Basic Epidemiology of ALS

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Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig’s disease, is a neurodegenerative disease. Motor neurons, which are responsible for controlling voluntary muscle movement, are affected and lead to the eventual weakening, atrophy, and paralysis of the muscles (What is ALS?). ALS affects about 18,000 people in the United States at any given time. The annual prevalence is about 5 out of every 100,000 people, and the average duration of this disease is three years before death (ALS (Lou Gehrig’s disease)). This disease is more common in males than in females, and the age of onset varies across individuals, ranging anywhere from 40 to 70 years old for the initial onset (ALS (Lou Gehrig’s disease)). Almost all, 90%, of cases of ALS occur without a known family history, highlighting that there are other risk factors besides genetics at play. There are still 10% of ALS cases that come from a mutated gene related to familial ALS (Who gets ALS?). Older research has demonstrated this may be related to the CRMP5 gene that is associated with ALS (Fauci, 2022). Additionally, mutations in the SOD1 gene and TARDBP on chromosome 1 are implicated (Andersen et al., 2002; Oyston et al., 2021).

ALS eventually causes loss of muscle control in those who have it. It usually starts with twitching or noticeable weakness in limbs. It may also impair the individual’s ability to speak, eat, and breathe (Amyotrophic lateral sclerosis (ALS) 2022). Symptoms that accompany ALS include difficulty walking, falling, weakness in legs or hands, slurred speech, trouble swallowing, as well as various cognitive and behavioral changes (Amyotrophic lateral sclerosis (ALS) 2022). In the early stages of the disease, symptoms tend to start in the hands, feet, or limbs and eventually progress to other parts of the body. This is because when the disease progresses, it increasingly deteriorates the motor neurons, leading to more muscles getting weaker. However, the beginning stages are not typically associated with any pain, and pain is not a frequent symptom of ALS (Amyotrophic lateral sclerosis (ALS) 2022). Some of the cognitive or behavioral symptoms are related to issues with language, decision-making, and the possibility of developing dementia over time (Amyotrophic lateral sclerosis (ALS)).

There are many additional risk factors that can contribute to the development and progression of ALS. These risks stem from a wide range of different factors, including lifestyle choices, environment or work, and other medical conditions. Lifestyle choices include smoking, physical health, and dietary factors. Smoking has been identified as a potential risk factor, but results are a bit conflicting regarding how influential this may be on the progression or onset of ALS. Some research has identified a causal link (Peters et al., 2019), while others have found no evidence for a causal relationship (Opie-Martin et al., 2020). Dietary factors can also play a protective role; a correlation link has been found between increased antioxidant intake and reduced risk of ALS (Caplilure-Llopis et al., 2019). Other genome-wide association studies have identified a link between ALS and having a higher body mass index, higher body fat percentage, and type 2 diabetes (Li et al., 2021). Environmental risk factors affect those who are exposed to higher levels of different chemicals and pesticides, such as military workers, carpenters, and construction workers, which may be linked with an increased risk of developing ALS (Beard & Kamel, 2014; Fang et al., 2009; Roos, 2017). Other medical conditions, such as entorhviral viruses, head trauma, cancer, and neuroinflammation, have been linked to ALS (Farace et al., 2020; Gu et al., 2021; Xue et al., 2018).

There is currently not a single method for definitively diagnosing ALS but instead requires a healthcare provider to conduct a physical and neurological exam to determine a diagnosis (Amyotrophic lateral sclerosis (ALS)). Some forms of imaging include electromyography, a nerve conduction study, magnetic resonance imaging, blood and urine tests, or muscle biopsies (Amyotrophic lateral sclerosis (ALS)). While there currently is no cure for ALS either, there are some treatments to help slow the progression of symptoms. There are currently three medications that have been approved by the FDA for treating ALS. These include Riluzole, which can improve life expectancy by 3 to 6 months but can cause functional changes in the liver (Amyotrophic lateral sclerosis (ALS) 2022). Edaravone is an intravenous medication or can be taken orally to help reduce the progression of symptoms, but it can also come with side effects such as headaches and shortness of breath (Amyotrophic lateral sclerosis (ALS) 2022). The third medication is Sodium phenylbutyrate and taurursodiol, which can slow the rate of decline, specifically when performing daily tasks (Amyotrophic lateral sclerosis (ALS) 2022).

