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Amyotrophic Lateral Sclerosis: II. Etiology and Action Mechanisms

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Abstract

Amyotrophic lateral sclerosis is driven by a complex interplay of excitotoxicity, mitochondrial dysfunction, oxidative stress, protein aggregation, and neuroinflammation, leading to motor neuron death and paralysis. Understanding these mechanisms has led to therapeutic strategies, though no cure exists yet. Motor neuron degeneration and death in ALS result from a combination of ten pathological mechanisms which this article will present. The disease's action mechanisms, the effects on motor neurons, and eventual treatments will be provided including effects on motor neurons and possible therapies.

Abbreviations

ALS: Amyotrophic lateral sclerosis; AMPA: Amino-methyl-propionic acid; ASO: Antisense oligonucleotides; ATP: Adenosine triphosphate; BBB: Blood-brain barrier; CNS: Central nervous system; DAMP: Damage-associated molecular patterns; DRP: Dipeptide repeat proteins; EAAT: Excitatory amino acid transporter; EMOP-RNAA: Excitotoxicity, mitochondria, oxidative, protein, RNA, neuroinflammation, axons, autophagy; FasL: Fas ligand; FTD: Frontotemporal dementia; FUS: Fused in sarcoma; GEMI: Genetic, excitotoxic, metabolic and inflammatory disruptions; IL: Interleukin; LMN: Lower motor neuron; NMDA: N-methyl-D-aspartate; NPC: Nuclear pore complex; NRDPM-OMCVE: Nucleocytoplasmic, RNA, proteostasis, DNA, mitochondrial, oligodendrocyte, microglial, cytoskeletal, vesicle, excitotoxicity; Pf: Protein-like profilin; RBP: RNA-binding protein; ROS: Reactive oxygen species; SG: Stress granules; SOD: Superoxide dismutase; TDP: Transactive response DNA-binding protein; TNF: Tumor necrosis factor; UMN: Upper motor neuron; UPS: Ubiquitin-proteasome system.

Keywords

Amyotrophic lateral sclerosis; apoptosis; autophagy

dysfunction; axonal transport deficit; C9orf72 repeat expansion toxicity; glutamate excitotoxicity accumulation; mitochondrial dysfunction; motor neuron degeneration; neuroinflammation; oxidative stress; nuclear RNA dysregulation; proteasomal pathways dysfunction; protein aggregation; toxic RNA dysregulation.

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that primarily affects motor neurons in the brain and spinal cord, leading to muscle weakness, atrophy, eventual paralysis, and ultimately respiratory failure and death. It results from a combination of interconnected mechanisms (Genetic, Excitotoxic, and Metabolic and Inflammatory disruptions - acronym GEMI) that ultimately lead to motor neuron degeneration. The disease's action mechanisms are complex and multifactorial, involving several interrelated pathological processes. Although the precise cause of ALS varies among individuals, the following mechanisms of action collectively contribute to the disease onset and progression. This article will discuss these several mechanisms beginning with an overall description of motor neuron degeneration and death to be followed by more detailed analyses.

Motor neuron degeneration and death

Motor neuron degeneration and death refer to the progressive loss of motor neurons—specialized nerve cells that control voluntary muscle movements. Progressive loss of these neurons leads to impaired muscle control, weakness, atrophy, and paralysis. This process is central to ALS. The degeneration selectively affects both upper motor neurons (UMNs) in the brain and lower motor neurons (LMNs) in the brainstem and spinal cord.

Motor neuron degeneration and death in ALS result from a combination of ten (10) pathological mechanisms (discussed below) - leading to progressive muscle weakness, paralysis, and ultimately respiratory failure:

1. Glutamate excitotoxicity,
2. Mitochondrial dysfunction,
3. Oxidative stress,
4. Protein aggregation,
5. Dysregulated nuclear RNA and toxic RNA accumulation,
6. Neuroinflammation,
7. Axonal transport deficit,
8. Dysfunction of autophagy and proteasomal pathways,
9. C9orf72 repeat expansion toxicity, and
10. Apoptosis.

Types of affected motor neurons

Both UMNs and LMNs are affected. Their respective characteristics are summarized below:

- Upper Motor Neurons (UMN):
  - Located in the motor cortex of the brain.
  - Transmit signals to lower motor neurons through the corticospinal tract.
  - Their degeneration leads to spasticity, hyperreflexia, and muscle stiffness.
- Lower Motor Neurons (LMN):
  - Located in the brainstem and spinal cord.
  - Directly innervate skeletal muscles via peripheral nerves.
  - Their loss leads to muscle weakness, atrophy, fasciculations and hyporeflexia.

Synopsis of the leading mechanisms

Several cellular and molecular mechanisms contribute to the progressive degeneration of motor neurons. They can be summarized by the acronym **EMOP-RNAA** (where **E** stands for Excitotoxicity, **M** for Mitochondria, **O** for Oxidative, **P** for Protein, **R** for RNA, **N** for

Neuroinflammation, **A** for Axons, and **A** for Autophagy):

Excitotoxicity (glutamate toxicity):

• Excessive stimulation: In ALS, motor neurons experience excessive stimulation by glutamate (a neurotransmitter).
• Impaired glutamate clearance by astrocytes via EAAT2 (excitatory amino acid transporter) dysfunction leads to toxic accumulation.
• Overactivation of glutamate receptors AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/NMDA (NMDA (N-methyl-D-aspartate) results in excessive calcium influx, triggering neuron damage.

Mitochondrial dysfunction and energy deficits:

• ROS production: Damaged mitochondria produce reactive oxygen species (ROS), leading to oxidative stress.
• Impaired motor neuron function: Energy deficits impair motor neuron function, making them more vulnerable to damage.

Protein misfolding and aggregation:

• Protein misfolding and aggregation: Mutant proteins, e.g., SOD1 (superoxide transmutase-1), TDP-43 (transactive response DNA-binding protein), and FUS (fused in sarcoma) misfold and aggregate inside motor neurons.
• Disruption of cellular function and death: These toxic protein clumps disrupt cellular functions and lead to neuron death.

RNA dysregulation and toxic RNA accumulation:

• RNA processing disruption: Mutations in TDP-43, FUS, and C9orf72 (chromosome 9, open reading frame 72) disrupt RNA processing, affecting essential cellular functions.
• Interference with neuron survival: In C9orf72 ALS, toxic RNA foci and abnormal DRPs (dipeptide repeat proteins) interfere with neuron survival.

Neuroinflammation (microglia and astrocyte activation):

• Release of inflammatory cytokines: Activated microglia and astrocytes release inflammatory cytokines TNF-α (tumor necrosis factor), and IL-1β (Interleukin) that exacerbate motor neuron damage.
• Accelerated neuronal loss: Astrocytes also fail to provide essential support, accelerating neuronal loss.

