the heavy metals mentioned here have been associated with multiple neurodegenerative diseases, confounding their roles in pathogenic processes.[68] In turn, this might be one of the reasons it has remained unclear how environmental exposures play a role in causing ALS.

Genetic components comprise an important, although variable part of pathogenesis. The first success in discovering causative genetic factors was achieved in 1993, with the discovery of 11 different mutations in the SOD1 gene.[69] Since then, numerous genetic variants have been implicated in ALS.[70] To highlight the sheer number, for SOD1 alone, 170 variants have been reported.[70] The genetic variants are major factors in fALS, which comprises 10% of all patients and is defined as having at least one other family member with the condition.[70] In these patients, few genes deliver a major contribution to pathology. Of these, C9ORF72 is found in 40% of fALS patients, SOD1 in 20%, TARDBP in about 5% and FUS in 3%.[70] Together, major mutations explain about 60% of fALS cases. However, the majority of all ALS patients does not have any apparent family history, with 90-95% defined as sALS.[70] There is a significant overlap with fALS, as C9ORF72 mutations are found in about 7% of sALS patients, SOD1 in 2%, TARDBP is 1% and FUS in less than 1%. In total, these genetic factors only cause up to 10% of sALS cases.[70] Furthermore, there is an increasingly large number of more rare genetic variants with varying clinical phenotypes, of which many have a less understood contribution.[4,70] Especially due to the mostly sporadic nature of the disease, and its relatively low prevalence, gene mapping and identification of genetic variants has remained challenging, calling for more cooperation and integration of research.[41]

Disease heterogeneity

The plurality of causative factors, whether known or not, leads to extreme variability in the manifestation of ALS. To this extent, there are major differences between sex, onset age, location and disease duration.[2] The age of disease onset is generally around 55 years, but ranges from 20 to 85, with fALS cases generally having an earlier onset.[2] Survival is generally short, ranging from 2 to 4 years, but in some cases up to 10. Here, longer survival is mostly seen in younger patients.[2] This phenomenon could be explained by the multistep hypothesis, as the normal aging process can only have a relatively small effect in younger individuals. Furthermore, men are twice as likely to contract the condition than women within sALS cases, while such a disparity is not seen in fALS.[2] One third of all cases has a bulbar onset, which can lead to emotional lability, whereas most cases begin in the limbs.[2] Additional behavioural changes are seen in some 20% of patients, when ALS occurs in concert with FTD, characterized by a cognitive decline and subsequent dementia.[2] It is suggested that the existing heterogeneity represents part of a continuum rather than distinct disease variants, further complicating efficient classification.[71] Taken together, the varying clinical manifestations are major confounding factors for finding biomarkers, assessing pathological mechanisms, and designing or interpreting clinical trials. Finally, a multitude of ALS-associated genes is known to be pleiotropic, implicated in other motor neuron diseases and neurodegenerative disorders.[71] It is debated whether related conditions with shared hallmarks, while involving different biological entities, should be regarded as a spectrum, emphasizing the extent of complexity observed in neurodegenerative diseases.[71]

The classifications of ALS are an attempt to structure the heterogeneity of the disease. However, the definition of familial and sporadic ALS contains an arbitrary element, posing a notable problem in the investigation of causative genetic variants. To highlight this, even sALS reportedly has a hereditary component in up to 60% of cases.[4] As the classification between sporadic and familial ALS is based on family history rather than a specific pheno- or genotype, differentiation is difficult, and could explain the overlap between groups.[70] In accordance with that, statistical models show that patients with a small family size or lowly penetrative genetic variants have a high risk of misclassification of fALS as sALS.[72] This sampling bias is known to confound genetic association studies, putting rare or lowly penetrative genetic variants at a disadvantage of being correctly identified.[41] Because of this, and in view of the fact that fALS and sALS are clinically indistinguishable, it might be better to create more

informative classifications. These could be based on definitions less prone to bias, such as clinical presentations or genetic signatures.

The incidence and causation of ALS additionally differ between geographical areas.[73,74] The reasons behind this are mostly unknown, although a founder effect has been identified for some mutations.[74] While differing genetic composition of patient populations between continents might not be a problem for domestic research and understanding of the condition, it is important to note that research and trial results will not be directly translatable between these patient populations. Furthermore, focusing on dominant native mutations may not contribute as much to a unifying hypothesis of ALS aetiology as a more holistic approach might.

