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Amyotrophic Lateral Sclerosis (ALS): Familial ALS, Genetic Mutation, and Possible Gene Therapy Cure

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ABSTRACT

In our world today, many neurodegenerative diseases are being studied and thoroughly evaluated. Scientists attempt to understand these diseases to a greater extent every day, researching possible causes and cures. In particular, a disease called Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that still elicits uncertainty, with many underlying causes and no current cure. Those with ALS experience motor neuron degeneration, the root source of ALS. This degeneration of motor neurons leads to paralysis, or the inability to move due to a loss of muscle function by impaired neural messaging. In a specific branch of ALS, familial ALS (fALS), motor neuron degeneration is caused by specific genetic mutations in an individual that were passed on by hereditary nature. This research identifies four genes that hold the genetic mutations responsible for fALS: SOD1, TARDBP, FUS, and C9orf72. Of these many genes, this research identified their corresponding mutational contributions to motor neuron degeneration like oxidative stress, protein aggregation, and other causes. Along with the analysis of the problematic genetic mutations, this study sheds light on next generation sequencing (NGS) as a way to efficiently scan for genetic mutations, as well as CRISPR-Cas9 gene therapy as a possible cure for fALS in the future of neurodegenerative study. Overall, this research aims to narrow in on fALS and demonstrates a comprehensive analysis of the specific gene mutations that take place, the significance of each in the overall progression of ALS and motor neuron degeneration, and future possibilities for next-generation sequencing and gene therapy.

Introduction

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that heavily affects brain and spinal cord nerve cells. The disease brings about motor neuron degeneration, reducing muscle functionality and movement. Motor neurons, a category of nerve cells, are the medium with which the brain can send signals to conduct motor activities such as walking or picking up an object. Fundamentally, the degeneration of motor neurons inhibits the brain from sending signals to the specified muscles, causing the muscles to atrophy, or essentially waste away due to their inability to contract. In its initial stages, ALS triggers slight, rapid twitches and cramps, still allowing for daily activities to be carried through. Over time, however, the progression of ALS in an individual typically reaches the stage of complete paralysis, inevitably restricting respiratory movement. Two different branches of ALS exist: familial ALS (fALS) and sporadic ALS (sALS). Familial ALS is genetically inherited from family members through specific genes passed on by DNA, while sporadic ALS can be attributed to various factors such as environment and lifestyle habits. In fALS, the genes passed along one's family tree are mutated, meaning that they do not function the way a normal gene would. Instead, these mutated genes lead to a plethora of problems, one being incorrect protein synthesis–a fatal issue for proper cell function. With this in mind, it is crucial to gain a deeper understanding of the mechanisms of each mutation, and how each plays a role in the progression of fALS through motor neuron damage.