Axonal transport deficits:

• Requirement for efficient transport: Motor neurons have long axons, requiring efficient transport of proteins and organelles.
• Accumulation of toxic cellular waste: In ALS, the transport machinery is impaired, leading to the accumulation of toxic cellular waste.

Defective autophagy and protein clearance:

• Removal prevention of damaged proteins: Impaired autophagy and proteasomal degradation prevent the removal of damaged proteins.
• Increased motor neurons stress: Accumulation of dysfunctional proteins further stresses motor neurons.

Consequences of motor neuron death

• Progressive muscular weakness: Loss of motor neurons leads to progressive muscle weakness, paralysis, and loss of voluntary movement.
• Muscle atrophy: As LMNs degenerate, muscles atrophy due to lack of stimulation.
• Spasticity and exaggerated reflexes: Loss of UMNs results in spasticity (muscle stiffness) and exaggerated reflexes.
• Respiratory failure and death: Eventually, respiratory

muscles are affected, leading to respiratory failure, the primary cause of death in ALS.

The above action mechanisms are further discussed in the sections below.

Glutamate excitotoxicity

Glutamate is the primary excitatory neurotransmitter in the brain and spinal cord, Glutamate excitotoxicity is a pathological process in which excessive or prolonged stimulation of neurons leads to neuronal damage and death. Excess glutamate overstimulates motor neurons, leading to toxic calcium (Ca^{2+}) influx. Motor neurons have low calcium-buffering capacity, making them highly vulnerable to excitotoxic damage.

This mechanism plays a significant role in ALS and other neurodegenerative diseases. Increased levels of glutamate (a neurotransmitter) in the synaptic cleft lead to excessive stimulation of motor neurons. Overactivation of glutamate receptors, e.g., AMPA/NMDA, causes calcium influx, oxidative stress, and neuronal damage.

Role of glutamate in normal neuronal function

• Glutamate is released by presynaptic neurons into the synaptic cleft.
• It binds to postsynaptic glutamate receptors (AMPA, NMDA, and kainate receptors), allowing calcium (Ca^{2+}) and sodium (Na^{+}) influx.
• After signal transmission, excess glutamate is cleared by astrocytes via EAAT2 to prevent toxicity.

How glutamate excitotoxicity occurs

- In ALS, glutamate accumulates in the synaptic cleft due to impaired clearance and excessive receptor activation, leading to excessive calcium influx and motor neuron damage.
- Reduced glutamate clearance

- Reduced glutamate clearance

Reduced glutamate clearance

• EAAT2 dysfunction: The glutamate transporter EAAT2, primarily located in astrocytes, is impaired in ALS.

• Astrocyte dysfunction: Reactive astrocytes fail to efficiently remove excess glutamate, leading to its accumulation.

Overactivation of glutamate receptors

• Excess glutamate overstimulates AMPA and NMDA receptors on motor neurons.

• This leads to prolonged calcium influx, overwhelming normal cellular processes.

Calcium toxicity and mitochondrial damage

• Motor neurons have low calcium-buffering capacity, making them highly susceptible to calcium overload.

• Excess calcium damages mitochondria, leading to:

- Increased ROS production → oxidative stress.
- Activation of caspases → cell apoptosis.
- Energy depletion → Neuronal dysfunction.

Oxidative stress and protein aggregation

• Elevated calcium levels lead to oxidative stress, damaging proteins, lipids, and DNA.

• Misfolded proteins (TDP-43, FUS, SOD1) aggregate, further impairing motor neurons.

Neuroinflammation and cell death

• Activated microglia release pro-inflammatory cytokines (TNF-α, IL-1β), exacerbating excitotoxicity.

• The combined effects of excitotoxicity, oxidative stress, and inflammation trigger motor neuron death.

Consequences of glutamate excitotoxicity

• Progressive motor neuron death: Leads to muscle weakness, atrophy, and paralysis.

• Increased vulnerability of motor neurons: Unlike other neurons, motor neurons have low calcium-buffering capacity, making them highly susceptible to damage.

• Disease progression: Excitotoxicity contributes to spread of neurodegeneration in ALS.

Therapeutic approaches targeting excitotoxicity

• Riluzole (FDA-approved for ALS):

o Inhibits glutamate release and enhances clearance, reducing excitotoxic damage.

• AMPA/NMDA receptor modulators:

o Reduce excessive calcium influx (e.g., Perampanel, Memantine).

• Antioxidants & mitochondrial protectors:

o Combat oxidative stress and calcium overload (e.g., Edaravone, Coenzyme Q10).

Mitochondrial dysfunction and energy deficits

Mitochondria are essential organelles responsible for energy production (adenosine triphosphate – ATP-synthesis), calcium homeostasis, and apoptosis regulation. Mitochondrial dysfunction is a major contributor to motor neuron degeneration in ALS, leading to energy deficits and increased production of ROS, contributing to oxidative stress and apoptosis (cell death).

Functions of healthy mitochondria in neurons

• ATP production: Mitochondria generate ATP via oxidative phosphorylation in the electron transport chain.

• Calcium homeostasis: Mitochondria help regulate intracellular calcium (Ca^{2+}) levels, preventing excitotoxicity.

• ROS management: Mitochondria naturally produce ROS, which are neutralized by antioxidants like SOD1

• Apoptosis regulation: Mitochondria control cell survival by releasing Cytochrome-c, which activates apoptosis (programmed cell death).

How mitochondrial dysfunction occurs

Mitochondria fail to produce enough ATP (energy), leading to energy deficits in motor neurons. Damaged mitochondria generate excessive ROS, increasing oxidative stress. Calcium overload disrupts mitochondrial function, triggering apoptosis.

Energy deficiency (ATP depletion)

• Motor neurons have high energy demands due to their long axons.

• In ALS, defective mitochondria fail to produce enough ATP, leading to neurodegeneration and muscle atrophy.

Oxidative stress and free radical accumulation

• Dysfunctional mitochondria produce excessive ROS, leading to oxidative damage to proteins, lipids, and DNA.

• Mutations in SOD1, an enzyme that detoxifies ROS, further exacerbate oxidative stress.

• Accumulation of damaged molecules triggers apoptosis and inflammation.

• High ROS levels generation: Motor neurons generate high levels of ROS due to their large size and high energy demands.

• Impaired ROS detoxification: Mutations in SOD1 – a key antioxidant enzyme - impair ROS detoxification, leading to protein, lipid, and DNA damage.

