Pathogenesis of amyotrophic lateral sclerosis

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ABSTRACT

Introduction

Amyotrophic lateral sclerosis (ALS) or motor neuron disease is a rapidly progressive neurodegenerative disorder. The primary involvement is of motor neurons in the brain, spinal cord, and peripherally. There is secondary weakness of muscles, and primary involvement of other brain regions, especially involving cognition.

Sources of data

Peer- reviewed journal articles and reviews. PubMed.gov

Areas of agreement

The pathogenesis of ALS remains largely unknown. There are a wide range of potential mechanisms, related to neurodegeneration. An increasing number of genetic factors are recognized.

Areas of controversy

There remains controversy, or lack of knowledge, in explaining how cellular events manifest as the complex human disease. There is controversy as to how well cellular and animal models of disease relate to the human disease.

Growing points

Large scale international collaborative genetic epidemiological studies are replacing local studies. Therapies related to pathogenesis remain elusive, with the greatest advances to date relating to provision of care (including multidisciplinary management), and supportive care (nutrition and respiratory support).

Areas timely for developing research

The identification of C9orf72 hexanucleotide repeats as the most frequent genetic background to ALS, and the association with frontotemporal dementia, gives the potential of a genetic background against which to study other risk factors, triggers, and pathogenic mechanisms, and to develop potential therapies.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) or motor neuron disease in its purest form is a readily identified clinical condition. It is a rapidly progressive degeneration of upper and lower motor neurons, that results in weakness and wasting of muscle in the arms, leg, trunk and bulbar region. There is associated spasticity in the arms, legs and bulbar regions. Clinical symptoms include loss of ambulation, loss of arm and hand function, difficulty with speech and swallowing, and breathlessness. Aspiration pneumonia and respiratory insufficiency are common terminal events. The characteristic onset is from symptoms and signs at a single site, progressing to contiguous regions, often leading to death within 3 to 5 years of symptom onset.

A wider range of presentation is recognized, and supported on a pathological and genetic basis. In particular the recognition of cognitive dysfunction, and specifically an association with behavioural variant frontotemporal dementia (FTD).

Despite increasing recognition of genetic and pathological contributions, the details of pathogenesis remain unclear. Disease modifying therapy is limited to riluzole (if nutrition and respiratory support are excluded), and there is no curative treatment. Key overriding considerations in pathogenesis include the contribution of environment and genetics, and the relatively selective vulnerability of motor neurons. The pathogenesis may be conceptualized to have a background vulnerability, a trigger factor, a propagation mechanism, and

the final manifestations of the clinical disease ALS. Each of these may involve different factors. This review explores recent evidence adding to our understanding of ALS pathogenesis, but is inevitably not comprehensive, the evidence may be conflicting, and there remains no fully confirmed pathogenic mechanism. The references tend to be reviews, where the prime publications in the field may be obtained, and are not comprehensive. Using statistical modelling, a six step process has been identified as underlying ALS pathogenesis, with conceptual similarities to cancer pathogenesis.[1] The findings at the late stage of disease probably do not represent, and may mask, factors earlier in the disease, which predates onset of any symptoms and signs.

PATHOGENESIS OF ALS

1. Background vulnerability

Epidemiological studies of ALS show a worldwide incidence of 2-3 per year per 100,000 population over the age of 15 years, and an overall lifetime risk of developing ALS of 1:350 for men and 1:400 for women. Risk increases with age, with a peak around 75 years.[2] Around 10% of individuals have a family history of ALS, and other neurodegenerative conditions including FTD. A study of twin data suggested that heritability contributes 60% to the risk of developing ALS, and environment 40%[3], but an analysis from three genome-wide association studies found a lower heritability of 21%. [4]

Genes

An increasing number of genes are recognized as associated, and causative of ALS.[5] The most common are C9orf72, SOD1, TARDBP, and FUS. Commonly proposed pathogenic mechanisms include RNA metabolism, and protein metabolism (Figure 1, Table 1).[6]