What is known about ALS
Neuroinflammation, glia, and motor neurons

ALS has been heavily researched over the past several decades in an attempt to learn more about the mechanisms underpinning the disease. Many biological processes implicated in ALS are related to neuroinflammation, or inflammation of the brain and nervous system (Ransohoff, 2016). This is linked to neurodegeneration, which is a gradual loss of neurons and the impairment of their normal functioning (Ransohoff, 2016). Neuroinflammation is thought to trigger a cascade of various immune responses that contribute to the subsequent neurodegeneration of neurons in the brain. These immune responses include the activation of immune cells, such as microglia, as well as the release of cytokines and chemokines, both of which can cause the death of neurons (Ransohoff, 2016). Microglia are the immune cells of the brain, and they can release pro-inflammatory cytokines and free radicals when overactivated, ultimately leading to damage and death of neurons (Ransohoff, 2016). One of these pro-inflammatory cytokines commonly produced during neuroinflammation is TNF-α. Mishra et al. used a rat model of ALS where they exposed rat astrogliosis to the cerebrospinal fluid from ALS patients. They found that this exposure increased the production and release of TNF-α, interleukin 6, cyclooxygenase-2, and prostaglandin E2, all inflammatory cytokines. This exposure also downregulated cytokine IL-10 and other anti-inflammatory trophic factors. Overall, it is evident that microglial cells play a key role in the pathogenesis of ALS (Mishra et al., 2017).

Neuroinflammation has been seen in the motor regions of the central nervous system in both familial and sporadic ALS patients (Liu & Wang, 2017). Additionally, alterations and dysfunctions in the astrocytes and microglia have been found in human and animal models of ALS (Obrador et al., 2021). In healthy patients, astrocytes provide support and regulate the homeostasis of neurons. However, in those with ALS, they have the potential to harm motor neurons. This is because, in ALS pathology, the astrocytes become enlarged and tend to abnormally proliferate towards reactive astrogliosis surrounding the degenerating motor neurons (Obrador et al., 2021). In mouse models that express a SOD1 (G93A) mutation, studies have shown that there is a spread of motor dysfunction that aligns with the proliferating astrocytes surrounding damaged motor neurons (Jiménez-Riani et al., 2017). This ends up acting like a scar around the degenerated motor neurons and produces inhibitory molecules that block the growth of damaged axons, keeping them short (Obrador et al., 2021).

In the central nervous system, microglia are the primary form of immune defense against any type of toxicity, injury, or infection (Filiano et al., 2015). When these get activated, they enlarge, migrate, and help remove toxic matter in the CNS through phagocytosis. However, they also can secrete pro-inflammatory cytokines. So, if they are unable to eliminate toxicity in the CNs, they remain active and continue to recruit astrocytes to the area of inflammation, resulting in an ongoing inflammatory process in the CNS (Dahike et al., 2015; Ransohoff, 2016). Dahike et al. examined the mechanisms underlying this inflammatory process using a wobbler mouse model of ALS. This is created through a ‘wobbler’ mutation of leucine-967 to glutamine, which destabilizes the Vps54 protein and GARP complex. This then leads to an impairment of vesicle trafficking and endosomes in the motor neurons (Dahike et al., 2015). Using this model, they were able to see that there was an abnormal density of Iba-1-positive microglial cells that express TNF-α and GFAP (glial fibrillary acidic protein). The motor neurons that they looked at had activation of caspase 3, which was indicative of ongoing neurodegeneration. This is what may have caused the motor dysfunction and eventual paralysis of their wobbler mouse model (Dahike et al., 2015). Additionally, they identified various forms of cellular degener-
Oxidative Stress

Another area of research into ALS has been oxidative stress, which is caused by the increased generation of reactive oxygen species (ROS) or a decreased level of antioxidants (Sies, 2015). Oxidative stress can have serious implications on the body as it has the ability to damage cells (Sies, 2015). While they might not be the cause of neurodegeneration, they have the potential to exacerbate the progression of the disease (Liu & Wang, 2017).