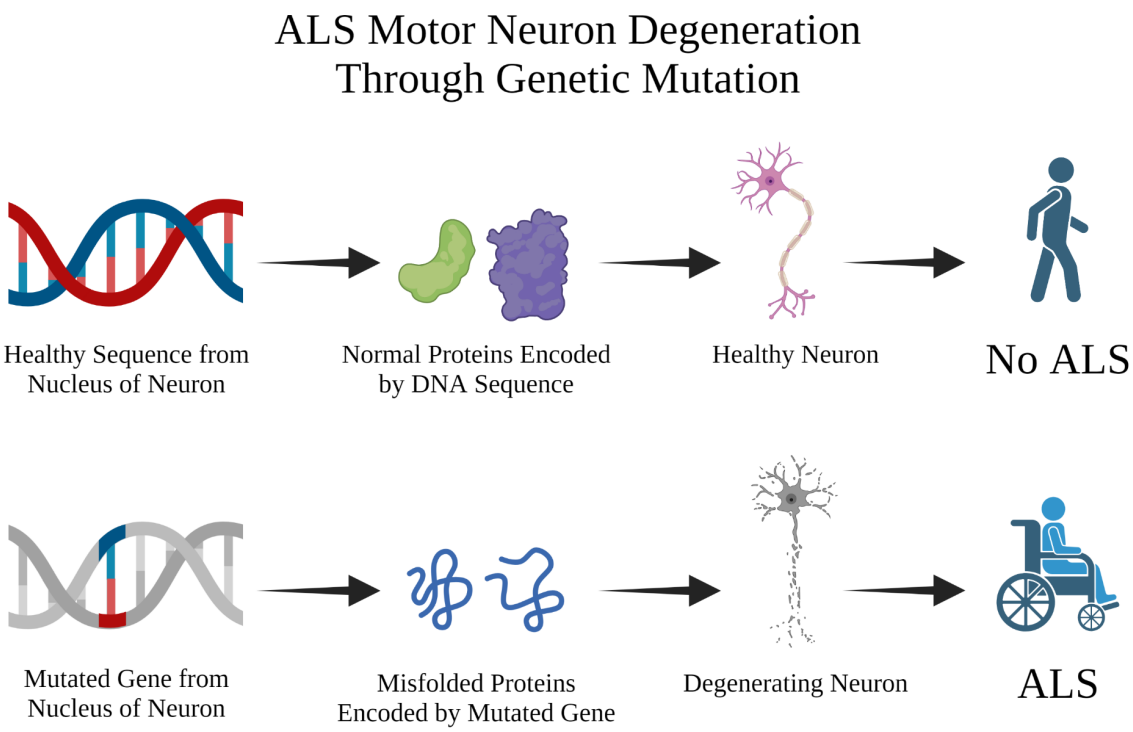
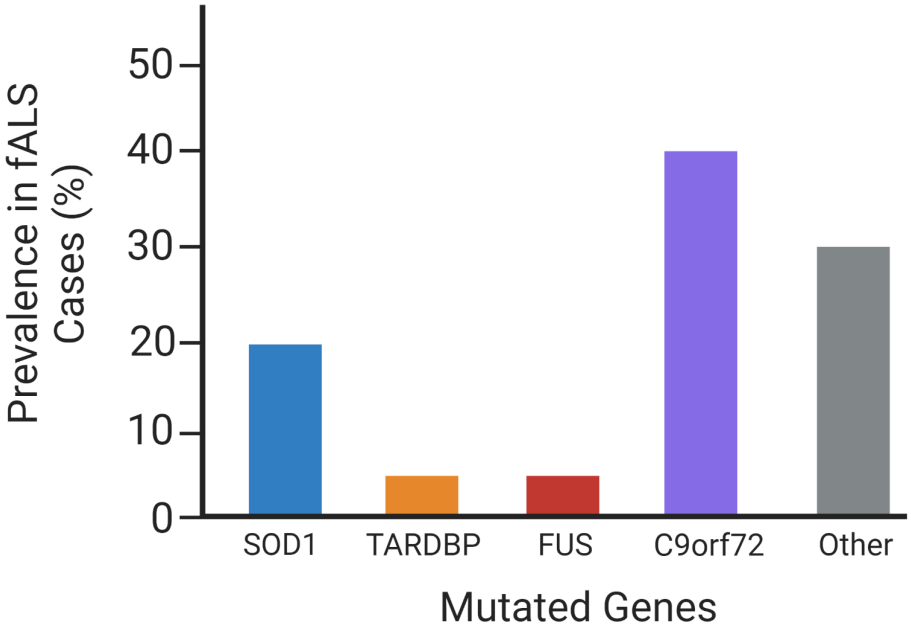


Figure 1. Schematic Representation of mutation leading to ALS-affiliated motor neuron degeneration. Created and Copyrighted by Soham Deshpande

Overview of Central Dogma

In order to understand how genetic sequences lead to protein misfolding and eventually cell death, it is crucial to evaluate the cellular mechanisms behind this process, otherwise known as the central dogma. Simply put, the DNA in the cell nucleus is copied into a one-stranded molecule called messenger RNA (mRNA) by several enzymes, a process known as transcription. This process is done in a complementary manner to the DNA's nucleotide bases (adenine (A), guanine (G), cytosine (C), and thymine (T)). The mRNA then exits the nucleus and resides in a ribosome in the cytoplasm of a cell to allow for protein synthesis, or translation. Through translation, the nucleotides on the mRNA strand (A, G, C, and Uracil (U)) are sequentially processed by transfer RNA (tRNA) molecules in a complementary fashion. The tRNA molecules scan the mRNA strand in groups of three nucleotides known as a codon. The tRNA molecules carry anticodons (three-letter codes) that match up correspondingly with the three-base codons on the mRNA strand to recognize the exact sequence of the amino acids that need to be linked together to create a cohesive, functional protein. As a result, it can be observed that even a minor mutation on a genetic strand can have a detrimental effect on the proper synthesis of proteins, which are necessary elements for vital cellular functions. Specific to ALS, many genes are found to be possible locales of mutation. Of those, four well-known genes are significant in their contribution to this neurodegenerative dilemma: SOD1, TARDBP, FUS, and C9orf72. By examining the role each gene and its mutation exhibits, we will be able to gain a broader understanding of the implications and mechanisms of ALS, and even assess possible cures relating to CRISPR-Cas9 gene therapy in the near future.



Graph 1. Prevalence of each mutation in fALS cases, SOD1 (~20%), TARDBP (~5%), C9orf72 (~40%), Various other mutations (~35%). Created and Copyrighted by Soham Deshpande