2.2 Technical issues

Difficult evaluation

Unreliable biomarkers

Effective biomarkers could be of great value for researchers and patients, as they can indicate key biological processes. They are generally classified into diagnostic, prognostic, predictive and pharmacodynamic markers.[41] As such, they can respectively aid in defining disease state, associated risks, treatability and the effectiveness of treatment.[41] Unfortunately, the biomarkers for ALS are unpractical and not always reliable, as they can yield variable results.[41] The source of biomarkers additionally complicates matters: while blood is relatively accessible and easy to handle, cerebrospinal fluid (CSF) is more accurate, as it is directly connected to the central nervous system.[41]

There are several biomarkers for ALS, divided in fluid-based and electrophysiological types, each with their own set of problems. For fluid biomarkers, such as neurofilament subunits and neurotrophin, notable differences can be introduced by disparities in sample collection and processing.[75] Additionally, diurnal fluctuations are observed, causing significant variation even within patients.[75] The specificity of several proteins is also a subject of debate, as neurofilament subunits, for instance, can be found in multiple conditions.[75] Finally, trial evaluation and other study results are confounded by the previously discussed heterogeneity and the possible presence of ALS-mimics that have not been successfully differentiated.[75] For electrophysiological markers, the most prominent problems are repeatability and reliability. Older methods have been reported to significantly vary dependent on the size and positioning of electrodes, as well as the temperature and positioning of the limb they are connected to.[75] While more modern methods are less prone to this variation, their reliability has not been evaluated as extensively.[75]

To solve the issues of biomarker development and usage, it will be necessary to come to a better understanding of ALS causation and heterogeneity, calling for a collaborative approach. Studies should be subjected to rigorous selection excluding possible ALS-mimics, and methods should be standardized.[75] To this extent, the FDA has already proposed specific guidelines that could provide uniform analyses.[75] Furthermore, collaboration in the form of consortia seen for other neurodegenerative diseases could combine expertise and provide the resources required for a task of this magnitude.

Diagnostic delay

Significant diagnostic delays are seen in ALS patients, with a median of about a year from initial onset of symptoms to a correct diagnosis.[76] This delay can be attributed to several factors. First, a patient may not want to seek out medical help for vague symptoms, rather waiting until they are decidedly ill.[76] As the early symptoms of ALS are variable, the second problem arises. Correct diagnosis can initially be challenging, with reportedly up to 50% of cases being misdiagnosed, possibly leading to

incorrect treatments and operations.[76] This confusion is understandable given the relatively low prevalence of ALS, and the initial symptoms resembling those of mononeuropathies, spinal cord diseases and some other neurological syndromes.[76] Third, the absence of sensitive and reliable biomarkers hinders early diagnosis, and may increase the frequency of misdiagnosis.[76] Fourth, due to the lack of biomarkers, presymptomatic diagnosis is currently impossible. [41] This can leave months between the start of neurodegeneration, subsequent emergence of symptoms, diagnosis and ultimately treatment. The disease may then have progressed too far for a trial to be effective, possibly confounding its results.[41] Besides the development of novel biomarkers, healthcare professionals may be trained to ensure early referral to neuromuscular specialists, enabling earlier accurate diagnosis.

Study design

Reproducibility

The reproducibility crisis is a central issue in science, and ALS research is no exception.[77] Within associational, pre-clinical and clinical studies in the field of ALS, improper study design, methodology and statistical analysis have been reported.[68,70,78,79] Additionally, methodological description can be incomplete, complicating reproduction of results further.[79] To emphasize the severity of low reproducibility, the supposed effect of riluzole was not seen in large mouse studies, and may actually be attributed to false positives or other statistical errors.[78] This stands in stark contrast with the previously published effects of the drug.[78] Similar disparities have been quantified for a host of seemingly advantageous drugs, none of which showed any survival benefits.[80] This highlights the bias towards positive results in science, as well as the necessity of robust design and publication of negative or toxic effects shown in well-designed studies.[78,80]

Potential reasons for trial failure have been classified into three categories: weak trial rationale, pharmacological problems, and issues in clinical trial design and methodology.[81] Trials with a weak rationale were usually continuations of inadequate animal model studies, which we will discuss in the next section.[81] Additionally, these preclinical studies were generally flawed in methodology, as therapy commenced before disease onset in animal models, yielding neuroprotection instead of actual treatment.[41,81] For these clinical studies, hypothetical targets were used in humans, which might not behave in the same fashion as observed in the animal models they were based on, possibly contributing to the ultimate failure of the drug.[81] Pharmacological issues include suspected insufficient dosage, bell-curved pharmacodynamics, and the lack of pharmacokinetic analysis.[81] Without robust knowledge and monitoring of dosages, the desired effects may not be acquired in patients. Furthermore, most studies supply a drug in combination with riluzole, without studying any possible interaction, allowing the actual effects of the additive drugs to go unnoticed.[81] Poor trial design and methodology further confound clinical outcomes, as budget and time constraints have been reported to make performance of trials extremely demanding.[81] Proper design of a clinical study is admittedly difficult, as it should account for the inherent heterogeneity of ALS while biomarkers to distinguish variants are lacking.[70,79] Additionally, ALS-mimics and late stage patients can confound results extensively if not identified timely and correctly.