C9ORF72

C9orf72 is the most frequent genetic change identified in patients with ALS. It is also specifically found in patients with FTD. Expansions are found in around 39% of familial ALS patients, and 7% of apparently sporadic cases, of European ancestry[7], but with significant differences between populations. For example, a study of 563 patients with ALS in Japan identified expansions in 0.4% of sporadic and 0% of familial ALS patients[8], and in less than 1% of Chinese sporadic ALS patients.[9] Patients with ALS often display cognitive changes, and there is an overlap between ALS and FTD.[10] Behavioural variant FTD is the most common clinical syndrome, with features of apathy, disinhibition, socially inappropriate behaviour, abnormal eating behaviours, loss of empathy, and perseverative, stereotyped or obsessive-compulsive behaviour. Hallucinations and delusions may occur. Within a family some individuals may have ALS, and some FTD. The genetic feature is a hexanucleotide repeat of GGGGCC, either in the promotor or intron 1 of the gene. The normal range of repeats is uncertain, but usually

considered up to 20 repeats, while patients with ALS and FTD usually have repeats of several hundred to thousands. Inheritance is autosomal dominant. There is no clear intergenerational anticipation, however, short expansions (45-78 repeats) have been associated with age at onset and C9orf72 families displayed possible anticipation. [11] There is TDP-43 pathology. There are also p62-positive neuronal cytoplasmic inclusions, composed of dipeptide repeat proteins (DPR) formed by translation from the abnormally expanded repeat in C9orf72. A particular feature of the C9orf72 expansion is repeat-associated non-ATG (RAN) translation, that can occur in all six sense and antisense frames, resulting in five different DPR proteins.[12] The mechanisms of toxicity remain unclear, but a richer understanding is gradually being achieved.[13]

SOD1

Copper zinc superoxide dismutase (SOD1) was the first genetic cause of ALS to be identified, in 1993.[14] Mutations are commonly estimated to be present in around 20% of familial patients, and 1% of apparently sporadic. The frequency will vary between populations, and in an Italian population SOD1 mutations were identified in 14% of familial ALS patients.[15] Founder haplotypes may have significant effects on frequency. The mutations are predominantly point mutations. Inheritance is autosomal dominant. There are over 170 different genetic alterations recognized, although many of these are private to individuals, and causation may be difficult to prove outside large families. Disease pathogenesis remains uncertain, but an unusual feature is the absence of TDP-43

pathology now thought to be typical of ALS otherwise. The disease mechanism appears to be a gain-of-function, and not a loss of enzyme function. Protein misfolding, and oxidative stress are amongst the possible mechanisms.

TARDBP

TAR DNA-binding protein 43 (TARDBP/TDP-43) mutations are found in around 5% of patients with familial ALS.[16] Inheritance is autosomal dominant. TDP-43 is the protein typically found in the tau- and alpha-synuclein-negative, ubiquinated, cytoplasmic inclusions or aggregates found in ALS, and a subset of FTD. TDP-43 is an RNA and DNA binding protein that regulates transcription, mRNA splicing, transport and stability. Current pathogenic concepts include a gain of function toxicity of mutant TDP-43 in disruption of stress granules, that aggregate and form cytoplasmic ubiqutinated protiein inclusions. Loss of function may occur through depletion of TDP-43 in the nucleus of the motor neuron, when the TDP-43 is included in the cytoplasmic ubiquitinated protein inclusions induced by mutant TARDBP, resulting in dysregulation of nuclear RNA metabolism.

FUS

Fused in sarcoma / translated in liposarcoma (FUS) is also found in around 5% of patients with familial ALS.[17] FUS is a nucleoprotein, that regulates RNA and DNA binding, gene expression, and mRNA splicing. FUS colocalises with TDP-43

to stress granules in the motor neuron. Potential pathogenic mechanisms are similar to those of TDP-43.

Other genes

Rarer genetic causes of ALS include vehicle-associated membrane protein B (VAPB); valosin-containing protein (VCP) (also found in patients with inclusion body myopathy, Paget disease and FTD (IBMPFD). Ubiquilin-2 (UBQLN2) is a rare form of X linked ALS. Alsin (ALS2) is associated with atypical juvenile forms of ALS, with recessive inheritance. Other genes include optineurin (OPTN), and spastacin (SPG11).[18] The role of genes such as angiogenin (ANG) remains controversial.

Other genes are being recognized as risk factors for ALS, including heavy chain neurofilament (NEFH), and progranulin (GRN). There are a number of reports of an intermediate length expansion of the CAG repeat in the ataxin-2 gene (ATXN2), that with more than 34 expansions cause spinocerebellar ataxia type 2 (SCA2), with repeat numbers of 27-33 found in 5% of patients with ALS.