Glial cells are major producers of ROS and reactive nitrogen species (RNS) in the central nervous system and contribute to the pathogenesis of diseases that affect motor neurons (D’Ambrosi et al., 2018). One of the major sources of ROS in the body is the mitochondria (D’Ambrosi et al., 2018). When mitochondria increase their production of ROS, this leads to irreversible damage of mitochondrial DNA, membrane lipids, and proteins, eventually causing motor neuron death (D’Ambrosi et al., 2018). Based on the current literature, mitochondrial alteration in ALS causes oxidative stress, playing a central role in the pathology of the disease. Oxidative stress generates motor neurons and leads to their death. Literature has shown that ALS is caused by many different molecular pathways in motor neurons. It is their interaction with surrounding glial cells that causes a cascade of downstream events, ultimately damaging the motor neurons (Smith et al., 2019).

Mitochondrial dysfunction

As previously mentioned, mitochondria are heavily implicated in ALS pathology as well, as they have many functions that help increase motor neuron survival (Obrador et al., 2021). One study looked at the structure and function of mitochondria and found that alterations in these are key in many different neurodegenerative diseases, including ALS (Calio et al., 2020). Some of these alterations in ALS include abnormal distribution of mitochondria, swollen mitochondria, a decrease in the DNA of mitochondria, or a decrease in the activity of the electron transport chain (Obrador et al., 2021). A study conducted by Mehta et al. used motor neurons derived from the iPSCs of patients expressing the C9orf72 repeat expansion associated with ALS. They looked at these motor neurons and identified issues with the mitochondria, including decreased mitochondrial membrane potential, issues with oxygen consumption, a decrease in the levels of ATP produced by mitochondria, as well as increased fragmented and decreased mitochondrial transport (Mehta et al., 2021). Issues with mitochondria also can affect the axons of motor neurons, as they play a role in regulating axonal homeostasis. The researchers found that these motor neurons have shorter axons, which may ultimately impair the axon’s ability to transport and lead to the accumulation of debris on the axons (Mehta et al., 2021). Recently, more in vivo research has been conducted. Sassani et al. used P-magnetic resonance spectroscopy in patients with ALS and showed that there was mitochondrial dysfunction present in the brains of those with ALS (Sassani et al., 2020).

RNA misprocessing

RNA misprocessing, specifically both the misfolding of proteins and the accumulation of them, is a major component of many neurodegenerative diseases. TDP-43, an RNA-binding protein, is implicated in the ubiquitin-positive neuronal inclusions in ALS. It helps to regulate the metabolism of target RNA and is responsible for mRNA stability and transport (Mathis et al., 2017). In most cases of ALS, there are TDP-43 mutations and misprocessing of RNA that lead to the development of ALS by disrupting the normal function of TDP-43 (Mathis et al., 2017). Another study reported that 97% of cases of ALS are associated with TDP-43 proteinopathies and with issues related to clearing TDP-43 from the nucleus (Giannini et al., 2020). This study used HeLa cells that have depleted levels of TDP-43, and they found that it increases R-loops and genome instability (Giannini et al., 2020). They also found that mutations and mislocalizations of TDP-43 from a lymphoblastoid cell line from an ALS patient cause the accumulation of R-loops, leading to the damage of DNA in ALS (Giannini et al., 2020).
**Protein Aggregation**

Related, abnormal protein aggregation is seen across many neurodegenerative diseases (Tanikawa et al., 2018). ALS is characterized by ubiquitinated proteins that aggregate in motor neurons, including FUS, TDP-43, OPTN, and UBQLN2, and are related to the C9orf72 gene (Chisholm et al., 2021). Recent research has also found the novel role of PAD4-mediated citrullination in protein aggregation in ALS. Tanikawa et al. found that many of the PAD4 substrates were highly expressed in the brain tissue and that they inhibited the aggregation of FET proteins, which are commonly found in neurodegenerative diseases. They also looked at FUS protein levels and saw that these increased in a PAD4 knockout mouse model, highlighting that PAD4-mediated citrullination plays a role in ALS pathogenesis (Tanikawa et al., 2018).

Overall, research has come a long way since the start of ALS research. There have been many technological advancements that bettered our understanding of the disease and the mechanisms underpinning it. Researchers have been able to identify the genetic roots of the disease and learned about the roles that mitochondrial dysfunction, oxidative stress, and neuroinflammation play, as well as the effects of RNA misprocessing and protein aggregation. Despite these findings, however, there is still a lot we do not understand about the cellular mechanisms of these processes.