Inadequate models

A wide variety of ALS models has been created to facilitate studying the disease. These include cell lines, primary cell cultures, rodents and higher mammal models. [5,41] While all of these have specific problems, the most prominent issue concerning models is the insufficient translatability of tested drugs to a clinical application. [5,41,67] Besides poor study design and analysis, this may be due to factors like internal variability and oversimplification of ALS pathology.[5,67]

The low success for animal models, especially the primarily used rodent models, is mainly due to variable phenotypes even for animals with the same mutation, as this is a major confounding factor.[5] To this extent, mice with mutations in genes homologous to those affected in patients have been described to have phenotypes unlike human patients.[5] Conversely, "wobbler" mice with a phenotype similar to ALS have a mutation in a gene that is not known to be related to the disease in humans.[5] Until now, no animal model is known to represent all features of ALS.[67] It has thus been proposed that extrapolations from animal models of the disease are too simplistic, focusing on individual features, rather than the entire pathological process.[67] Importantly, these models merely reflect genetic factors, disregarding the sporadic nature of ALS and the effects of other internal or external factors.[67] Indeed, in case ALS pathogenesis is a multi-step process, adequate animal models should reflect this.[6,67] Higher mammal models, like canine, porcine, and simian, are phenotypically closer to humans, enabling more accurate comparison.[5] However, these models are also based on genetic factors, have a greatly increased generation time, and are ethically less favourable.[5,41]

Patient-derived cell lines and primary cell cultures may constitute all features present in respective patients, but do not represent the complete neural environment.[5] In that sense, they are still a simplified model. Additionally, induced pluripotent stem cell (iPSC)-derived motor neurons undergo epigenetic changes removing any previous signatures, and may undergo genetic changes further undermining their use.[5] Directly reprogrammed motor neurons remove this issue, as the iPSC step is completely skipped, thus maintaining the hallmarks of aging.[5] The most promising cellular models may be olfactory stem cell cultures, as these can be directly and relatively non-invasively derived from the nasal cavity of patients.[5] The cells can be differentiated into glia and neurons, and could be particularly useful in delineating processes before or early in pathogenesis.[5] However, olfactory stem cell models have not been validated extensively, and a standard method for genesis and characterization should be developed.[5]

2.3 Structural problems

As we have seen in the previous section, ALS is an extremely heterogenous disease. It can manifest virtually any time during adult life, initially bulbar or peripheral, and concurrently with FTD; it varies in aggressiveness, how it affects different sexes and different geographical populations. Individuals may have associated or causative genetic variants with varying penetrance, affecting their predisposition to other associated environmental and aging processes. Due to the heterogenous nature and complex causality of ALS, it has remained impossible to pose a unifying hypothesis. It has also prevented the discovery of general biomarkers, and the development of models that reproduce the disease with all the features observed in humans.

The absence of suitable biomarkers and disease models have complicated research further. The fact that most ALS models are based on variants of the four major causative genes (SOD1, C9ORF72, TARDBP and FUS) is a vast misrepresentation of the heterogenous patient population, and an extreme reductionist view of the complex causality of ALS.[70] As such, the translatability of supposedly beneficial therapies remains poor. Additionally, a large number of preclinical studies has flaws in design and methodology, which have made reproduction of results unfeasible.[81] Moreover, negative results to provide some measure of counterbalance have mostly remained unpublished, allowing drugs with dubious efficacy to move forward to clinical trials.[78] Thus, with few exceptions, drugs that were effective in preclinical trials had little to no effect in patients. For clinical trials, flaws in design and methodology might have yielded positive results in exploratory trials, only to fail in confirmatory trials.[81] On the other hand, these flaws might have caused drugs with small effects, or effects on subgroups of patients, to go unnoticed.

Solutions addressing these problems have been posed. Predominantly, the current simplistic approach should be altered into more integrated, holistic research.[67] While there might be more simple causative factors explaining a fraction of cases, a unifying hypothesis can only be formulated by expanding our knowledge of the different pathogenic factors and their interplay. This will require large-scale and long-term studies, subsequently analysed with sophisticated computational tools. It might also demand a transition towards more exploratory research instead of hypothesis testing. Additionally, increased understanding of pathological processes may allow development of improved models and biomarkers, which would in turn increase research efficiency. However, there are several structural characteristics of research that inhibit this transition, especially in how resource allocation is performed by funding agencies. We will argue that at least for complex diseases like ALS, the current funding structure slows progression in several ways. Finally, we will offer suggestions that could lead to improvements.

First and foremost, competition can reportedly have deleterious effects.[82] The reward structure of science is based on recognition, mainly that of being the first to make a discovery.[82,83] Although this incentivizes the sharing of discoveries, it also gives rise to competition. While this might be beneficial for other sectors, competition in science has been connected to withholding information and materials from peers.[82] Subsequently, results cannot be adequately confirmed and progress is impeded. Other detrimental effects of competition have been observed in the form of sabotage, biased peer- and grant-review and other perverse practices.[82] As the economics of science are described as 'winner-take-all', it can lead to great inequality between research groups.[83] This inequality is argued to lead to a cumulative advantage for groups that are successful, increasing inequality even further compared to groups that might initially not be as convincing to grant reviewers, or as successful in their research in a short-term period.[83]