Next-generation sequencing technologies allow rapid and relatively inexpensive sequencing of ALS-related genes. Sequencing 17 known genes, 64% of familial and 28% of sporadic ALS patients had potentially pathogenic novel or rare coding variants. 4% of patients had variants in more than one gene. This gives further insight to the oligogenic pathogenesis of ALS, and complicates the clinical interpretation of genetic analysis of patients.[19,20]

Epigenetics

Epigenetics provides a potential link between environmental and genetic pathogenic mechanisms. Environmental factors switch genes on and off and affect how cells express genes, rather than changes in a DNA sequence. This includes DNA methylation, histone modification, and noncoding RNAs such as microRNA (miRNA). A range of epigenetic mechanisms and findings have been reviewed.[21]

2. Trigger factors

Investigating the earliest stages of ALS in patients is difficult, but pathological studies point to a focal onset, occurring at an apparently random location, and progressing contiguously.[22] This may be an explanation for the range of clinical presentations, i.e. ALS or progressive muscular atrophy (PMA); bulbar or limb onset; ALS or FTD; on the background in some instances of a strong genetic mutation. Inevitably, autopsy data represents the end stage of the disease, and it is difficult to extrapolate to the onset of disease. An alternative theory to contiguous propagation is a network propagation i.e. through axonal and synaptic connections.[23] Protein misfolding and templating of pathogenic proteins, a prion-like mechanism, is currently being explored in ALS and many other neurodegenerative diseases. An EMG approach to determining

propagation did not support contiguous spread, and suggested "multifocal hits with local propagation".[24]

Imaging studies are becoming increasingly sophisticated. Longitudinal analysis of MRI scans of patients with ALS identified a core white matter pathology – that appeared to be stable – and reflecting upper motor neuron clinical features, but with a progressive grey matter pathology that was widespread, and interpreted as representing a widespread cortical network degeneration.[25] This has implications for developing and monitoring potential therapies.

Epidemiological studies are difficult to perform in ALS, and some results are contradictory. Physical activity, including sports and repeated traumatic events was not found to be a risk factor for ALS, and could be protective.[26] Repeated head trauma was associated with risk of ALS,[20] and intriguingly TDP-43-positive inclusions have been identified in the frontotemporal brain and spinal cord of people with chronic traumatic encephalopathy.[27] There are studies suggesting associations with football, smoking, heavy metals, pesticides and chemicals, occupation, military service, electric shock, geography, and cyanotoxins, but the evidence is not clear, and proof of causation not available.[2,28]

3. Propagation

There are a wide range of potential pathogenic mechanisms, reflecting the range and fashion of mechanisms of neurodegenration. A pathway analysis of results

from two ALS genome-wide association studies (GWAS) supported shared genetic pathways with Alzheimer's disease and Parkinsons's disease.[29] A range of neurodegenerative diseases, beyond ALS an FTD, are more frequent than expected in individuals with large C9orf72 hexanucleotide repeat expansions.[30] It remains probable that ALS is the final end point of a number of different pathogenic pathways, converging at the clinical presentation. Different individuals may have contributions from different pathways, as supported by the range of potential genetic backgrounds (Figure 2).[31]

RNA processing

The dominant theory of ALS pathogenesis at present relates to RNA processing. In particular this is due to the finding of TDP-43 pathology in most ALS cases, and genes including TARDBP and FUS being genetic causes of ALS. These genes are both involved in pre-mRNA splicing, RNA transport and RNA translation. The pre-mRNA containing the C9orf72 repeat expansion may sequester nuclear RNA-binding proteins that are then unavailable for correct splicing of other mRNAs. MicroRNAs (miRNA) are dysregulated in FTD-ALS, and may be disrupted by C9ORF72, TDP-43, FUS, and other genetic pathways associated with ALS.[32]

Protein aggregation

Intraneuronal protein aggregates, including TDP-43, FUS, and SOD1 are well recognised in pathological studies of patients with ALS, and in animal models of

the disease. It is proposed that the aggregates disturb normal protein homeostasis (proteostasis) and induce cellular stress. The aggregates may sequester RNA and other proteins essential for normal cellular function. The physical effect of the aggregates may cause impaired axonal transport. There may also be impaired protein degradation, related to failure of ubiquitin-dependent protein degradation. Energetic exhaustion of motor neurons may relate to the turnover of misfolded proteins.[33]

Oxidative stress

Oxidative stress is implicated in the pathogenesis of neurodegenerative diseases, including ALS. When first identified, SOD1 mutations suggested a possible primary role in oxidative stress related to the superoxide dismuatase function. This does not appear to be the case, and oxidative stress is probably a secondary component of pathogenesis.

Mitochondria

Mitochondrial dysfunction is a common feature of many neurodegenerative disorders. There is a body of evidence to support impaired mitochondrial bioenergetics, but also possible roles related to mitophagy and quality control[34], endoplasmic reticulum stress and calcium signalling.[35] Mutant SOD1 accumulates in the intermembrane space of mitochondria, leading to mitochondrial dysfunction.[36] Mitochondrial dysfunction has been hypothesized to explain a range of clinical features of ALS, including motor neuron

hyperexcitability, fasciculations, and differential motor neuron vulnerability.[37]

Mutations of the CHCHD10 gene have been identified in patients with ALS.