**Current treatments for ALS**

**Functional Analysis**

Functional analysis refers to the analysis of a behavior to identify what sustains that behavior related to a disease (Apa Dictionary of Psychology). In the current context, ALS functional analysis broadly looks at the function of muscles and motor neurons. There are several different areas that are looked at in connection to ALS, including electromyography, muscle strength testing, pulmonary function, and cognitive and speech tests.

Electromyography is a test that looks at the electrical activity of muscles, and because motor neurons and muscles are affected by the degeneration of motor neurons in ALS, it is used to monitor symptoms (Bashford et al., 2020). Bashford et al. conducted a systematic review of the use of EMG in ALS. It was originally and continually used because of its non-invasive and practical manner (Bashford et al., 2020). Practitioners opt to use EMGs when trying to collect patient data over extended periods of time, being able to re-test the same muscle groups repeatedly (Bashford et al., 2020). These can be used to test muscle function either weeks, months, or years in the future to identify any changes that may have been caused by neuronal death. Specifically, they can measure the electrical activity of various limbs, motor nerves, and sensory nerves (Bashford et al., 2020). There are also subtypes of EMGs that can be used without electrical stimulation, such as motor unit decomposition. This has great potential to be used for at-home data collection because, without electrical stimulation, you would not need a clinician or technician to run the tests, ultimately providing us with a much larger data set regarding muscle function in ALS patients. There is also MUNIX, which is used in clinical trials and can help model biomarker development and eventually can be used to monitor symptoms (Bashford et al., 2020).

Another functional test used is to measure muscle strength. This involves measuring the strength of muscle groups through different kinds of tests, again to help monitor the progression of the disease (Shefner, 2016). This type of testing is not specific to ALS; rather, it can be used in many different scenarios across many different diseases that affect motor neurons and subsequent motor function (Shefner, 2016). Muscle strength testing can be incorporated into different studies or clinical trials that look at the use of treatment options and their effects on muscle function. ALS causes motor dysfunction, so having a reliable way to test changes in motor function, whether that be to slow down or recover dysfunctions, can be helpful (Shefner, 2016).

Pulmonary function testing is another common type of functional testing. ALS affects the muscles, including those that we use for breathing. Because of this, pulmonary function testing can be used to test and monitor lung function (Lechtzin et al., 2018). Respiratory pulmonary function tests (PFTs) can be used to make decisions based on respiratory function, such as whether or not to place someone on ventilation. This technique is not as commonly used or studied, so the contexts in which it may be helpful are not well understood (Lechtzin et al., 2018). However, this does hold the potential to be used in clinical trials, like muscle strength, to test how a drug treatment affects pulmonary function.

Other types of functional tests include cognitive testing to look at memory or attention, as ALS affects the normal cognitive functioning in some ALS patients (Chio et al., 2019). Additionally, speech or swallowing assessments may also be used, as ALS can affect the muscles used for these as well (Hirakoa et al., 2017). Overall, in terms of being able to better diagnose and monitor symptoms and disease progression in ALS, functional analysis may be used. It may be more beneficial if used to assess treatment outcomes but can also be used in tandem with other forms of supportive care.

**Cellular Based Treatments**

There are currently several different ALS treatments circulating, each based on different cellular aspects of the disease. It is important to note that while many of these studies are emerging both in experimental studies and clinical trials, there is a lot more work that needs to be done in this area. The first FDA-approved drug is Riluzole. It reduces the release of glutamate, which is thought to play a role in motor neuron degeneration and death. It is the most widely used treatment. However, it was approved by the FDA before ALS mouse models were created, so it hasn’t been as well studied as other drugs being approved now (Hogg et al., 2017). There have been mixed findings regarding the efficacy and safety of this drug. Sala et al. conducted a study using the SH SY5Y cell line, which over-expressed G93A SOD1 mutations, creating an ALS model. They had exposed these cells to Riluzole for 24 hours. What they found was that this exposure eventually led to a decrease in cell death and a decrease in ROS levels. As we know, oxidative stress plays a key role in contributing to motor neuron death in ALS patients, so reducing the ROS levels may help restore the antioxidant/ROS balance. They repeated the experiment with a cellular model of familial ALS, but this drug was ineffective. Together, these results highlight that this drug may be effective in preventing neuronal degeneration seen in ALS (Sala et al., 2019). Another study looked at Riluzole in three separate mouse models of ALS. They created these three models with the SOD1G93A mutation, the TDP-43A315T mutation, and the FUS (1-359) mutation. All three of these received riluzole treatment, either through their drinking water or a vehicle, after symptoms were already present. What they found was that it had no significant benefits for the mice. It did not improve their performances on rotarod tests or their stride length, two measures of motor function, and it did not extend the lifespan of the mice either. This highlights that the efficacy of this drug may be called into question, and more clinical trials should be conducted (Hogg et al., 2017).