Calcium dysregulation

• Motor neurons have low calcium-buffering capacity, making them vulnerable to Ca^{2+} overload.

• Mitochondrial dysfunction leads to calcium accumulation, activating destructive enzymes (calpains, caspases) that degrade proteins and DNA.

• Elevated Ca^{2+} also disrupts mitochondrial membrane potential, triggering apoptosis.

Impaired axonal transport

• Mitochondria must be transported along axons to provide energy at synapses and neuromuscular junctions.

• In ALS, defective mitochondrial transport causes localized energy deficits, leading to synaptic dysfunction and muscle weakness.

Apoptotic cell death

• ALS mitochondria release cytochrome-c and pro-apoptotic factors (Bax, Bak), activating caspases that initiate motor neuron death.

Consequences of mitochondrial dysfunction

Energy depletion in motor neurons, increased oxidative damage, and activation of cell death pathways:

- Increased neuronal stress → leads to progressive motor neuron death.
- Loss of neuromuscular function → results in muscle weakness, atrophy, and paralysis.
- Acceleration of disease progression → due to widespread energy deficits and oxidative stress.

Potential therapies targeting mitochondrial dysfunction

• Antioxidants (Edaravone, Co-enzyme Q10): Antioxidants reduce oxidative stress and ROS damage.

• Mitochondrial stabilizers (Cyclosporine A, Olesoxime): Prevent mitochondrial membrane damage and apoptosis.

• Calcium regulators (Riluzole, Memantine): Reduce excitotoxic calcium overload.

• Energy boosters (Creatine, Nicotinamide, Ketogenic diet): Enhance ATP production and support mitochondrial function.

• Co-enzyme Q10, Nicotinamide: Support mitochondrial health.

In summary, mitochondrial dysfunction in ALS leads to energy depletion, oxidative stress, calcium dysregulation, and apoptosis, all of which contribute to motor neuron death and disease progression. Targeting mitochondrial health is a promising strategy to slow ALS progression and protect neurons.

Oxidative stress and free radical damage

Oxidative stress is a condition in which reactive oxygen species (ROS) and reactive nitrogen species (RNS)

accumulate, causing cellular damage. In ALS, oxidative stress plays a critical role in motor neuron degeneration, leading to progressive muscle weakness and paralysis. Mutations in SOD1 and other genes disrupt cellular antioxidant mechanisms. Accumulation of ROS damages proteins, lipids, and DNA, further accelerating neuronal degeneration.

What are reactive oxygen and nitrogen species?

These molecules are normally produced during metabolism but are balanced by antioxidant defenses to prevent damage:

- ROS: Superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•).
- RNS: Nitric oxide (NO•), peroxynitrite ($ONOO^-$).

How does oxidative stress occur?

In ALS, the balance between ROS production and antioxidant defenses is disrupted, leading to excessive oxidative stress. Excess ROS and RNS cause DNA, protein, and lipid damage. Mutations in SOD1 reduce antioxidant defenses, leading to increased oxidative stress.

Mitochondrial dysfunction

• Damaged mitochondria produce excess ROS, overwhelming antioxidant defenses.

• ATP depletion and oxidative stress impair motor neuron function.

Mutations in antioxidant enzymes

- SOD1 mutations reduce the ability to neutralize ROS, leading to oxidative damage.
- Other ALS-linked proteins (TDP-43, FUS) may also impair antioxidant defenses.

Glutamate excitotoxicity

• Excess glutamate leads to calcium overload, which further damages mitochondria and increases ROS production.

Protein aggregation

• Misfolded proteins (e.g., SOD1, TDP-43, FUS) become oxidized, forming toxic aggregates that disrupt cellular functions.

Inflammation and microglial activation

• Activated microglia release pro-inflammatory cytokines and ROS, worsening neuronal stress and damage.

Consequences of oxidative stress

Protein misfolding and aggregation, cellular damage and inflammation, and increased motor neuron vulnerability.

- DNA and RNA damage → Leads to genetic instability and impaired protein production.
- Lipid peroxidation → Destroys cell membranes, causing neuronal dysfunction.
- Protein oxidation and aggregation → Leads to toxic clumps that disrupt motor neuron function.
- Neuroinflammation → ROS activates inflammatory pathways, worsening neuronal loss.
- Motor neuron death → Ultimately, oxidative stress triggers apoptosis, accelerating ALS progression.

Therapeutic strategies to reduce oxidative stress

Antioxidants:

- Edaravone (FDA-approved for ALS) reduces ROS and oxidative damage.
- Co-enzyme Q10, Vitamin E, and N-acetylcysteine (NAC) act as free radical scavengers.

Mitochondrial protectors:

Creatine, Nicotinamide, and MitoQ help maintain mitochondrial function and reduce oxidative stress.

Glutamate modulators:

- Riluzole reduces glutamate excitotoxicity, indirectly lowering oxidative stress.

Anti-inflammatory agents:

- Minocycline, Resveratrol, and Curcumin help suppress inflammation and ROS production.

In summary, oxidative stress in ALS results from mitochondrial dysfunction, impaired antioxidant defenses, excitotoxicity, and neuroinflammation, leading to motor neuron damage and death. Therapies targeting oxidative stress aim to slow disease progression and protect motor neurons.

Protein misfolding and aggregation

Protein misfolding and aggregation, particularly involving TDP-43, SOD1, FUS, and C9orf72 DPRs, are key pathological processes in ALS, contributing to motor neuron degeneration. They aggregate inside motor neurons, disrupting cellular functions. Misfolded proteins lose their normal function and form toxic aggregates, disrupting essential cellular processes, leading to motor neuron degeneration and disease progression and triggering neuronal death. Impaired protein degradation systems (proteasome and autophagy) allow toxic aggregates to accumulate. Targeting these aggregates is a promising therapeutic approach.

Understanding RNA dysregulation in ALS is crucial for developing RNA-targeted therapies, such as antisense oligonucleotides (ASOs), which aim to modify RNA processing and reduce disease progression.

How proteins normally fold

Proteins must fold into specific 3D structures to function properly. This process is assisted by:

- Chaperone proteins (e.g., heat shock proteins) that help in proper folding.
- Proteasome and autophagy systems that degrade misfolded or damaged proteins.

In ALS, these mechanisms fail, leading to the accumulation of misfolded and aggregated proteins.

Key misfolded and aggregated proteins

Several proteins linked to ALS become misfolded and aggregate inside motor neurons:

TDP-43 aggregation

- Most common protein aggregate in ALS (~97% of cases).
- Normally involved in RNA processing and gene regulation.
- In ALS, TDP-43 mis-localizes from the nucleus to the cytoplasm, becomes hyperphosphorylated, ubiquitinated, and aggregates, leading to cellular dysfunction and toxicity.