Research output and its perceived impact, or bibliometrics, have long been the dominant way of measuring scientific quality, illustrated by the rise of 'hyperprolific' authors.[84] This brings about another problem: more novel research is known to gain recognition relatively slowly.[85] In turn, the lack of recognition can influence future performance of research groups exploring novel approaches.[85] It has been suggested this is due to the reluctance of incumbent paradigms to recognize novelty. Additionally, delayed recognition is reported to generate fewer short-term citations and publication in journals with a lower impact factor.[85] As funding agencies also rely on bibliometrics, decisions about resource allocation will be detrimental to groups applying novel approaches.[85] It has been reported that chances for acquiring funding are better indicated by previous performance than grant proposal quality, highlighting the uphill battle for novel approaches.[86,87]

Within the competitive environment, the qualitative manner in which scientists are awarded recognition ensures that resources will not generally be shared, inhibiting cooperation in favour of splintered research.[82] As increased funding will directly affect scientists' individual research output, researchers are not generally inclined to share their funds.[82] Besides that, the qualitative focus in science steers toward faster, cheaper research, increasing throughput at the cost of validity.[88] Indeed, while ALS models have enabled extensive fast screening, their predictive validity has been dismal.[41,78] Furthermore, negative results may not be published as these will not generally improve the competitive position of a group, besides not being as interesting to journals due to a relatively low impact.

Reliance on bibliometric indicators also seems to steer away from "high-risk, high-gain" research, inhibiting more innovative ventures in favour of safe research.[85] Given the ease of reliably assessing the predicted success of hypothesis testing, this promotes hypothesis-driven research above exploratory research.[89] Where hypothesis-driven research may be well suited for progressively

deepening current knowledge, exploratory research can reveal entirely new directions, instead widening our understanding of complex pathological processes, consequently improving our ability to develop therapeutics.[89] However, as the outcome of exploratory research cannot be predicted reliably, such ventures are regarded as inherently risky. In turn, the preference of funding agencies for hypothesis testing contributes to simplifying hypotheses and splintering research, inhibiting innovative and combined approaches.[89]

For some neurodegenerative conditions, like Alzheimer, proposals have been made to shift funding priority from research to prevention and patient care.[90,91] Given the poor track record of research and the extensive burden that long neurodegenerative diseases cause to the healthcare system, re-evaluation of our priorities is definitely worth considering. For ALS, however, these proposals are less pronounced.[35] Perhaps this is a result of the fast progression and relatively low prevalence, stemming the burden on our healthcare system, or the fact that ALS prevention is impossible, as pathogenic factors remain poorly understood. Still, as the current scientific climate is largely unsuitable for researching complex diseases, the outlook on effective therapeutics is grim, and it remains important to debate the balance between funding for prevention, care, and cure.

In summary, the structure of research funding incentivizes practices that limit progress, predominantly materialized in the competition for funds and quantitative recognition. These factors limit cooperation and integrated approaches. Additionally, new groups or approaches suffer from increasing inequality relative to their vested counterparts. Research is steered towards faster, cheaper, more quantifiable output, outcompeting qualitative or exploratory research, inhibiting innovation and development of novel models and techniques. This phenomenon is especially deleterious to research of complex disorders such as ALS, where success relies on qualitative research, comprising integrated and holistic approaches.

Private funding agencies are aware of the structural issues concerning science, as explained by a senior research coordinator (S. van den Berge, personal communication, November 13th, 2020). Within private agencies, guidelines have been implemented in an effort to alleviate pressure on scientists that would not be favoured by traditional research funding. This is facilitated further by the ability of these organisations to set their own priorities and appoint review boards accordingly. As a result, they can spread expertise to allow more interdisciplinary research, and bar members with competing interests from decisions, decreasing bias. Furthermore, this ability enables private funds to be more flexible, fostering collaboration by promoting aligned research efforts and co-financing large-scale or long-term studies with similar agencies. One example of a such a venture is project MinE, which will survey genetic and environmental factors in 15,000 ALS patients and half as many control subjects.[92] One of the initiating researchers, a professor in neurogenetics, emphasizes that a project of such an unprecedented magnitude would not have been feasible without private co-financing, risking splintering the research (J.H. Veldink, personal communication, November 6th, 2020).

Like their private counterparts, some public funding agencies acknowledge the existing issues in funding decisions, exemplified by the National Institutes of Health announcing a high-risk, high-reward funding programme in 2019.[93] However, while such grants do allow more risky approaches, this does not mean they are any less competitive, and proven records, competences and preliminary data are still required. In effect, these programmes thus offer some symptomatic relief, but accomplish little in addressing the core problems decelerating innovation, calling for systemic changes.

To combat competition, one could think of more general funding. This would provide a financial baseline for research groups regardless whether they favour previous or novel approaches, possibly jumpstarting many innovative projects. It would also allow more time-consuming or risky projects to

be performed, where these would not be supported by the current system. Thus, it could promote development of new models and techniques that are less erroneous than the existing ones. Additionally, more general funding would lead to less biased and arbitrary funding decisions. There are proposals for modified funding structures, where an interdisciplinary panel would judge possible merits of research and its technical soundness.[67,85,94] This would ensure recognition of good practices, while limiting personal or professional bias in the decision process.