SOD1

The SOD1 gene, known as Superoxide Dismutase 1, is a gene that "encodes for the detoxifying copper/zinc binding SOD1 enzyme" (Huai & Zhang., 2019). In focus, this enzyme is necessary for breaking down harmful superoxide radicals, which are essentially highly reactive oxygen molecules that contain an additional electron. Superoxide radicals are part of a group named ROS (Reactive Oxygen Species). ROS molecules originate from and are byproducts of intracellular processes like cellular respiration by the mitochondria (the organelle in a cell responsible for energy production). As the mitochondrial electron transport chain (ETC) works to produce ATP energy for the cell to utilize, it occasionally releases radical molecules derived from oxygen, known as superoxide radicals. The SOD1 enzyme works to catalyze these highly reactive oxygen particles into more usable and less dangerous particles for the cell, "converting superoxide to molecular oxygen and hydrogen peroxide" (Bunton-Stasyshyn et al., 2014). Superoxide radicals are oxidant particles, meaning they possess the ability to react and oxidize with other substances, and in this case, other cellular molecules like lipids and proteins. Since metals like copper and zinc are imperative parts of the active site of the SOD1 enzyme, superoxide molecules react with the metallic active site of the enzyme to initiate an important chemical reaction, the products of which are oxygen and hydrogen peroxide (Buettner, 2011). It can be observed that the SOD1 enzyme plays a crucial role in maintaining a healthy, functional cell by targeting radical oxidative molecules that could deal a great amount of damage to a cell's essential properties.

SOD1 Mutations

With ALS, however, the gene encoding the enzyme, SOD1, is mutated, greatly impacting the extent to which cells are protected from ROS. In humans, the SOD1 gene is located on chromosome 21. Often, the mutation

that occurs at a SOD1 gene is called a missense mutation. Missense mutations occur when one amino acid is replaced with another in the formation of the SOD1 protein (enzyme) due to a changed nucleotide base, resulting in a loss of function of the enzyme to execute its allotted task. Most frequently, the mutations found on the SOD1 gene are the D90A, G93A, and A4V mutations. The D90A mutation occurs at position 272 on the SOD1 gene and is a recessive mutation. As for its mechanisms, the D90A mutation exhibits a change at codon 90, where the amino acid of aspartic acid is swapped to alanine due to a nucleotide base change of A to C (Pansarasa et al., 2018). This phenomenon demonstrates a missense mutation, as there is a change in amino acids during the process of transcription. Likewise, the other mutations, G93A and A4V, exhibit different amino acid substitutions. A4V, the most common mutation causing ALS in the United States, exhibits a substitution from alanine to valine at codon 4, and the G93A mutation changes glycine 93 to alanine at codon 93 (Pansarasa et al., 2018). As a result of these dramatic amino acid sequence alterations, the synthesis of the SOD1 enzyme is flawed, causing a misfolded shape of the resulting enzyme. When a protein is stripped away of its 3D configuration, it loses its ability to perform its assigned responsibility in a cell's processes. In this case, since the SOD1 enzyme is irregularly synthesized and sequenced, it becomes misfolded and loses its enzymatic function of breaking down the excess ROS. The excess ROS in a cell thereby harms its surrounding cellular properties, leading to what is known as oxidative stress.

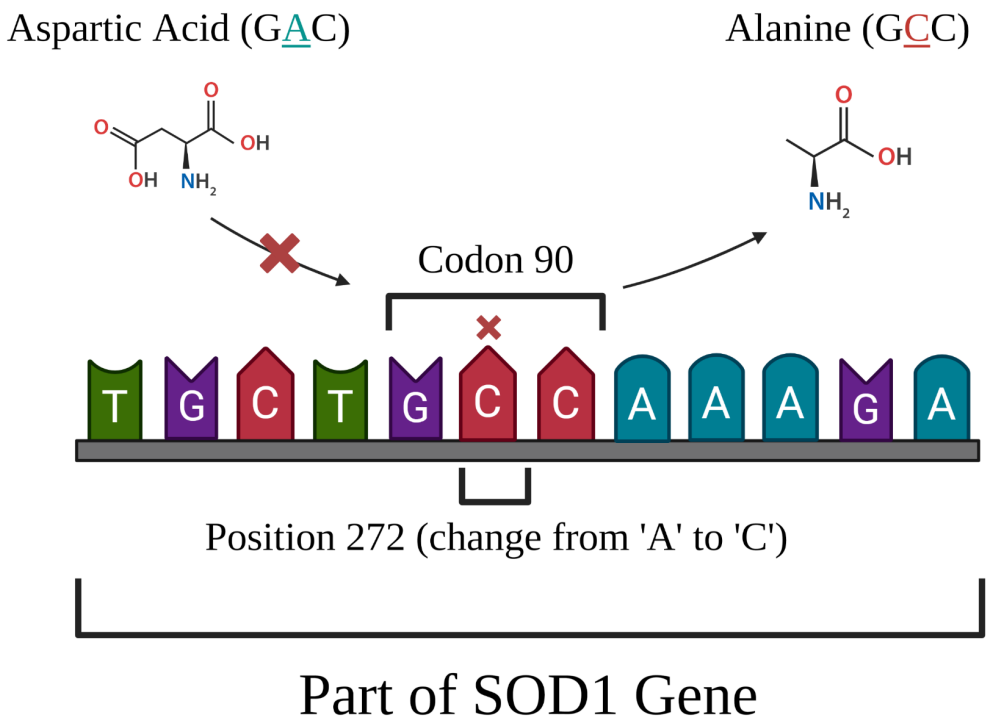


Figure 2. D90A Mutation, Transformation of amino acids from native genes, position 272 mutated to alanine from aspartic acid. Created and Copyrighted by Soham Deshpande