CHCHD10 is a mitochondrial protein located in the intermembrane space and

enriched at cristae junctions. The phenotype of the patients included FTD.[38]

Excitotoxicity

Glutamate excitotoxicity has long been implicated in ALS pathogenesis. This was

supported by neurochemistry studies, and is a potential explanation of selective

neuronal vulnerability. This was the basis of trialling riluzole as a treatment for

ALS, as a glutamate-release inhibitor. The mechanism of riluzole is unclear, but

also has an effect on sodium channels, and calcium-activated potassium

channels.[39]

Growth factors

Growth factors are essential in neuronal growth, maintenance and repair. In

previous decades there was interest related to their potential therapeutic use,

but without success. There is current interest in a Nogo-A monoclonal antibody,

to encourage nerve growth.[40] Nogo-A is a neurite outgrowth inhibitor that has

been shown to be overexpressed in skeletal muscle in ALS.

Axoplasmic flow

A unique feature of spinal nerves is the long axon. Essential to the maintenance

of the axon structure, and transport of proteins, mitochondria, and other

important traffic between the nucleus and cell body, and the neuromuscular

junction or other synapses, are neurofilaments. Neurofilaments are the most

abundant cytoskeletal protein in motor neurons, and determine axon diameter.

There are well demonstrated defects of axonal transport in animal models, and

human pathological findings of impaired transport. In the majority of patients

disturbed axoplasmic flow is probably a secondary, but important, effect.

Templating

The concept of a proteinopathy, or protein-misfolding disorder, is an attractive

hypothesis for neurodegenerative disorders, including ALS.[41,42] Inclusions or

misfolded proteins are clearly a common feature of ALS pathology, whatever the

genetic or environmental basis. The protein misfolding, and templating, could be

an explanation for contiguous propagation. Calling ALS a prion disease has

probably confused the issue, and templating may be a preferred term, as this is

not proposed to be a prion protein. There is no evidence of infectivity of ALS,

although conjugal cases occur rarely. If templating is a propagation mechanism,

this should be amenable to potential therapeutic approaches.

Inflammatory

An inflammatory basis to neurodegeneration is well recognised. In ALS there is

an understandable focus on the motor neurons, but evidence exists that this is a

non-cell-autonomous disease, and non-neuronal cells, in particular microglia may have an important role in pathogenesis. This is a complex area, and much of the data is based on animal models.[43] Nevertheless, inflammatory pathways are a potential therapeutic target, especially in influencing rate of progression.[44]

Myogenic

There has been a long standing debate between the primary neurogenic – and now motor cortex and networks, and the primary myogenic – or neuromuscular junction – origin of ALS. The potential pathogenesis of ALS is, at present broad. For the patient it is the muscle weakness that causes major disability, and if the muscle weakness could be prevented or reversed, much of the disability would resolve. Muscle weakness unifies the wider presentations of spinal muscular atrophy, spinobulbar muscular atrophy, and the other age-related cachexias. Growth factors, axonal transport, mitochondrial dysfunction, and other factors impact on this potential cause, contributor or consequence.[45,46]

A number of genes that cause ALS also affect muscle, including VCP, MATR3, and CHCHD10, with differing phenotypes. VCP (Valosin-containing protein) mutations may present as ALS (with or without frontotemporal dementia), Charcot-Marie-Tooth disease type 2Y, or inclusion body myopathy with early-onset Paget disease and frontotemporal dementia.[47] MATR3 (Matrin 3) mutations may present as ALS, or a distal myopathy.[48] CHCHD10 (Coiled-coil-helix-coiled-coil-helix domain-containing protein 10) mutations may present as

ALS and/or frontotemporal dementia, spinal muscular atrophy Jokela type, or an autosomal dominant isolated mitochondrial myopathy.[38]

4. Final clinical manifestations

The final clinical manifestations relate to systems biology, and difficulties in understanding relate to the limited knowledge we have of the human nervous system in health and disease. A key question remains as to why the motor neurons are selectively involved. The presentation of limb weakness, muscle wasting, fasciculation, spasticity, and difficulties with speech and swallowing are more readily understood.[49].