The focus on quantitative output should be converted to value research quality. The way recognition and funding are acquired currently delivers a perverse incentive to cut corners and perform highly simplified research, wasting resources on ill-conceived and inadequate inquiries. A focus on qualitative appreciation of science could allow exploratory research over simple hypothesis testing, and may enable more large-scale and long-term approaches that are unfavourable in the current system. Naturally, qualitative recognition will require structural changes to how we value science on all levels, from departmental to international, and may be measured according to area-specific guidelines. A wider view than bibliometric indicators should unquestionably be used, taking into account overall research quality and societal impact.

A start has been made by initiatives like the San Francisco Declaration on Research Assessment formulated in 2013, recognizing the quantitative focus of research assessment and the derivative scientific environment.[95] The declaration proposes guidelines for improving reward systems in funding and publishing, and is signed by some 2000 institutions worldwide, publicly declaring their commitment. Although these initiatives are most certainly commendable, changes are implemented slowly, as is demonstrated by the association of Dutch universities and funding agencies proposing policy changes only as recent as 2019.[96] Indeed, it is encouraging to see institutions publicly acknowledge issues and their commitment to improving upon the current situation. However, changing how we value science remains an extensive process. As institutions cannot implement drastic measures on their own, in fear of losing their competitive position, major changes will require a shift in the mentality of the majority of the scientific community, funding agencies and governmental bodies. It will take time to reach a consensus on how we should design a new system for assessing value, and to draw up and implement policies ensuing this. Ultimately, a fairer system of accreditation will benefit the scientific community and society at large, giving rise to more qualitative research, in turn providing a more suitable environment for the study of complex disorders like ALS.

3. Conclusion

ALS is a complex, heterogeneous condition that is currently poorly understood. Despite extensive research, therapies only have marginal effects. An integrated, holistic approach could lead to increased understanding, novel biomarkers, and improved models, which would amplify research efficiency and aid in developing new therapeutic strategies. Such an approach is inhibited by structural issues in allocation of funding, as time-consuming and large-scale studies suffer from competition and the quantitative focus of scientific and funding institutions. More generalized funding would limit competitiveness by undercutting the importance of grant proposal and ensuing inequality. A focus on qualitative contributions to science and society, rather than a quantitative appraisal, may yield more fair recognition and increase innovation.

"Let us keep looking in spite of everything. Let us keep searching, for it is indeed the best method of finding. And perhaps thanks to our efforts, the verdict we will give such a patient tomorrow will not be the same we must give this man today."

Jaen Martin Charcot, lessons of February 1889

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References

1. Siddique, N. & Siddique, T. *Amyotrophic Lateral Sclerosis Overview*. *GeneReviews* (University of Washington, Seattle, 2019).
2. Brown, R. H. & Al-Chalabi, A. Amyotrophic Lateral Sclerosis. *N. Engl. J. Med.* **377**, 162–172 (2017).
3. Chio, A. *et al.* Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J. Neurol. Neurosurg. Psychiatry* **82**, 740–746 (2011).
4. Pensato, V. *et al.* Sorting Rare ALS Genetic Variants by Targeted Re-Sequencing Panel in Italian Patients: OPTN, VCP, and SQSTM1 Variants Account for 3% of Rare Genetic Forms. *J. Clin. Med.* **9**, 412 (2020).
5. Mejzini, R. *et al.* ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? *Front. Neurosci.* **13**, (2019).
6. Chiò, A. *et al.* The multistep hypothesis of ALS revisited: The role of genetic mutations. *Neurology* **91**, e635–e642 (2018).
7. Tyler, H. R. & Shefner, J. Amyotrophic lateral sclerosis. in *Handbook of clinical neurology* vol. 59 169–215 (Elsevier, 1991).
8. North, C. S. The Classification of Hysteria and Related Disorders: Historical and Phenomenological Considerations. *Behav. Sci.* **5**, 496–517 (2015).
9. Goetz, C. G. Amyotrophic lateral sclerosis: Early contributions of Jean-Martin Charcot. 8 (2000).
10. Goetz, C. G. Chapter 15 Jean-Martin Charcot and the anatomo-clinical method of neurology. in *Handbook of Clinical Neurology* vol. 95 203–212 (Elsevier, 2009).
11. Charcot, J. M. Des amyotrophies chroniques - atrophie musculaire progressive spinale prototypique. (English: Chronic spinal amyotrophies. Protopathic spinal progressive muscular atrophy.). in *Oeuvres Comple`tes (Lectures on diseases of the nervous system)* vol. 2 221-233 (163–191) (Bureaux du Progres Medical (New Sydenham Society), 1874).
12. Charcot, J.-M. & Joffroy, A. *Deux cas d'atrophie musculaire progressive: avec lésions de la substance grise et des faisceaux antérolatéraux de la moelle épinière*. (Masson, 1869).
13. Mott, F. W. The Croonian Lectures on Degeneration of the Neurone: Delivered at the Royal College of Physicians of London. *BMJ* **2**, 13–18 (1900).
14. Tandan, R. & Bradley, W. G. Amyotrophic lateral sclerosis: Part 1. Clinical features, pathology, and ethical issues in management. *Ann. Neurol.* **18**, 271–280 (1985).
15. Tandan, R. & Bradley, W. G. Amyotrophic lateral sclerosis: Part 2. Etiopathogenesis. *Ann. Neurol.* **18**, 419–431 (1985).
16. Plaitakis, A. & Caroscio, J. T. Abnormal glutamate metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.* **22**, 575–579 (1987).
17. Rothstein, J. D. *et al.* Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann. Neurol.* **28**, 18–25 (1990).