Impact of SOD1 Mutations on Motor Neuron Degeneration

Of the many ramifications of the SOD1 gene mutation, oxidative stress is one of the most prevalent causes of motor neuron degeneration, leading to the onset of ALS. As the influx of ROS continues to increase in a cell, vital macromolecules like lipids, proteins, and even DNA are at a greater risk of damage. In general, oxidative stress produces an imbalance of oxidants and antioxidants, which "occurs as a result of the excess level of

oxygen species (ROS) or improper functioning of the antioxidant system" (Singh et al., 2019). In order to evaluate how ROS actually affect a cell's properties, it is imperative to understand how they are formed. ROS originate from the mitochondrial ETC, which is responsible for oxidative phosphorylation in a cell, or essentially how the mitochondria use oxygen molecules to help convert nutrients to usable ATP energy. In the ETC, electrons flow through complexes, or energy-converting enzymes. During the passage of electrons through these complexes, some electrons may escape and react with oxygen molecules, forming superoxide radicals. These highly reactive radicals are then able to exit the mitochondria and freely roam a cell. Specifically, ROS attack membrane proteins and facilitate the mutilation of the neuronal membrane, which is rich in polyunsaturated fatty acids, making it ideal for the ROS to react with. This is due to lipid peroxidation, a process in which oxidant radicals "attack lipids containing carbon-carbon double bonds, especially polyunsaturated fatty acids" (Ayala et al., 2014). This mutilation and decay of essential macromolecules and membrane molecules leads to necrosis, or cell death via disease and degeneration. As a result, it can be clearly observed that the progression of oxidative stress is likely a cause of mutations in the SOD1 gene, due to its release of dysfunctional SOD1 enzymes. Since the enzymes are unable to catalyze the harmful ROS as a substrate, these ROS are able to expand in numbers within a cell, eventually leading to neuronal necrosis. Eventually, this necrosis then leads to ALS, as action potential signals are not able to be passed through the damaged neurons, inhibiting electrical impulses and kickstarting muscle atrophy.

TARDBP

While SOD1 mutations definitely play a role in the progression of ALS, mutations in another gene also remain suspect of ALS: TARDBP. The TARDBP gene, otherwise known as the transactive response DNA binding protein gene, regulates the encoding and formation of the TDP-43 protein, or the transactive response DNA binding protein 43 kDa. A healthy TDP-43 protein works to regulate protein-protein interactions and transcription, a process in the central dogma as discussed previously. Specifically, this protein binds to DNA and is responsible for processing mRNA. By cutting and splicing various segments of the mRNA strand, this protein holds an essential role in the arrangement of amino acids and the synthesis of other proteins. Furthermore, TDP-43 is a highly localized protein, as it "is predominantly localized to the nucleus, but is also present in the cytoplasm and mitochondria" (Suk & Rousseaux., 2020). This is due to its vitality in DNA transcription in the nucleus. Without this protein's proper oversight of transcription, the transcriptional activity becomes impaired, and the protein diverges from its primary location (mislocalization). TARDBP being the root source of TDP-43 synthesis, it is crucial to recognize its mutation mechanism.

TARDBP Mutations and Pathways

Firstly, the TARDBP gene is located on chromosome 1. The gene contains a glycine-rich domain in which most of the mutations occur. Specifically, these mutations are often found on the C-terminus of the gene, or the end of an amino acid chain terminated by a free carboxyl group. In this glycine-rich domain of the gene, most mutations occur on exon 6, which "encodes ~60% of the TDP-43 protein and more than 70% of the entire mRNA transcript" (Pesiridis et al., 2009). Several mutations are responsible for different causes of TDP-43-induced neurodegeneration like the alteration of phosphorylation states, mislocalization, and aggregation. Of these mutations, one is prevalent in the pathogenesis of fALS: A382T. The A382T mutation is the mutation of amino acid 382, alanine, to threonine. This mutation has been found to "decrease the nuclear localization of [TDP-43], to increase its phosphorylation status, and to favor the formation of C-terminal fragments deriving from proteolytic digestion of the full-length proteins" (Zanini et al., 2022). All in all, the A382T mutation is known to trigger all 3 degenerative processes in a neuron (alteration of phosphorylation, mislocalization, and

aggregation), but several other mutations are identified as specific contributors to TDP-43-induced neurodegeneration. For example, mutations like S393L, S379C, and S379P damage essential phosphorylation sites used to facilitate the natural function of TDP-43, leading to mislocalization and hindered protein signaling. Other mutations like G348C "may be predisposed to ALS by increasing the propensity for TDP-43 to aggregate through disulfide bond formation" (Pesiridis et al., 2009). Overall, numerous mutations in the glycine-rich domain of the TARDBP gene assist in the progression of neuron degeneration, either by altered phosphorylation, mislocalization, or aggregation. However, it is imperative to realize the actual damage being done to neurons as a result of these issues, and how that damage leads to the progression of ALS.