SOD1 misfolding

• Mutations in the SOD1 gene (~2% of ALS cases) cause the enzyme to misfold.

• Misfolded SOD1 aggregates disrupt mitochondrial function and increase oxidative stress, leading to motor neuron death.

FUS aggregation

- Involved in RNA transport and processing.
- Mutations cause FUS to mis-localize and aggregate, impairing RNA metabolism and stress responses.

C9orf72 dipeptide repeat proteins (DPRs)

- Most common genetic cause of ALS (C9orf72 expansion).
- Leads to the production of toxic dipeptide repeat proteins, which aggregate and disrupt cellular functions.

How protein aggregation causes motor neuron degeneration

The consequences of protein misfolding and aggregation are blockage of RNA processing and protein transport, neuroinflammation, and mitochondrial dysfunction and apoptosis.

Loss of protein function

• Essential proteins (TDP-43, FUS) are trapped in aggregates, leading to RNA and protein processing defects.

Toxic gain of function

Aggregates interfere with mitochondria, RNA metabolism, and protein degradation pathways.

Disruption of cellular transport

Aggregates impair axonal transport, blocking delivery of essential molecules to motor neurons.

Proteasome and autophagy dysfunction

• ALS aggregates overwhelm protein degradation systems, causing further accumulation of toxic proteins.

Neuroinflammation activation

- Aggregates trigger microglial and astrocyte activation, leading to chronic neuroinflammation.
- Potential therapies targeting protein aggregation
- Chaperone proteins (Arimoclomol): Enhance proper protein folding.
- Proteasome activators: Boost degradation of misfolded proteins.
- Autophagy enhancers (Rapamycin, Trehalose): Clear protein aggregates.
- Anti-aggregation compounds (Tolcapone, Anle138b): Prevent protein clumping.

In summary, protein misfolding and aggregation in ALS, particularly involving TDP-43, SOD1, FUS, and C9orf72 DPRs, disrupt essential cellular functions, leading to motor neuron degeneration and disease progression. Targeting these aggregates is a promising therapeutic approach.

Dysregulated nuclear RNA and toxic RNA accumulation

Dysregulated nuclear RNA (nRNA) processing in ALS refers to abnormalities in how RNA is transcribed, spliced, transported, and degraded in neurons, contributing to the disease's progression. Growing evidence suggests that defects in RNA metabolism play a major role in ALS. In this process, RNA-binding

proteins (TDP-43, FUS, C9orf72) become mis-localized, and dysfunctional. The consequences are:

- Disruption of RNA processing,
- Transport and translation leading to cell stress and dysfunction,
- Neurodegeneration,
- Abnormal protein production,
- Increased stress granules, and
- Motor neuron degeneration.

Key aspects of dysregulated nuclear RNA processing

Abnormal RNA-binding proteins (RBPs)

- Proteins like TDP-43, FUS, and RBPs normally regulate RNA splicing, transport, and stability.
- In ALS, these RBPs mis-localize (often moving from the nucleus to the cytoplasm) and form toxic aggregates, disrupting RNA processing.
- Defective RNA splicing
- RNA splicing is altered, leading to the production of abnormal proteins or loss of essential ones.
- Mutations in TDP-43 and FUS cause global splicing defects, affecting many neuronal genes.
- Impaired RNA transport
- Proper transport of mRNA from the nucleus to the cytoplasm is crucial for neuron function.
- In ALS, RBPs like TDP-43 and FUS fail to regulate this process, causing mRNA mis-localization and loss of synaptic function.
- Disrupted stress granules and RNA aggregation
- RNA and RBPs abnormally accumulate in stress granules (SGs), which are transient RNA-protein complexes that form in response to cellular stress.
- Persistent SG formation is linked to neurodegeneration in ALS.
- Nuclear pore dysfunction
- The C9orf72 repeat expansion, a common genetic cause of ALS, disrupts nuclear-cytoplasmic transport, preventing proper RNA export.

Aberrant RNA stability and decay

- Normally, cells regulate RNA degradation to maintain homeostasis.
- In ALS, defects in RNA degradation pathways lead to the accumulation of toxic RNA species.

Consequences for ALS pathogenesis

- Loss of motor neuron function due to defective RNA metabolism.
- Accumulation of toxic RNA-protein aggregates, contributing to neuronal death.
- Widespread gene expression changes, affecting pathways like neuroinflammation, mitochondrial function, and axonal transport.
- Potential therapeutic approaches
- Gene therapies targeting RNA dysfunction (ASOs for C9orf72, TDP-43, SOD1).
- Targeting RNA-binding proteins (e.g., stabilizing TDP-43 localization).
- Correcting RNA splicing defects using antisense oligonucleotides (ASOs).
- Enhancing nuclear-cytoplasmic transport to prevent RBP mis-localization.
- Reducing toxic RNA accumulation using small-molecule drugs or gene therapy.

Neuroinflammation (microglia and astrocyte activation)

Neuroinflammation is a critical area of research in ALS, as controlling it may slow the progression of the disease and improve outcomes. It refers to the chronic activation of the brain's immune system, particularly the glial cells (microglia and astrocytes), which play a significant role in the disease's progression. While inflammation is typically a protective response to injury or infection, in ALS, this process becomes dysregulated, contributing to the damage and death of motor neurons. It affects the response in the central nervous system (CNS) that contributes to motor neuron degeneration. This involves activation of immune cells like microglia, astrocytes, and peripheral immune cells, leading to chronic inflammation that exacerbates disease progression.

Activated microglia (immune cells in the CNS) and astrocytes become overactivated and release pro-inflammatory cytokines (TNF-α, IL-1β) that contribute to motor neuron damage. Further, dysfunctional astrocytes, which normally support neurons, become reactive, fail to support motor neurons, exacerbate disease progression, and contribute to motor neuron toxicity. The consequences are chronic inflammation worsens oxidative stress and excitotoxicity and increased neuronal stress and death.

Key components of neuroinflammation

Microglial activation

Microglia, the primary resident immune cells in the brain and spinal cord, are activated in ALS and shift from a protective state to a toxic, pro-inflammatory state. These cells become overactive and release pro-inflammatory molecules, such as cytokines (e.g., TNF-α, IL-1β), chemokines, and reactive oxygen species (ROS), which can damage neurons. The chronic activation of microglia is linked to neuronal death and progression of ALS.

- Early-stage ALS: Microglia help clear debris and support neurons.
- Late-stage ALS: Microglia become hyperactivated, releasing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and ROS, leading to neurotoxicity.