18. Spreux-Varoquaux, O. *et al.* Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. *J. Neurol. Sci.* **193**, 73–78 (2002).
19. Cleveland, D. W. & Rothstein, J. D. From charcot to lou gehrig: deciphering selective motor neuron death in als. *Nat. Rev. Neurosci.* **2**, 806–819 (2001).
20. Rothstein, J. D. *et al.* Knockout of Glutamate Transporters Reveals a Major Role for Astroglial Transport in Excitotoxicity and Clearance of Glutamate. *Neuron* **16**, 675–686 (1996).
21. Rothstein, J. D. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann. Neurol.* **65**, S3–S9 (2009).
22. Rothstein, J. D., Van Kammen, M., Levey, A. I., Martin, L. J. & Kuncl, R. W. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann. Neurol.* **38**, 73–84 (1995).
23. Rothstein, J. D. *et al.* Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **433**, 73–77 (2005).
24. Wang, R. & Zhang, D. Memantine prolongs survival in an amyotrophic lateral sclerosis mouse model. *Eur. J. Neurosci.* **22**, 2376–2380 (2005).
25. Berry, J. D. *et al.* Design and Initial Results of a Multi-Phase Randomized Trial of Ceftriaxone in Amyotrophic Lateral Sclerosis. *PLOS ONE* **8**, e61177 (2013).
26. Cudkowicz, M. E. *et al.* Efficacy and safety of ceftriaxone for amyotrophic lateral sclerosis: results of a multi-stage, randomised, double-blind, placebo-controlled, phase 3 study. *Lancet Neurol.* **13**, 1083–1091 (2014).
27. de Carvalho, M. *et al.* A randomized, placebo-controlled trial of memantine for functional disability in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis.* **11**, 456–460 (2010).
28. Pascuzzi, R. M. *et al.* A phase II trial of talampanel in subjects with amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis.* **11**, 266–271 (2010).
29. Bensimon, G., Lacomblez, L. & Meininger, V. A Controlled Trial of Riluzole in Amyotrophic Lateral Sclerosis. *N. Engl. J. Med.* **330**, 585–591 (1994).
30. Bellingham, M. C. A review of the neural mechanisms of action and clinical efficiency of riluzole in treating amyotrophic lateral sclerosis: what have we learned in the last decade? *CNS Neurosci. Ther.* **17**, 4–31 (2011).
31. Boillée, S., Vande Velde, C. & Cleveland, D. W. ALS: A Disease of Motor Neurons and Their Nonneuronal Neighbors. *Neuron* **52**, 39–59 (2006).
32. Bruijn, L. I. *et al.* ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**, 327–338 (1997).
33. Wong, P. C. *et al.* An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* **14**, 1105–1116 (1995).
34. Klivenyi, P. *et al.* Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med.* **5**, 347–350 (1999).
35. 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**, (2017).
36. Hirano, A., Donnenfeld, H., Sasaki, S. & Nakano, I. Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* **43**, 461–470 (1984).