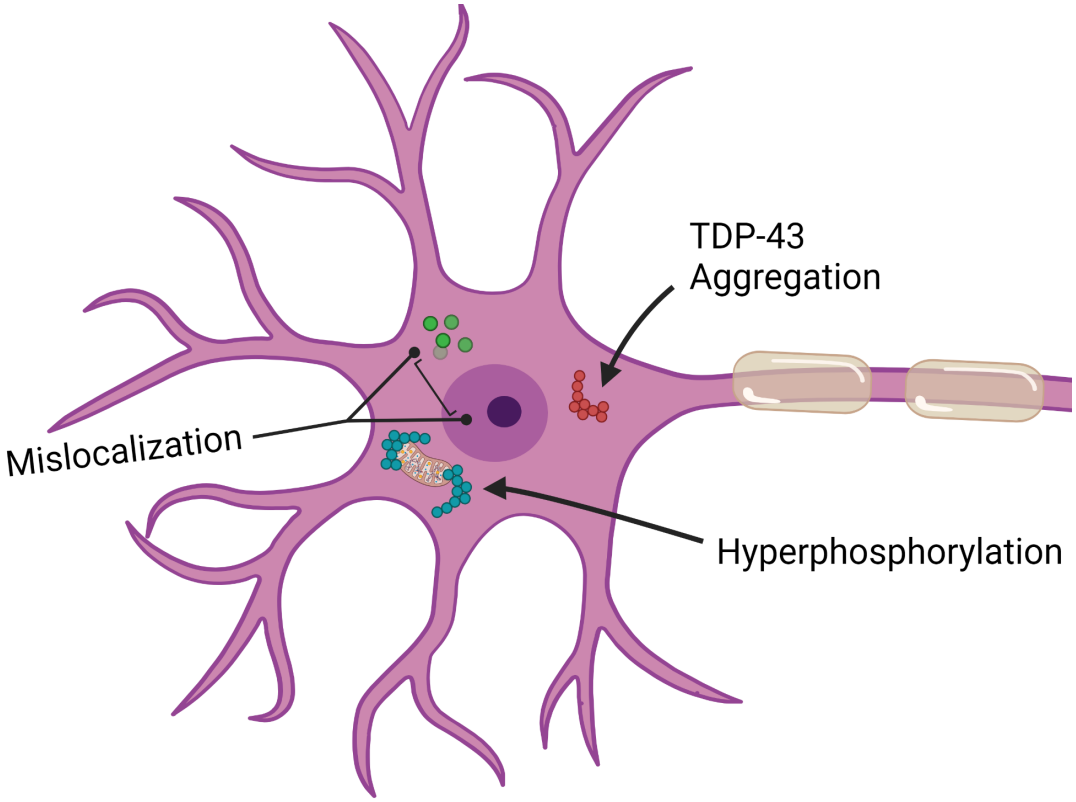


Figure 3. Mislocalization, aggregation, and hyperphosphorylation of TDP-43 in a neuron. Created and Copyrighted by Soham Deshpande

Resulting TDP-43 Neuronal Damage

Although the alteration of phosphorylation sites, mislocalization of TDP-43, and aggregation are all labeled as relatively separate causes of neurodegeneration, oftentimes, altered phosphorylation and mislocalization actually lead to aggregation. Protein aggregation, or the abnormal clumping of misfolded proteins within cells, is found to be one of the primary causes of neuron degeneration in neurodegenerative diseases. In the case of fALS, TDP-43 is misfolded and dysfunctional due to mutations in the TARDBP gene, forming toxic clumps within neurons and disturbing various cellular processes. For example, a mouse model studying the impact of TDP-43 without nuclear localization signal (NLS) sequences (sequences of amino acids that act as signals for a protein to be transported into the cell nucleus) showed the profound impact altered TDP-43 had on biological processes in neurodegenerative diseases. It demonstrated that "hTDP-43ΔNLS expression showed cytoplasmic accumulation of insoluble, phosphorylated TDP-43…[exhibiting] significant neurodegeneration with neuron

loss, muscle denervation, and progressive muscle impairment" (Wood et al., 2021). This indicates that the mis-localization and aggregation of TDP-43 are shown to have a significant influence on the progression of neuro-degenerative diseases like ALS, and it can be said that the pathogenesis of TDP-43 from mutated TARDBP is a key factor in motor neuron degeneration.

Fused in Sarcoma (FUS) and Its Similarity

Fused in sarcoma (FUS) is another gene responsible for mutations leading to fALS. FUS encodes for the FUS protein, and similar to TDP-43, the FUS protein plays an important role in RNA-binding and splicing, and has a propensity for aggregation. On the FUS gene, "the majority of FALS-related mutations are more commonly found in the G-rich region, the 2nd [Arg-Gly-Gly] region, and the NLS" (Shang & Huang., 2016). These mutations lead to the misfolded FUS protein being highly susceptible to aggregation in the neuronal cytoplasm. FUS-related mutations have been found to elicit neurodegeneration, especially in rodent models. For example, transgenic rats and mice "expressing mutant FUS-R521C proteins [developed] early onset ALS-like symptoms, including hindlimb paralysis, muscle wasting, and reduced innervation at the neuromuscular junction (NMJ)" (Shang & Huang., 2016). Other models of the same type of mice showed decreased dendritic arborization in spinal motor neurons. Overall, mutations in the FUS gene have been found to have some contribution to fALS pathology as well, and, notably, it can be recognized that FUS proteinopathy is largely similar to that of TDP-43.