Genotype-phenotype correlations are difficult to define, especially given the rarity of some specific mutations. There is an observation that patients with SOD mutations have predominantly lower limb onset, and with TARDP mutations have upper limb onset. Patients with FUS mutations have an earlier age of onset, and shorter lifespan.[50]

CONCLUSION

Therapeutic implications

The lack of a clear pathogenesis has hampered the development of an effective therapy. On the other hand, the abundance of potential mechanisms has led to many attempts at treatments which usually fail in clinical trials. [51] The potential

reasons for failure are many, and include the wrong target, inappropriate pharmacodynamics, route of administration, outcome measures, publication bias, and the probability that there may be different pathogenic mechanisms at play in different patients. The genetics of ALS has shown that this represents a plethora of diseases, rather than a single biological entity. The relevance of therapeutic benefit in cell and animal models of ALS remains uncertain.[52,53] In one individual there may be different mechanisms active at once, or at different stages of the disease. Upstream targets include genetic mechanisms such as SOD1. 21 ALS patients with a range of SOD1 mutations participated in a Phase 1 study of intrathecal antisense oligonucleotide ISIS 333611.[54] There are more than 150 different SOD1 mutations, some family specific. The antisense oligoneucleotide used in this study was designed to activate RNase H mediated degradation of all SOD1, rather than a specific mutant, allowing for a wider potential use, and possibility of an effective clinical trial. C9orf72 creates the potential for a more generic gene therapy.[55] Downstream targets, if identified, allow a more universal therapy, and may be common to other neurodegenerative diseases. ATXN2 (ataxin) remains another potential therapeutic target.[56]

Diagnostic implications

The identification of significant genetic contributions, allows genetic definition of a disease. This has widened our understanding of clinical presentations, including more focal, slowly progressive, and atypical forms, that would not fit classical diagnostic criteria.

Genetic implications

The identification of genes has the potential for greater understanding of ALS, and prevention or treatment in the future. At present, however, there is uncertainty of the significance of many reported mutations, given the age-dependent nature of ALS, the incomplete penetration of causal genes, and varying clinical presentations, including apparently different neurodegenerative diseases with C9orf72. There is much discussion of how best to approach this from a clinical point of view, and there are a range of theories.[57] At one extreme the identification of a gene may be helpful for decision making in families with clear multigeneration autosomal dominant inheritance, such as some families with SOD1 mutations. Given the potential future developments related to our understanding of ALS pathogenesis, genetics, and therapy, there is a strong argument of preserving DNA from affected individuals for future research. Some suggest that all patients with ALS should be tested for all genes and be provided with the relevant information, with appropriate explanation and support. Others suggest that only patients with a clear family history of ALS should be offered the possibility of genetic testing. This is further complicated by the current availability of laboratory testing for genes, and the associated cost. Advances in genetic technology are rapidly solving this issue, with large numbers of genes being sequenced at a relatively low cost. There is a range of clinical views on how to approach this, and a broad view is required.[58] We have achieved the situation where there are a large number of potential genes for ALS, some individuals may have more than one gene, some none, there is variable penetrance, and we do not know how these mutations cause the disease. From

being potentially potent in determining outcome, genetic analysis is now but another risk factor – although not to be dismissed. The situation would be transformed if we had preventive or curative approaches based on this knowledge.

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Table 1

Genes implicated in causation of ALS.

(Many of these are very rare causes, and the function given is putative.[5]

RNA metabolism

C9ORF72

TARDBP

FUS

hnRNPA1

hnRNPA2B1

SETX

TAF15

ANG

Antioxidant

SOD1

Cellular transport

ALS2

VAPB

Protein metabolism

SQSTM1

VCP

UBQLN2

OPTN

Axonal outgrowth

PFN1

Glutamatergic signaling

DAO

Genes that appear to modify ALS risk or progression

RNA metabolism

ATXN2

Cytosketal protein

NEFH

Protein metabolism

GRN

Angiogenesis

VEGF

Neurotransmission

UNC13A

Figure 1.

Approximate frequency of gene mutations in familial ALS (other known affected family members) and apparently sporadic ALS (no other known family members).

Figure 2.

An illustration of the complexity of ALS pathogenesis. The inner circle includes

the associated genes with highest frequency (C9orf72, SOD1, TDP-43/TARDBP,

and FUS). The second order ring includes the large number of genes with a

lower frequency of association. The third order ring includes the possible

pathogenic mechanisms that are hypothesised to be associated with these genes.

The outer ring includes the other diseases that may be associated with these

genes. The complex relationship between genes associated with

neurodegeneration, mechanisms of neurodegeneration, and clinical disease

phenotypes is apparent. Red = major genes; Black = minor genes; Orange =

disease mechanisms; Blue = associated diseases. Mechanistic connections are

illustrated by orange lines, and disease associations by blue lines.