37. Xu, Z., Cork, L. C., Griffin, J. W. & Cleveland, D. W. Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* **73**, 23–33 (1993).
38. Lee, M. K., Marszalek, J. R. & Cleveland, D. W. A mutant neurofilament subunit causes massive, selective motor neuron death: implications for the pathogenesis of human motor neuron disease. *Neuron* **13**, 975–988 (1994).
39. Couillard-Després, S. *et al.* Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc. Natl. Acad. Sci.* **95**, 9626–9630 (1998).
40. Williamson, T. L. & Cleveland, D. W. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat. Neurosci.* **2**, 50–56 (1999).
41. Katyal, N. & Govindarajan, R. Shortcomings in the Current Amyotrophic Lateral Sclerosis Trials and Potential Solutions for Improvement. *Front. Neurol.* **8**, 521 (2017).
42. Niedzielska, E. *et al.* Oxidative Stress in Neurodegenerative Diseases. *Mol. Neurobiol.* **53**, 4094–4125 (2016).
43. Cozzolino, M. *et al.* Oligomerization of Mutant SOD1 in Mitochondria of Motoneuronal Cells Drives Mitochondrial Damage and Cell Toxicity. *Antioxid. Redox Signal.* **11**, 1547–1558 (2009).
44. Cohen, T. J. *et al.* An acetylation switch controls TDP-43 function and aggregation propensity. *Nat. Commun.* **6**, 5845 (2015).
45. Pickles, S. *et al.* ALS-linked misfolded SOD1 species have divergent impacts on mitochondria. *Acta Neuropathol. Commun.* **4**, 43 (2016).
46. Andrus, P. K., Fleck, T. J., Gurney, M. E. & Hall, E. D. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J. Neurochem.* **71**, 2041–2048 (1998).
47. Bruijn, L. I. *et al.* Elevated free nitrotyrosine levels, but not protein-bound nitrotyrosine or hydroxyl radicals, throughout amyotrophic lateral sclerosis (ALS)-like disease implicate tyrosine nitration as an aberrant in vivo property of one familial ALS-linked superoxide dismutase 1 mutant. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7606–7611 (1997).
48. Beal, M. F. Coenzyme Q10 as a possible treatment for neurodegenerative diseases. *Free Radic. Res.* **36**, 455–460 (2002).
49. Ferrante, K. L. *et al.* Tolerance of high-dose (3,000 mg/day) coenzyme Q10 in ALS. *Neurology* **65**, 1834–1836 (2005).
50. Yoshino, H. & Kimura, A. Investigation of the therapeutic effects of edaravone, a free radical scavenger, on amyotrophic lateral sclerosis (Phase II study). *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis.* **7**, 241–245 (2006).
51. Abe, K. *et al.* Confirmatory double-blind, parallel-group, placebo-controlled study of efficacy and safety of edaravone (MCI-186) in amyotrophic lateral sclerosis patients. *Amyotroph. Lateral Scler. Front. Degener.* **15**, 610–617 (2014).
52. Tanaka, M., Akimoto, M., Palumbo, J. & Sakata, T. A Double-Blind, Parallel-Group, Placebo-Controlled, 24-Week, Exploratory Study of Edaravone (MCI-186) for the Treatment of Advanced Amyotrophic Lateral Sclerosis (ALS) (P3.191). *Neurology* **86**, (2016).
53. Tanaka, M., Sakata, T., Palumbo, J. & Akimoto, M. A 24-Week, Phase III, Double-Blind, Parallel-Group Study of Edaravone (MCI-186) for Treatment of Amyotrophic Lateral Sclerosis (ALS) (P3.189). *Neurology* **86**, (2016).
54. Marin, B. *et al.* Variation in worldwide incidence of amyotrophic lateral sclerosis: a meta-analysis. *Int. J. Epidemiol.* **46**, 57–74 (2017).

55. Bruijn, L. I. *et al.* Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854 (1998).
56. Bruening, W. *et al.* Up-regulation of protein chaperones preserves viability of cells expressing toxic Cu/Zn-superoxide dismutase mutants associated with amyotrophic lateral sclerosis. *J. Neurochem.* **72**, 693–699 (1999).
57. Niwa, J.-I. *et al.* Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity. *J. Biol. Chem.* **277**, 36793–36798 (2002).
58. Fornai, F. *et al.* Lithium delays progression of amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci.* **105**, 2052–2057 (2008).
59. Aggarwal, S. P. *et al.* Safety and efficacy of lithium in combination with riluzole for treatment of amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **9**, 481–488 (2010).
60. Verstraete, E. *et al.* Lithium lacks effect on survival in amyotrophic lateral sclerosis: a phase IIb randomised sequential trial. *J. Neurol. Neurosurg. Psychiatry* **83**, 557–564 (2012).
61. UKMND-LiCALS Study Group. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Neurol.* **12**, 339–345 (2013).
62. Komine, O. & Yamanaka, K. Neuroinflammation in motor neuron disease. *Nagoya J. Med. Sci.* **77**, 537–549 (2015).
63. Liddelow, S. A. *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature* **541**, 481–487 (2017).
64. Liu, J. & Wang, F. Role of Neuroinflammation in Amyotrophic Lateral Sclerosis: Cellular Mechanisms and Therapeutic Implications. *Front. Immunol.* **8**, 1005 (2017).
65. Trias, E. *et al.* Post-paralysis tyrosine kinase inhibition with masitinib abrogates neuroinflammation and slows disease progression in inherited amyotrophic lateral sclerosis. *J. Neuroinflammation* **13**, 177 (2016).
66. Mora, J. S. *et al.* Masitinib as an add-on therapy to riluzole in patients with amyotrophic lateral sclerosis: a randomized clinical trial. *Amyotroph. Lateral Scler. Front. Degener.* **21**, 5–14 (2020).
67. Riancho, J., Gil-Bea, F. J., Santurtun, A. & López de Munaín, A. Amyotrophic lateral sclerosis: a complex syndrome that needs an integrated research approach. *Neural Regen. Res.* **14**, 193–196 (2019).
68. Belbasis, L., Bellou, V. & Evangelou, E. Environmental Risk Factors and Amyotrophic Lateral Sclerosis: An Umbrella Review and Critical Assessment of Current Evidence from Systematic Reviews and Meta-Analyses of Observational Studies. *Neuroepidemiology* **46**, 96–105 (2016).
69. Rosen, D. R. *et al.* Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62 (1993).
70. Su, X. W., Broach, J. R., Connor, J. R., Gerhard, G. S. & Simmons, Z. Genetic heterogeneity of amyotrophic lateral sclerosis: Implications for clinical practice and research. *Muscle Nerve* **49**, 786–803 (2014).
71. Saberi, S., Stauffer, J. E., Schulte, D. J. & Ravits, J. Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. *Neurol. Clin.* **33**, 855–876 (2015).
72. Al-Chalabi, A. & Lewis, C. M. Modelling the Effects of Penetrance and Family Size on Rates of Sporadic and Familial Disease. *Hum. Hered.* **71**, 281–288 (2011).
73. Zou, Z.-Y. *et al.* Genetic epidemiology of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* **88**, 540–549 (2017).