C9orf72

In the pathogenesis of fALS, mutations in the gene C9orf72 are said to be the most prevalent abnormality in patients. C9orf72, or chromosome 9 open reading frame 72, is a gene located on chromosome 9, as implied by the name. C9orf72 is a well-known gene in the pathogenesis of fALS due to its repeated hexanucleotide expansion of GGGGCC. Essentially, the nucleotide strand on the C9orf72 gene has repeated sequences of GGGGCC, consisting of 4 guanines and 2 cytosines. When the sequence is abnormally expanded in excess, protein synthesis is profoundly impaired. Specifically, the repeated expansion induces "the production of dipeptide repeat proteins (DPRs) through repeat-associated non-AUG (RAN) translation" (Schmitz et al., 2021). Dipeptide repeat proteins (DPRs) are synthesized by the C9orf72 hexanucleotide repeat expansion and are toxic to neurons. This is because the DPRs are synthesized without a start codon, leading to the production of short dipeptide sequences that are highly repetitive. Currently, 5 different DPRs have been identified: glycine-arginine (GR), glycine-alanine (GA), glycine-proline (GP), proline-alanine (PA), and proline-arginine (PR).

GA Pathology and Inclusions

The hexanucleotide sequence is repeated normally in healthy individuals, but it is in excess in patients with ALS. Healthy individuals usually carry up to 23 hexanucleotide repeats, however, patients with C9orf72-mediated ALS are usually found to have greater than 30 hexanucleotide repeats (Freibaum & Taylor, 2017). As these irregular repeats incur the production of DPRs, it's important to understand the pathology of these DPRs in neural degeneration. Usually, DPRs clusters have been found in various regions of the brain and spinal cord in ALS patients, including the hippocampus and basal ganglia to name a few. Of the DPRs, GA is "the most readily visible in p62/ubiquitin-positive inclusions in the brain and spinal cord of patients with ALS" (Freibaum & Taylor, 2017). Essentially, this means that in inclusions involving the p62 protein and the molecule-tagging protein Ubiquitin within neurons, GA is observed in high amounts. Moreover, GA affects neurons adversely in

many ways. Fundamentally, Poly-GA "[inhibits] dendritic arborization and [induces] apoptosis in primary neurons" (May et al., 2014). This means that Poly-GA proteins play a big role in cell death as well as the degeneration of dendritic branches that allow a neuron to communicate with other neurons. Due to these findings, aggregation is found to be the primary cause of DPR-induced neurodegeneration from the C9orf72 repeat expansion mutation.

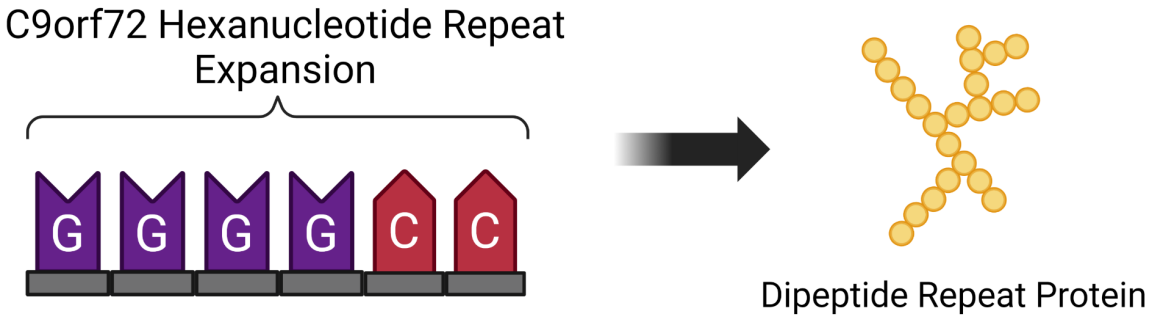


Figure 4. Hexanucleotide Repeat Expansion of C9orf72 Mutation resulting in the creation of Dipeptide Repeat Proteins. Created and Copyrighted by Soham Deshpande