Astrocyte dysfunction

Astrocytes, another type of glial cell, normally support motor neurons and help maintain the brain's homeostasis. In ALS, they become reactive, contributing to inflammation, secreting harmful substances that can damage motor neurons, and contribute to neurodegeneration. In addition, this leads to secretion of toxic factors as reactive astrocytes release inflammatory cytokines and kill motor neurons.

Mutations in ALS-related genes, like SOD1, TDP-43, and FUS, can make astrocytes more toxic to motor neurons, exacerbating disease progression.

Peripheral immune system infiltration

• CNS infiltration: Immune cells from the periphery such as T-cells and monocytes infiltrate the CNS, worsening inflammation. These cells amplify the inflammatory response, which worsens neuronal damage and accelerates the disease.

• Dysregulated T-cell responses: While regulatory T-cells (Tregs) normally suppress inflammation, they become dysfunctional in ALS.

• Monocytes/macrophages: Release inflammatory molecules, contributing to motor neuron death.

Complement system activation

• The complement system, part of the immune system that helps fight infections, becomes overactivated in ALS. This overactivation can lead to synaptic damage, neuronal injury, and further inflammation.

Role of C9orf72 & TDP-43 mutations in inflammation

• C9orf72 repeat expansion, the most common genetic cause of ALS, disrupts immune signaling and activates microglia.

• TDP-43 pathology, found in most ALS cases, triggers inflammatory pathways and cytokine release.

Effects of neuroinflammation

- Motor neuron degeneration: Chronic inflammation accelerates cell death.
- Blood-brain barrier (BBB) disruption: Increased permeability allows harmful immune cells to enter the CNS.
- Toxic cycle of inflammation and neuronal death: Dying neurons release danger signals that further activate immune cells.

Potential therapeutic approaches

- Anti-inflammatory drugs: Targeting microglia with drugs like Ibudilast.
- Regulatory T-cell (Treg) therapy: Enhancing Treg function to suppress inflammation.
- Gene therapy: Targeting mutations that drive inflammation (e.g., C9orf72).
- Glial cell modulation: Reducing astrocyte toxicity.

Neurotoxic effects of protein aggregates

• ALS is characterized by the accumulation of misfolded proteins like TDP-43, FUS, and SOD1. These protein aggregates can trigger an inflammatory response by acting as damage-associated molecular patterns (DAMPs), which activate immune cells and exacerbate neuroinflammation.

Consequences of neuroinflammation

• Motor neuron death: Chronic inflammation leads to oxidative stress, excitotoxicity (damage from excessive glutamate), and apoptosis (programmed cell death) of motor neurons.

• Progression of disease: Neuroinflammation not only accelerates the death of motor neurons but also contributes to the spread of ALS pathology to other regions of the brain and spinal cord.

• Impaired immune function: While inflammation can be protective, in ALS, the immune system becomes dysregulated, impairing the body's ability to protect neurons and contributing to neurodegeneration.

Potential treatments targeting neuroinflammation

• **Edaravone:** A drug that reduces oxidative stress and, by extension, neuroinflammation in ALS.

• **Masitinib:** A tyrosine kinase inhibitor that specifically targets microglial activation and has been studied as an anti-inflammatory therapy in ALS.

Cytokine inhibition: Targeting inflammatory cytokines like IL-1β, TNF-α, or IL-6 to reduce neuroinflammation.

Regulatory T-cell therapy: Boosting Tregs (immune cells that suppress inflammation) may help control the neuroinflammatory process.

Axonal transport deficits

Axonal transport deficits in ALS refer to disruptions in the movement of essential cellular components—such as proteins, organelles (e.g., mitochondria), RNA, and signaling molecules—along the long projections (axons) of neurons. Since motor neurons extend over long distances (e.g., from the spinal cord to muscles),

they depend heavily on efficient transport to maintain cellular function. Axonal transport is crucial for maintaining the health and function of neurons, especially motor neurons, which have very long axons that stretch from the brain to the muscles. When it is impaired, neurons degenerate, leading to muscle weakness and paralysis. In ALS, transport proteins (dynein, kinesin) become dysfunctional, causing axonal degeneration. Here is a closer look at the role of axonal transport in ALS and how it is disrupted.

Types of axonal transport and their disruption

We distinguish between anteretrograde and retrograde transports. Both types of transport are essential for the proper functioning of neurons and require motor proteins like kinesins (for anterograde transport) and dyneins (for retrograde transport), along with other cargo adapters.

Anterograde transport (Cell body → Axon terminal)

• Function: Transports essential materials from the soma (cell body) to the axon terminal, including synaptic vesicles, mitochondria, and RNA.

• Motor protein: Kinesin (moves along microtubules toward the axon terminal).

ALS-related deficits:

- Reduced kinesin function leads to insufficient delivery of essential cargo.
- Synaptic dysfunction due to impaired neurotransmitter supply.

Retrograde transport (Axon terminal → Cell body)

Function: Returns damaged organelles, misfolded proteins, and signaling molecules to the soma for repair or degradation.

Motor protein: Dynein (moves toward the cell body).

ALS-related Deficits:

Impaired dynein activity causes toxic protein accumulation.

Reduced neurotrophic factor signaling (e.g., BDNF, NGF), which is necessary for neuron survival.

Molecular causes of axonal transport deficits

TDP-43 & FUS pathology

- TDP-43 mislocalization: Disrupts RNA regulation, affecting transport-related genes.
- FUS mutations: Interfere with RNA granule transport, impairing synaptic function.

C9orf72 repeat expansion

C9orf72 mutations: Disrupt nucleocytoplasmic transport, leading to defects in RNA and protein transport along axons.

Cytoskeletal disruptions

Microtubules act as "tracks" for axonal transport. In ALS, proteins-like profilin-1 (Pf1).

Types of axonal transport deficits

In ALS, axonal transport becomes disrupted in the following ways:

Motor protein dysfunction

Motor proteins such as kinesin and dynein are responsible for the movement of materials along microtubules. In ALS, mutations in ALS-related genes (like SOD1, TDP-43, and FUS) can impair the function of these motor proteins.

Defective motor proteins lead to slower or failed transport, disrupting the flow of materials to and from the axon terminals.

Accumulation of cargo in axons

- When transport is impaired, cargo such as vesicles, mitochondria, RNA, and proteins accumulates in the axons or cell body, leading to axon swelling and neuron dysfunction.

- For example, mitochondria, which provide energy to the axon, fail to reach the axon terminals, leading to energy deficits and increasing oxidative stress.

Protein aggregates and toxicity

- Misfolded proteins (such as TDP-43, SOD1, and FUS) are known to accumulate and form aggregates in ALS.