74. Zarei, S. *et al.* A comprehensive review of amyotrophic lateral sclerosis. *Surg. Neurol. Int.* **6**, 171 (2015).
75. Benatar, M. *et al.* ALS biomarkers for therapy development: State of the field and future directions. *Muscle Nerve* **53**, 169–182 (2016).
76. Kraemer, M., Buerger, M. & Berlit, P. Diagnostic problems and delay of diagnosis in amyotrophic lateral sclerosis. *Clin. Neurol. Neurosurg.* **112**, 103–105 (2010).
77. Baker, M. 1,500 scientists lift the lid on reproducibility. *Nat. News* **533**, 452 (2016).
78. Scott, S. *et al.* Design, power, and interpretation of studies in the standard murine model of ALS. *Amyotroph. Lateral Scler.* **9**, 4–15 (2008).
79. Gordon, P. H. & Meininger, V. How can we improve clinical trials in amyotrophic lateral sclerosis? *Nat. Rev. Neurol.* **7**, 650–654 (2011).
80. Perrin, S. Preclinical research: Make mouse studies work. *Nat. News* **507**, 423 (2014).
81. Mitsumoto, H., Brooks, B. R. & Silani, V. Clinical trials in amyotrophic lateral sclerosis: why so many negative trials and how can trials be improved? *Lancet Neurol.* **13**, 1127–1138 (2014).
82. Fang, F. C. & Casadevall, A. Competitive Science: Is Competition Ruining Science? *Infect. Immun.* **83**, 1229–1233 (2015).
83. Stephan, P. E. The Economics of Science. *J. Econ. Lit.* **34**, 1199–1235 (1996).
84. Ioannidis, J. P. A., Klavans, R. & Boyack, K. W. Thousands of scientists publish a paper every five days. *Nature* **561**, 167–169 (2018).
85. Wang, J., Veugelers, R. & Stephan, P. Bias against novelty in science: A cautionary tale for users of bibliometric indicators. *Res. Policy* **46**, 1416–1436 (2017).
86. Győrffy, B., Herman, P. & Szabó, I. Research funding: past performance is a stronger predictor of future scientific output than reviewer scores. *J. Informetr.* **14**, 101050 (2020).
87. Boudreau, K. J., Guinan, E. C., Lakhani, K. R. & Riedl, C. Looking Across and Looking Beyond the Knowledge Frontier: Intellectual Distance, Novelty, and Resource Allocation in Science. *Manag. Sci.* **62**, 2765–2783 (2016).
88. Scannell, J. W. & Bosley, J. When Quality Beats Quantity: Decision Theory, Drug Discovery, and the Reproducibility Crisis. *PLOS ONE* **11**, e0147215 (2016).
89. Haufe, C. Why do funding agencies favor hypothesis testing? *Stud. Hist. Philos. Sci. Part A* **44**, 363–374 (2013).
90. Klimova, B., Maresova, P. & Kuca, K. Non-Pharmacological Approaches to the Prevention and Treatment of Alzheimer's Disease with Respect to the Rising Treatment Costs. *Curr. Alzheimer Res.* **13**, 1249–1258 (2016).
91. Livingston, G. *et al.* Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *The Lancet* **396**, 413–446 (2020).
92. Project MinE | Project MinE. https://www.projectmine.com/.
93. Britt, R. 2019 NIH Director's awards for High-Risk, High-Reward Research Program Announced. *National Institutes of Health (NIH)* https://www.nih.gov/news-events/news-releases/2019-nih-directors-awards-high-risk-high-reward-research-program-announced (2019).
94. Fang, F. C. & Casadevall, A. Research Funding: the Case for a Modified Lottery. *mBio* **7**, e00422-16, /mbio/7/2/e00422-16.atom (2016).
95. DORA – San Francisco Declaration on Research Assessment (DORA). https://sfdora.org/.
96. VSNU, NFU, KNAW, NWO & ZonMw. Ruimte voor ieders talent: naar een nieuwe balans in het erkennen en waarderen van wetenschappers. (2019).