DPR-Induced Motor Neuron Degeneration

DPR aggregations result in neurotoxicity in numerous ways. These include impaired cellular trafficking, cellular mutilation, and synaptic dysfunction. For example, Poly GR/PR proteins "target nucleopore complexes and affect the nuclear–cytoplasmic trafficking of RNA and proteins" (Xu & Xu, 2018). As a result, cytoplasmic processes are disrupted and proteins essential for cellular functions are mislocalized. Additionally, GR/PR proteins affect the formation of stress granules, which are membrane-less organelles composed of proteins and RNAs formed to protect RNA information when a cell is under some form of stress. Furthermore, not only might DPRs impact nuclear-cytoplasmic trafficking and the formation of stress granules, but DPRs have also been found to crease and fragment the nuclear membrane of a cell. According to an experiment done using immunofluorescence imaging on cells, "a large proportion of cells containing GA, GR or PR inclusions had misshapen nuclei, indicating that DPRs cause structural abnormalities in the nucleus" (Ryan et al., 2022). Lastly, synaptic processes are highly impacted by DPRs, especially GA. As discussed previously, GA inhibits dendritic arborization and synaptic function. As discovered in a study, the "evaluation of cortical neurons expressing GA peptides revealed a striking abrogation of synaptic vesicle release" (Jensen et al., 2020). This simply means that GA peptides that were present in neurons showed to repeal synaptic vesicles from delivering neurotransmitters, which are essential for neuronal communication. Overall, it can be observed that the hexanucleotide expansion mutation in the C9orf72 gene contributes greatly to the pathogenesis of ALS, as it initiates the creation of harmful DPRs in a cell. Although more information needs to be gathered on the effect DPRs have on a cell, it can be said definitively that DPRs play an essential role in the degeneration of neurons.

Next-Generation Sequencing (NGS)

The ability to determine genetic sequence mutations is vital to the oversight of fALS. Without the ability to do so, patients with fALS-inherited genes may not realize they carry the disease before it is too late and symptoms already begin to show. The process of observing one's genomic sequence for mutations is known as genetic

testing. Genetic testing is a highly reliable and quick method of checking for mutations. Next-generation sequencing, or NGS, is an efficient method of genetic testing that allows scientists to test genetic sequences with high scalability, throughput, and especially speed, as "the speed of execution and the amount of data output generated with NGS is exponentially greater than with Sanger sequencing" (Pecoraro et al., 2020). Specifically, NGS is used to scan the order of nucleotides on a gene to check for abnormalities like variants and mutations. Moreover, its high processing speed allows it to check many genomes over a short period of time. In the case of ALS, NGS technology "identified 51 new or rare variants in 18 different genes…associated with ALS" (Pecoraro et al., 2020). It is notable, however, that NGS cannot actually pick up expansions in the C9orf72 gene, needing specific Polymerase Chain Reaction (PCR) techniques. Although the interpretation of NGS test results sometimes casts uncertainty, NGS technology proves widely helpful in genetic sequencing and is a powerful tool in identifying possible genetic etiologies of fALS.

Gene Therapy Overview

As the utilization of NGS continues to grow within the field of medicine, it is possible that this technology can go hand-in-hand with gene therapy. Essentially, results from NGS testing can be used to greatly increase gene therapy's efficiency and effectiveness. Currently, ALS does not have a specific cure. Though it may have some possible treatment options to slow the progression of the disease, no definitive cure is present. Theoretically, since fALS is genetically based, gene therapy may prove a viable solution to this neurodegenerative disease. In order to acknowledge how gene therapy may be a cure for fALS, it's important to understand how it actually works. Gene therapy is a technique used to modify an individual's genes to cure disease, which can be through modifying existing genes, introducing a new gene, or replacing a diseased gene with a healthy one. In the past, this method has been used to treat various diseases such as cancer, AIDS, hemophilia, and cystic fibrosis. Gene therapy has also been applied to several neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Although gene therapy in relation to ALS hasn't been thoroughly studied, research regarding such is promising.

AAV Vector and CRISPR-Cas9 Application

CRISPR-Cas9, a genetic editing tool in the field of gene therapy, is widely used to treat many different genetic diseases today. This technology is well known for its efficiency and accuracy in DNA cutting and consists of two parts: the Cas9 enzyme and a guide RNA. Simply, when entered into a specific cell, the guide RNA directs the Cas9 enzyme to the specific genetic sequence that needs to be edited. Then, after being led to the specific area, the Cas9 enzyme cuts out the mutated sequence, leaving the DNA's natural repair processes to properly splice together the area. Although CRISPR-Cas9 is observed to be an effective tool for gene therapy, it has its limitations. One of which is the possibility for off-site edits, which is when the Cas9 enzyme accidentally acts upon untargeted genetic areas, resulting in unintended cleavages. Through increased research on genetic editing techniques for neurodegenerative diseases, AAV-mediated methods have been found to effectively deliver genetic material for the purpose of genetic modification. AAV-mediated approaches involve the use of Adeno-Associated Viruses (AAV) that act as vectors to target the desired cells and their DNA. In the field of genetic therapeutics, wild AAV are not used, but rather recombinant AAV (rAAV). rAAV lack their viral DNA, essentially making them "protein-based nanoparticle[s] engineered to traverse the cell membrane, where [they] can ultimately traffic and deliver its DNA cargo into the nucleus of a cell" (Naso et al., 2017). In order to use AAV to deliver CRISPR-Cas9, dual AAV has to be used. Essentially, "one AAV expresses Cas9 while the other expresses the sgRNA and donor template DNA" (Xu et al., 2019). This way, both components of the CRISPR-Cas9 complex are effectively transmitted through the use of AAV. Furthermore, a recent study done on mice