- These aggregates can physically obstruct axonal transport and contribute to axonal blockage, which exacerbates neuron dysfunction and toxicity.

Defective retrograde transport

• Retrograde transport is critical for bringing signals (like growth factors) from the synapse back to the cell body to maintain neuron health.

• In ALS, disruptions in retrograde transport prevent the delivery of important survival signals to motor neurons, which can lead to cell death.

Microtubule instability

• Microtubules serve as the “tracks” for motor proteins during axonal transport.

• In ALS, microtubule destabilization occurs, which impairs the integrity of axonal transport and contributes to motor neuron dysfunction and degeneration.

Consequences of axonal transport deficits

• Neurodegeneration: Impaired transport leads to the accumulation of toxic substances, oxidative stress, and energy depletion, ultimately resulting in motor neuron death.

• Synaptic dysfunction and muscle weakness: Failure to deliver necessary signaling molecules or neurotransmitters to synapses contributes to impaired synaptic function and communication between motor neurons and muscles.

• Distal axon degeneration: The axons that are farthest from the cell body are often the first to degenerate, leading to the characteristic distal-to-proximal pattern of muscle weakness seen in ALS.

• Increased motor neuron stress.

Research into therapies targeting axonal transport

1. Gene therapy: Efforts to correct genetic mutations that impair motor proteins (like SOD1 or TDP-43) are being investigated.

2. Small molecule drugs: Compounds that enhance motor protein function or stabilize microtubules could help restore axonal transport.

3. Mitochondrial therapy: Improving mitochondrial function or delivering them to axons may help provide energy and reduce oxidative stress in motor neurons.

4. HDAC inhibitors (Tubastatin A): Support axonal transport.

Dysfunction of autophagy and proteasomal pathways

In ALS, the autophagy and proteasomal pathways—both crucial for clearing damaged proteins and organelles—are disrupted. This leads to the accumulation of toxic protein aggregates, which contribute to motor neuron degeneration. Here, defective protein degradation pathways lead to the accumulation of toxic proteins and impaired clearance of damaged organelles exacerbates cellular stress.

How Autophagy Works?

Initiation: Damaged proteins or organelles are tagged.

Autophagosome formation: A membrane engulfs the damaged material.

Fusion with lysosome: The autophagosome merges with a lysosome for degradation.

Degradation and recycling: Enzymes break down the contents for reuse.

Autophagy dysfunction

Autophagy is a cellular recycling process that degrades damaged proteins and organelles through lysosomal digestion.

Autophagy defects

• Impaired autophagosome formation: Proteins like TDP-43 and FUS disrupt autophagy-related genes (ATG genes).

• Defective cargo recognition: Mutations in p62/SQSTM1 (a key autophagy receptor) prevent proper clearance.

• Blocked lysosomal degradation: C9orf72 mutations impair lysosomal function, leading to toxic accumulation.

Proteasomal pathway dysfunction

The ubiquitin-proteasome system (UPS) is responsible for degrading short-lived and misfolded proteins.

How the proteasomal pathway works

1. **Ubiquitination:** Damaged proteins are tagged with ubiquitin.

2. **Recognition by proteasome:** The proteasome identifies and unfolds the tagged proteins.

3. **Degradation:** The protein is broken into smaller peptides for recycling.

Proteasomal defects

• TDP-43 and FUS aggregates: These interfere with ubiquitin signaling, preventing proper degradation.

• Impaired proteasome function: Mutations in UBQLN2 (Ubiquilin-2) disrupt protein clearance.

• C9orf72 expansion: Leads to the accumulation of dipeptide repeats, overwhelming the proteasome.

How defective protein degradation pathways drive ALS progression

• Failure to clear misfolded proteins: Leads to protein aggregation (e.g., TDP-43, FUS, SOD1).

• Disrupted proteostasis (protein balance): Causes stress on motor neurons.

• Increased oxidative stress & mitochondrial damage: Worsen neurodegeneration.

Consequences of autophagy dysfunction and proteasomal pathways

Ubiquitin-positive inclusions: A hallmark of ALS, accumulate, further stressing neurons.

Toxic protein aggregates: Accumulate, stressing motor neurons and promoting degeneration.

Potential therapeutic approaches

Autophagy activators: Drugs like Rapamycin enhance autophagy to clear toxic proteins.

Proteasome enhancers: MG-132 inhibitors improve protein degradation.

Gene therapy: Targeting C9orf72 to restore lysosomal function.

C9orf72 repeat expansion toxicity

C9orf72 repeat expansion toxicity is the most common genetic cause of ALS and frontotemporal dementia (FTD). It results from an abnormal G4C2 hexanucleotide repeat expansion (GGGGCC) in the C9orf72 gene, leading to toxic gain-of-function and loss-of-function effects that drive neurodegeneration and produce toxic RNA foci and abnormal dipeptide repeat proteins. These contribute to neuronal dysfunction and cell death.

Mechanisms of toxicity in C9orf72-ALS

Loss of C9orf72 function

• The C9orf72 gene normally plays a role in autophagy and immune regulation.

• Expanded repeats reduce C9orf72 protein levels, impairing lysosomal function and increasing neuroinflammation.

Toxic RNA accumulation (RNA foci formation)

• Mutant C9orf72 RNA forms nuclear RNA foci, which trap essential RNA-binding proteins like RNPs, TDP-43, and FUS.

• This disrupts RNA processing, leading to defects in splicing, transport, and translation.

Dipeptide repeat protein toxicity

The repeat expansion undergoes repeat-associated non-AUG (RAN) translation, producing toxic dipeptide repeat (DPR) proteins:

- Poly-GA, Poly-GP, Poly-GR, Poly-PR, and Poly-PA
- These DPRs aggregate in neurons, leading to:
- Protein misfolding and proteasome dysfunction.
- Mitochondrial damage and oxidative stress.
- Disruption of nucleocytoplasmic transport.

Impaired nucleocytoplasmic transport

• The nuclear pore complex (NPC) regulates RNA and protein transport between the nucleus and cytoplasm.

• DPR proteins (especially Poly-GR and Poly-PR) interfere with NPC function, leading to TDP-43 mis-localization, a hallmark of ALS.

Consequences of C9orf72 toxicity

• Motor neuron degeneration: Due to toxic protein aggregates and defective RNA processing.

• Neuroinflammation: From impaired C9orf72 function.

• Synaptic dysfunction: Caused by RNA-binding protein sequestration.

Potential therapeutic approaches

Antisense oligonucleotides (ASOs): Target C9orf72 RNA to reduce toxic repeat expression (e.g., Tofersen).

Small molecule inhibitors: Block RAN translation to prevent DPR protein formation.