Another FDA-approved drug is Edaravone, which has shown some promising benefits for ALS patients. It is a member of the substituted 2-pyrrozolin-5-one class and can be provided through injection (Cruz, 2018). The mechanism of action is still unknown; however, it does have antioxidant properties. As previously mentioned, oxidative stress contributes heavily to the pathophysiology of ALS, so restoring the balance between antioxidants and ROS levels in the body may help to reduce the adverse effects of oxidative stress (Cruz, 2018). It has been used in many clinical trials, which have shown that it helped prevent the degeneration of motor function seen in ALS, more so compared to control groups (Cruz, 2018). It’s important to note that this came with some severe side effects. How it affects patients, such as renal impairment, end-stage renal disease, and hepatic impairment (Cruz, 2018). Thus, we need to conduct further studies to assess the efficacy and safety of using this drug in ALS patients.

Other treatment options use stem cells in ALS patients. Stem cell therapy involves taking healthy stem cells and transplanting them into a patient to try and act as a regenerative therapy (Answers to your questions about Stem Cell Research 2022). One type of stem cell used in ALS is mesenchymal stem cells (MSCs). They are known to regulate immune cells, specifically regulatory T cells (Treg), which are implicated in ALS.
Researchers believe that these have neuroprotective effects and may help prevent motor neuron death and dysfunction (Kim et al., 2018). This study looked at a single cycle of repeated injections of BM-MSCs to assess its clinical efficacy in ALS patients. They found that there were positive effects for up to 6 months, without any adverse side effects, and it helped to change the cerebrospinal fluid environment from pro to anti-inflammatory (Kim et al., 2018). Future research is required, but this acts as a good starting point for stem cell therapy in ALS.

Gene therapy is also being used as a treatment option for those with ALS. This is a technique that targets specific gene mutations in a disease that may be causing dysfunction in proteins and includes either turning off specific genes, replacing them, or modifying mutated genes (Gene therapy). Most utilized for gene therapy in ALS are mutations in the SOD1 gene, as they are the most widely studied. Biferi et al. examined Adeno-associated virus serotype rh10 vectors (AAV10). This specific therapy was used to target exon skipping of hSOD1 mRNA through the expression of specific targeting exon-2 antisense sequencing in nuclear RNA. What they found was that injecting this into SOD1 G93A mice, both at birth and at 50 days of age, had effective effects on the mouse model of ALS. It acted protectively against issues with motor function and weight loss (Biferi et al., 2017).

Neurotrophic factors are also used in different treatment options for ALS, as they are proteins used to support neurons (Thomsen et al., 2017). A study conducted by Thomsen et al. looked at glial cell line-derived neurotrophic factor (GDNF). It’s been used previously in research on many different neurodegenerative diseases, such as Parkinson’s disease and Huntington’s disease, as well as in cell and animal models of ALS. This study, in particular, used tail vein injections of AAV9-GDNF into young adult rats, and their mouse model was the SOD1 G93A. They conducted grip strength tests and assessed the effects on forelimb paralysis, which is seen in mice models of ALS. They ultimately found that these injections improved overall motor function and grip strength and delayed the onset of forelimb paralysis. However, it had no effects on the length of survival compared to control groups and also resulted in other complications related to working memory and overall activity levels (Thomsen et al., 2017). This demonstrates that while this may be a novel treatment option, further research still needs to be done in order to figure out whether it is safe to use in clinical trials.

We can see that there is a wide range of treatment options available, either in research or clinically, for those with ALS. There is no cure currently, and many of these treatments are understudied. Further research needs to be done in order to determine whether these treatments are safe clinically and if they really provide benefits that outweigh the risks associated with them. It’s also important to note that each individual may respond differently to treatment, so personalized medicine may also be of interest when working with patients with ALS.

References