with the SOD1-G93A mutation demonstrated that an "AAV-mediated siRNA delivery led to a 39% survival benefit" (Fang et al., 2022). Many serotypes of central nervous system (CNS) AAV vectors exist, such as AAV1, AAV2, AAV4, AAV5, AAV8, and AAV9. Although many of these AAV serotypes can be used in CNS motor neurons, AAV9 is used most commonly to treat neurological disorders. Nevertheless, the use of AAV vectors through CRISPR-Cas9 technology to transport genetic material has been regarded highly as a reliable and efficient method, encouraging its future study.

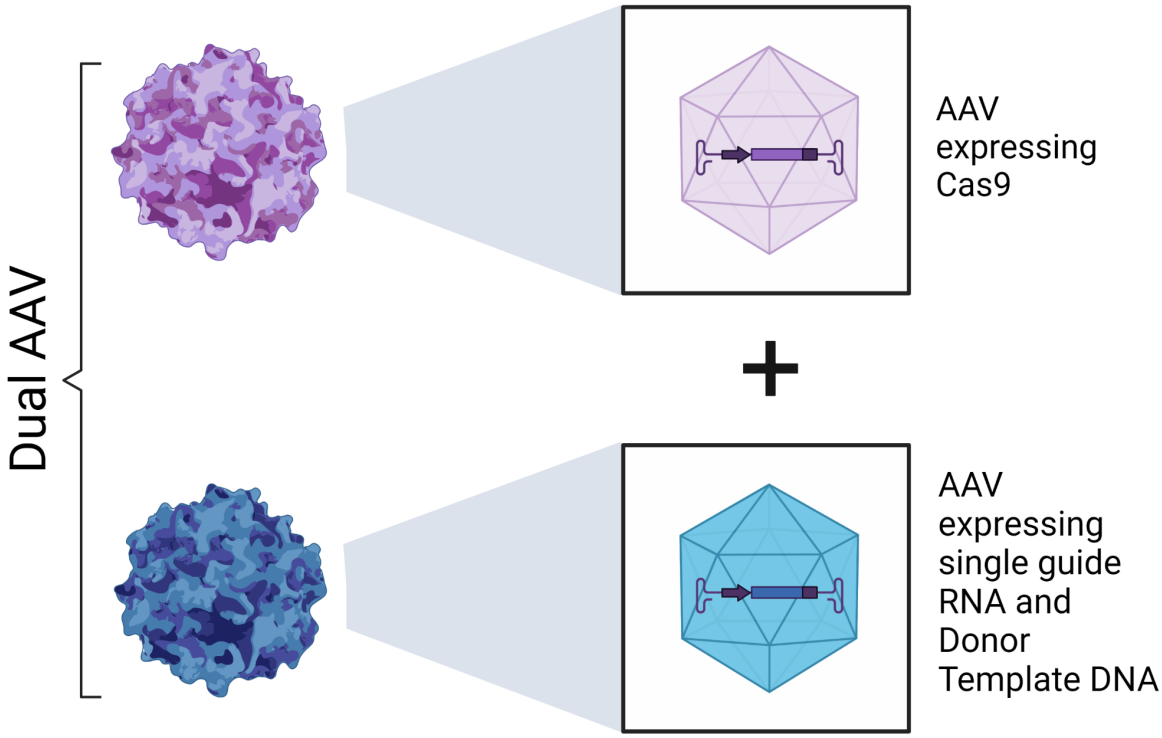


Figure 5. Dual AAV-mediated CRISPR-Cas9 application. Created and Copyrighted by Soham Deshpande

Conclusion

Overall, it can be seen that several genetic mutations are greatly responsible for the progression of fALS, inducing profound motor neuron degeneration through various ways. Four genes were significant in their possession of these mutations and contribution to this disease: SOD1, TARDBP, FUS, and C9orf72. It was found that these mutations cause motor neuron degeneration by producing misfolded proteins, specifically dysfunctional enzymes resulting in the lack of catalyzation of superoxide radicals (SOD1), protein mislocalization and aggregation (TARDBP/FUS), and toxic DPRs from hexanucleotide repeat expansions (C9orf72). With the examination of each mutation's role in the progression of motor neuron degeneration, fALS can be better comprehended and tended to in the future of medicine. Additionally, NGS techniques and CRISPR-Cas9 applications need to be further analyzed as possible cures to fALS, with the lack of such stimulating urgency in this area of research. With ALS cases on the rise in modern diagnoses, its pathogenesis needs to be understood to a greater extent in order to gain more knowledge about its oversight, treatment, and possible cure. As more research aims to uncover ALS, information about this disease may also yield promising discoveries in the future of neurodegenerative diseases, and in the advancement of medicine as a whole.

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