Enhancing nucleocytoplasmic transport: Restoring NPC function to prevent TDP-43 mislocalization.

Autophagy activators: Promote clearance of toxic DPR aggregates.

Apoptosis

Apoptosis is a programmed cell death process that plays a significant role in motor neuron degeneration in ALS. While apoptosis is a normal mechanism for removing damaged cells, in ALS, it becomes excessively activated, contributing to the progressive loss of motor neurons.

Mechanisms of apoptosis

Apoptosis in ALS is triggered by various cellular stressors, including protein aggregation, oxidative stress, mitochondrial dysfunction, and excitotoxicity. These stressors activate two major apoptotic pathways:

Intrinsic (mitochondrial) pathway

Triggered by mitochondrial dysfunction and oxidative stress.

Key Steps:

Mitochondrial damage: Releases cytochrome c into the cytoplasm.

Cytochrome-c activates caspase-9: Activates in turn caspase-3.

Caspase-3 initiates cell death: Leads to motor neuron apoptosis.

ALS-related mitochondrial defects:

Mutations in SOD1, TDP-43, and FUS disrupt mitochondrial function.

Accumulation of toxic proteins increases mitochondrial permeability, leading to cytochrome-c leakage.

Extrinsic (death receptor) pathway

Activation by external signals binding to death receptors (e.g., Fas, TNF receptor).

Key Steps:

1. Fas ligand (FasL) or TNF-α: Binds to its receptor on the motor neuron membrane.
2. Caspase-8 is activated: This activates caspase-
3. Caspase-3 induces apoptosis: Causes motor neuron death.

ALS-related extrinsic apoptosis triggers:

- Increased levels of TNF-α and FasL in ALS patients.
- Chronic neuroinflammation upregulates death receptor signaling.

Factors contributing to apoptosis

- TDP-43 & FUS protein aggregation: Disrupt RNA processing and mitochondrial function, triggering apoptosis.
- C9orf72 repeat expansion: Produces toxic dipeptide repeat proteins (DPRs) that damage neurons.
- Excitotoxicity: Excess glutamate signaling overstimulates neurons, leading to calcium overload and apoptotic signaling.
- Oxidative stress: Increased reactive oxygen species (ROS) damage DNA and proteins, triggering apoptosis.
- Neuroinflammation: Activated microglia and astrocytes release pro-apoptotic cytokines like TNF-α and IL-1β.

- Mitochondrial damage, oxidative stress, and excitotoxicity: Activate apoptosis pathways in motor neurons. Pro-apoptotic proteins (Bax, Bak) cause mitochondrial membrane damage, leading to cytochrome-c release and cell death.

Therapeutic strategies to prevent apoptosis

- Caspase inhibitors: Blocking caspase activation (e.g., VX-765) may reduce apoptosis.
- Mitochondrial protectors: Drugs like Riluzole and Edaravone help prevent mitochondrial damage.
- Mitochondrial stabilizers: Cyclosporine A (Olesoxime).
- Anti-inflammatory agents: Targeting TNF-α and FasL signaling to reduce death receptor activation.
- Glutamate modulators: Preventing excitotoxicity (e.g., AMPA receptor inhibitors) may reduce apoptosis.
- Bcl-2 mimetics: Preventing apoptosis activation.

Summary of ALS action mechanisms

The summary of ALS action mechanisms is provided in Table 1 below;

Mechanism	Effect on motor neurons	Possible treatments
1. Glutamate excitotoxicity	Excessive Ca^{2+} influx & neuron death	o *Riluzole* o *Memantine*
2. Mitochondrial dysfunction	Energy failure, oxidative stress	*o Edaravone* *o CoQ10*
3. Oxidative stress	DNA, lipid, protein damage	o *NAC* o *Antioxidants*
4. Protein aggregation	TDP-43, SOD1, FUS clumps	o Autophagy enhancers
5. RNA metabolism defects	Disrupted gene regulation	o ASO therapies
6. Neuroinflammation	Chronic immune activation	o *Minocycline*
7. Axonal transport defects	Impaired synaptic function	o HDAC inhibitors
8. Apoptosis activation	Motor neuron loss	o Bcl-2 mimetics
9. C9orf7 repeat expansion toxicity	Neuronal dysfunction and cell death	o Antisense oligonucleotides o Small molecule inhibitors o Enhancing nucleocytoplasmic transport o Restoring NPC function o Autophagy activators
10. Apoptosis	Progressive loss of motor neurons	o Caspase inhibitors. o Mitochondrial protectors (*Riluzole, Edaravone*) o Mitochondrial stabilizers (*Cyclosporine A, Olesoxime*) o Anti-inflammatory agents. o Glutamate modulators o Bcl-2 mimetics

Table 1: ALS action mechanisms and possible treatments

Van Damme, Robberecht, and Van Den Bosc (2017) have also proposed a slightly different list of ten mechanisms for ALS with the associated genes (Figure 1). The first three of these mechanisms relate directly to the cell's body whereas the remaining mechanisms concern the cell extensions.

