CellPress

Review

The 'Omics' of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease that primarily affects motor neurons and is accompanied by sustained unregulated immune responses, but without clear indications of the ultimate causative mechanisms. The identification of a diverse array of ALS phenotypes, a series of recently discovered mutations, and the links between ALS and frontotemporal degeneration have significantly increased our knowledge of the disease. In this review we discuss the main features involved in ALS pathophysiology in the context of recent advances in 'omics' approaches, including genomics, proteomics, and others. We emphasize the pressing need to combine clinical imaging with various different parameters taken from omics fields to facilitate early, accurate diagnosis and rational drug design in the treatment of ALS.

ALS: A Dire Paradigm for an Intricate Disease

Despite its first description over 150 years ago by Charcot, ALS (also known as Lou Gehrig's disease) remains a pathology of significant challenges. There is a very limited knowledge of the relevant events transpiring before the disease becomes clinically evident, which has largely restricted the possibilities for prevention and therapy. Although ALS is a rare disease, with an incidence of 1–2:100 000 and a prevalence of 4–6:100 000, once diagnosed ALS is typically lethal within the next 5 years [1]. At the cellular level, ALS is characterized by the death of upper and lower **motor neurons** in the motor cortex, brainstem, and spinal cord, in what has been described as a **dying-back** (see Glossary) phenomenon from the most distal end of axons [2]. The arrangement of symptoms displayed by patients during the course of the disease reflects this progressive loss of motor neurons.

The majority of advances in our understanding of the cellular and molecular mechanisms underlying ALS are the result of efforts made over the past 20 years. An important discovery was the unveiling of missense mutations in the gene encoding the protein **superoxide dismutase 1** (SOD1) that are present in a significant percentage of familial cases. This led to the subsequent description of cellular dysfunction mechanisms triggered by mutant SOD1, and the development of the first transgenic mouse model $SOD1^{G93A}$ for basic research and drug testing. More recently, the presence of mutant **TAR DNA-binding protein 43** (TDP-43) in sporadic and familial cases, and the description of a hexanucleotide repeat expansion in **chromosome 9 open reading frame 72** (*C9ORF72*) in both familial and sporadic cases around the world have been reported [3]. This increased pace in the discovery of genomic anomalies in ALS is a reflection of the technological advances, particularly of those collectively known as 'omics'.

Trends

ALS, or amyotrophic lateral sclerosis, is a progressive neurodegenerative disease that affects motor neurons. There is no cure for ALS. Although ALS is a brain disease closely related to Parkinson's, Alzheimer's, and Huntington's diseases, so far the complex descriptions of ALS-associated damage have not clarified the ultimate causative mechanisms.

Current interventions are the result of unintentional discoveries or the non-specific application of cell-based therapies whose effects are not completely understood. However, research on ALS is currently thriving and the body of knowledge on the subject has increased remarkably in recent years.

The emergence of functional immunomics for ALS from established omics technologies are opening new therapeutic avenues based on the smart manipulation of the immune system.

Molecular imaging in the field of ALS is evolving. Thus, a combination of omics technologies and clinical imaging may very well be the key for breaking-down ALS.

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These findings have highlighted the long known complexity of ALS, with up to 21 ALS variant phenotypes described thus far. Importantly, new mutations have been linked to the familial and sporadic forms of the disease, and the limits between these artificial categories have therefore become harder to define. Adding further complexity are the immune manifestations accompanying the disease, such as sustained inflammation and its associated damage, as well as the cellular compensatory efforts that can drive progression instead of halting it. As such, **structural genomics** and the so-called functional genomics, transcriptomics, proteomics, **metabolomics**, and other omics seem well suited for the task of unmasking various mechanisms of ALS pathogenesis and progression, and for uncovering suitable targets in diagnosis, prophylaxis, and treatment.

Genomics of ALS

Structural genomics have largely contributed to the discovery of genes with mutations responsible for two thirds of familial ALS (fALS) cases, and to approximately 11% of sporadic ALS (sALS) [4]. This has underlined the complexity of ALS at the genomic level because fALS is now viewed as a polygenic rather than a monogenic disease [5]. Genomics studies are often preceded by the observation of a disease transmission pattern as seen in fALS because these inheritance patterns are suggestive of a genetic cause. Thus, mutations in the gene encoding SOD1 were the first genetic anomaly linked to fALS identified by linkage mutation analysis [6], and are currently considered to be gain of function mutations. To date, more than 150 mutations in the *SOD1* gene have been described. While the original finding of mutated *SOD1* shaped ALS basic and preclinical research up to the mid-2000s, the discovery of new mutations has opened up new research avenues.

Similarly to the identification of SOD1, mutations in the **fused in sarcoma** (*FUS*) gene were identified using the loss-of-heterozygosity mapping in a Cape Verdean family suffering from ALS [7]. While linkage analysis and gene mapping have been commonly used to find additional ALS related mutations [8], novel techniques such as **exome sequencing** have also been successful. Mutations have now been found in **profilin 1** (*PNF1*) [9], **ubiquilin 2** (*UBQLN2*) [10], **heterogeneous nuclear ribonucleoproteins** A1 and A2/B1 (*HNRNPA1* and HNRNP*A2B1*) [11], although the role of the latter is still unclear [12]. Very recently, exome sequencing has identified genes related to autophagosome maturation and the selective disposal of protein aggregates in a large ALS sample set [13]. These findings may lead to new therapeutic approaches because they highlight poorly studied associations between autophagy and neuroinflammation in the pathogenesis of ALS.

In other cases, the identification of aberrant or misplaced proteins found in ALS patients has led to the screening of mutations of the corresponding genes in both fALS and sALS. *TARDBP* was discovered by genome sequence analysis after TDP-43 protein inclusions were detected in biopsies of ALS patients [14]. This prompted the search for different mutations in the gene coding for this protein, *TARDBP* [15,16]. A key feature of ALS-associated TDP-43 seems to be its aggregated state and abnormal location in the cell (cytoplasmic instead of nuclear) in both sporadic and familial cases [17].

One of the biggest successes from ALS genomics has been the detection from two independent research teams, of a massive hexanucleotide repeat (GGGGCC) expansion in the *C9ORF72* gene, located in the noncoding region of chromosome 9, in some ALS patients [18,19]. Its importance is based on the presence of this mutation in 25–40% of fALS cases. The clue leading to this discovery was the observation that frontotemporal dementia (FTD) and ALS were frequently inherited together [20]. Three pathogenic mechanisms are currently thought to account for its deleterious effect: (i) accumulation of RNA transcripts that can act as a trap for RNA-binding proteins [21]; (ii) expression of dipeptides $(Gly\text{-}Arg)_n$, $(Gly\text{-}Pro)_n$, and $(Pro\text{-}Arg)_n$ that can bind to the heterogeneous