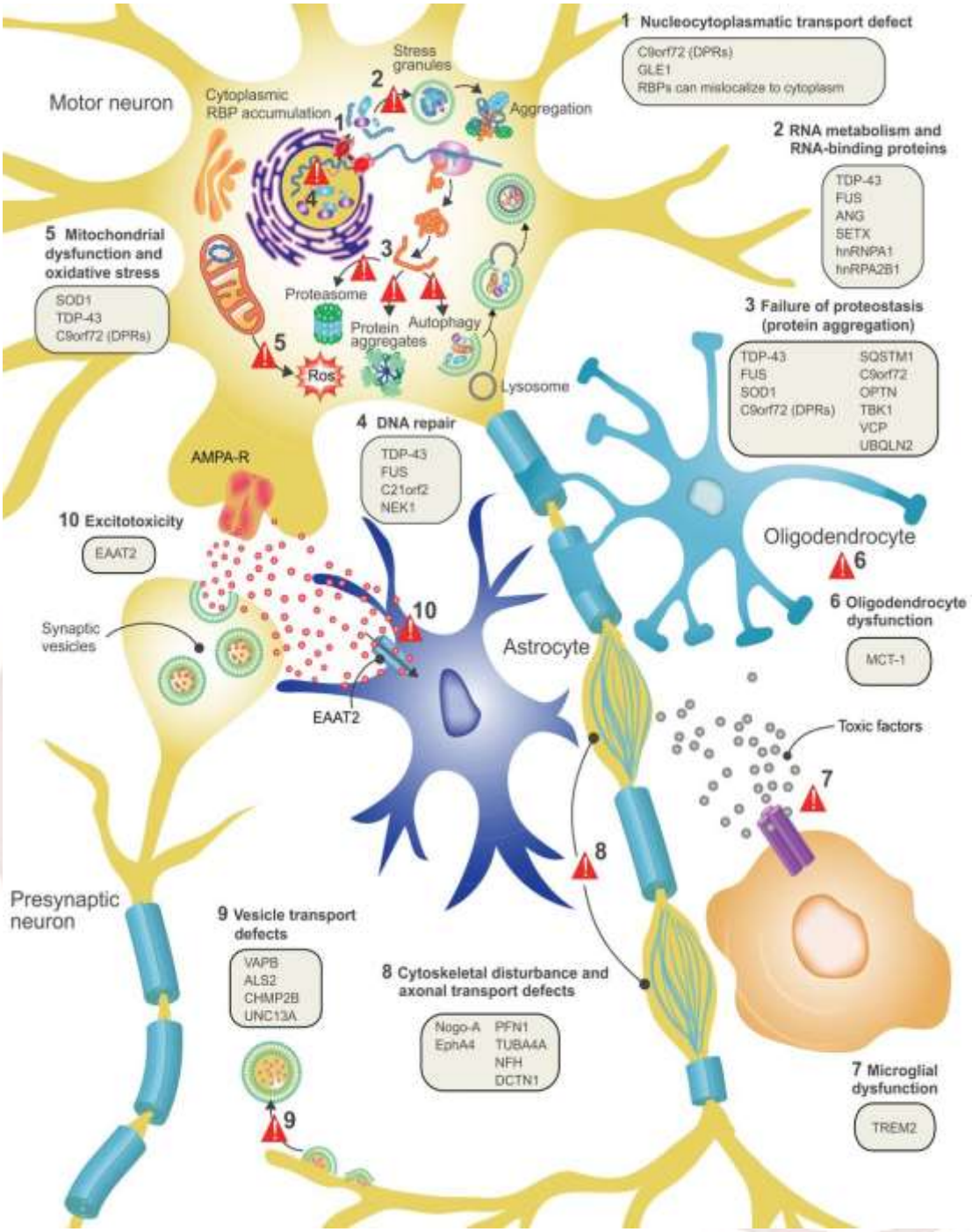
These are:

1. Nucleocytoplasmic transport defect;
2. RNA metabolism and RNA binding proteins
3. Failure of Proteostasis
4. DNA repair
5. Mitochondrial dysfunction and oxidative stress
6. Oligodendrocytes
7. Microglial dysfunction
8. Cytoskeletal disturbance and axonal transport defects
9. Vesicle transport defects; and
10. Excitotoxicity.

(mnemonic: NRPDM-OMCVE)

Conclusions and take-aways

- ALS is driven by a complex interplay of excitotoxicity, mitochondrial dysfunction, oxidative stress, protein aggregation, and neuroinflammation, leading to motor neuron death and paralysis. Understanding these mechanisms has led to therapeutic strategies, though no cure exists yet.
- Motor neuron degeneration and death refer to the progressive loss of motor neurons—specialized nerve cells that control voluntary muscle movements. Progressive loss of these neurons leads to impaired muscle control, weakness, atrophy, and paralysis. This process is central to ALS The degeneration selectively affects both upper motor neurons in the brain and lower motor neurons in the brainstem and spinal cord.
- Motor neuron degeneration and death in ALS result from a combination of ten (10) pathological mechanisms: glutamate excitotoxicity, mitochondrial dysfunction, oxidative stress, protein aggregation, dysregulated nuclear RNA and toxic RNA accumulation, neuroinflammation, axonal transport deficit, dysfunction of autophagy and proteasomal pathways, C9orf72 repeat expansion toxicity, and apoptosis. This process leads to progressive muscle weakness, paralysis, and ultimately respiratory failure.



Reference: Van Damme, Robberecht, and Van Den Bosc (2017)

Figure 1: Ten proposed disease mechanisms for ALS and associated genes

- Glutamate excitotoxicity is a pathological process in which excessive or prolonged stimulation of neurons leads to neuronal damage and death. Excess glutamate overstimulates motor neurons, leading to toxic calcium influx. Motor neurons have low calcium-buffering capacity, making them highly vulnerable to excitotoxic damage.

- Mitochondria are essential organelles responsible for energy production (ATP synthesis), calcium homeostasis, and apoptosis regulation. Mitochondrial dysfunction is a major contributor to motor neuron degeneration in ALS, leading to energy deficits and increased production of reactive oxygen species, contributing to oxidative stress and apoptosis.

- Oxidative stress plays a critical role in motor neuron degeneration, leading to progressive muscle weakness and paralysis. Accumulation of ROS damages proteins, lipids, and DNA, further accelerating neuronal degeneration.

- Misfolded proteins lose their normal function and form toxic aggregates, disrupting essential cellular processes, leading to motor neuron degeneration and disease progression and triggering neuronal death. Targeting these aggregates is a promising therapeutic approach.

- Understanding RNA dysregulation in ALS is crucial for developing RNA-targeted therapies, such as antisense oligonucleotides, which aim to modify RNA processing and reduce disease progression.

- Dysregulated nuclear RNA processing in ALS refers to abnormalities in how RNA is transcribed, spliced, transported, and degraded in neurons, contributing to the disease's progression. Defects in RNA metabolism play a major role in ALS.

- Neuroinflammation is a critical area of research in ALS, as controlling it may slow the progression of the disease and improve outcomes. It refers to the chronic activation of the brain’s immune system, particularly the glial cells (microglia and astrocytes), which play a significant role in the disease's progression.

- Dysfunctional astrocytes, which normally support neurons, become reactive, fail to support motor neurons, exacerbate disease progression, and contribute to motor neuron toxicity. The consequences are chronic inflammation worsens oxidative stress and excitotoxicity, and increased neuronal stress and death.

- Axonal transport deficits in ALS refer to disruptions in the movement of essential cellular components along the long projections (axons) of neurons. It is crucial for maintaining the health and function of motor neurons. When it is impaired, neurons degenerate, leading to muscle weakness and paralysis.

- Dysfunction of autophagy and proteasomal pathways lead to the accumulation of toxic protein aggregates, which contribute to motor neuron degeneration.

- C9orf72 repeat expansion toxicity is the most common genetic cause of ALS and frontotemporal dementia, contributing to neuronal dysfunction and cell death.

- Apoptosis plays a significant role in motor neuron degeneration in ALS. While apoptosis is a normal mechanism for removing damaged cells, in ALS, it becomes excessively activated, contributing to the progressive loss of motor neurons.

- A summary of ALS action mechanisms, effects on motor neurons and possible treatments was provided including effects on motor neurons and possible therapies.

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