Convergence in Motor Nerve Diseases: Relating ALS Etiology to Diagnosis in MND Mimics

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive, paralytic disorder characterized by degeneration of motor neurons in the brain and spinal cord, with the eventual atrophy of most muscles, including those of the diaphragm. Among the motor nerve diseases (MNDs), ALS is the most common, amounting to 80% of cases, and has an overall prevalence of about 1 to 9 cases per 100,000 across the globe. Research into the etiological factors contributing to the disease has shown that multiple factors are causative for ALS, with three major domains of cellular processes - RNA and protein processing and homeostasis, cytoskeletal mechanics, and neuroinflammation - among the predominant factors discovered to date. Conversely, much less is known of the non-ALS MNDs, which variously mimic ALS symptoms, anatomical targeting and disease progression. Based on their resemblance and recent discoveries of shared causative mechanisms, knowledge of their etiology and degenerative events could benefit from ALS findings, which can be expected to be mutually beneficial for ALS diagnosis and therapy. Accordingly, this paper presents current genetic, mechanistic, and biomarker findings of shared dysfunctions of ALS and the non ALS MNDs, illustrating a number of converging mechanisms that could be targeted to assess prognosis and therapeutic intervention.

Keywords: *Motor Nerve Diseases; Amyotrophic Lateral Sclerosis; Spinal Muscular Atrophy; ASC 1 Complex; Primary Lateral Sclerosis; Biomarkers; Neurofilament Protein*

Introduction

Amyotrophic lateral sclerosis is a progressive, paralytic disorder characterized by degeneration of motor neurons in the brain and spinal cord, with the eventual atrophy of most muscles, including those of the diaphragm [1]. Death due to respiratory paralysis typically occurs within three to five years. Among the motor nerve diseases (MND), ALS is the most common, amounting to 80% of cases, and has an overall prevalence of about 1 to 9 cases per 100,000 across the globe. In the advanced countries of United States and Europe, the cumulative lifetime risk of ALS is about 1 in 400, which translates to 800,000 persons who are now alive who will die from ALS in the United States alone. About 10% of these individuals are expected to have familial ALS, usually inherited as dominant traits, and the remaining 90% of cases will be sporadic, that is, occurring without a family history. ALS tends to peak in the sixth decade, although young adults have been diagnosed, who typically belong to the familial group.

Clinically, there is considerable heterogeneity with respect to the populations of motor neurons involved and symptomatic expression. Failure of corticospinal (upper) motor neurons, for example, results in muscle stiffness and spasticity, whereas the failure of lower motor neurons exhibits excessive electrical irritability that is seen in spontaneous muscle twitching termed fasciculations. With neuron degeneration and loss of synaptic connectivity to target muscles, muscle atrophy invariably ensues. Although most cases of ALS typically present initially in the limbs, about one third of the cases are bulbar, seen, for instance, in difficulties with chewing, speaking, or swallowing. About 15 to 20% of persons with ALS have progressive cognitive abnormalities, often accompanied by distinct behavioral changes resulting in dementia [2], which have been shown to correlate with degeneration of the frontal and temporal lobes.

While a diagnosis of ALS is increasingly facile as the disease progresses, early diagnoses are prone to error. Its presentation with such non-specific symptoms as limb weakness, fasciculations, and fatigue, which can comprise both classic upper/bulbar and lower motor neuron manifestations, is variously mimicked by other motor neuron diseases [3]. In primary lateral sclerosis (PLS), for example, there is selective involvement of corticospinal and corticopontine motor neurons, with few findings of lower motor neuron dysfunction. Consistent with a largely upper motor nerve dysfunction, PLS exhibits severe spastic muscle stiffness and little muscle atrophy. The disorder, moreover, overlaps clinically with a broad group of corticospinal disorders termed hereditary spastic paraplegias, which feature symmetrical onset, slow progression, and possible sensory losses. In progressive muscular atrophy (PMA), on the other hand, lower motor neuron involvement is predominant, with little spasticity. Like ALS, its pathophysiology manifests similar muscle atrophy and electromyography. Additionally, histopathology reveals a gradual neuronal degeneration within the pyramidal tracts. Several additional MNDs also act as confounders for ALS. Diseases such as Spinal Muscular Atrophy (SMA), Spinal and Bulbar Muscular Atrophy (SBMA), Postpolio Syndrome (PPS), and Progressive Bulbar Palsy display closely allied symptomatology. Despite these similarities, overall the MNDs are sufficiently distinct as to warrant classification as mimics of ALS with only two, PMA and PBP occasionally designated ALS variants.

Currently, neuroimaging plays a chief role in the diagnosis and exclusion of ALS [3]. Conventional magnetic resonance imaging (MRI) is used, for instance, in excluding ALS MND mimics in addition to other non MNDs, such as multiple sclerosis or other inflammatory conditions, which can resemble ALS. Radiographic MRI sequences for ALS demonstrate bilateral symmetric T2 and FLAIR hyperintensities anywhere along the corticospinal tract superiorly from the cortices extending caudally to the brainstem. Generalized decreases in cerebral volume have also been reported, especially in the gray matter of the frontal and temporal lobes. Other imaging techniques, including positron emission tomography (PET), diffusion tensor imaging (DTI), and MR spectroscopy (MRS) also play significant diagnostic functions that can exhibit decreased radiotracer uptake, neuronal loss in the dorsal prefrontal cortices, or alterations in metabolic substances, respectively.

Despite the utility of neuroimaging, the need for early diagnosis, with the prospect of long term neurological sequelae in its absence, has been the stimulus for early detection methods that can elicit more rapid therapeutic intervention. Only two drugs, riluzole and edaravone, for example, have been used for patients and both are more effective at the early stages of ALS. Research into the etiological factors of ALS and their contribution to disease progression, by contrast, is yielding a substantial body of findings at genetic, cellular, and systemic levels. These have shown that multiple factors are causative for ALS, including mechanisms linked to protein processing and homeostasis, cytoskeletal mechanics, and neuroinflammation among the dominant causes discovered to date. Benefitting from these studies, for instance, biomarkers have become the focus of intense research with the hope that they may aid early identification of ALS vis a vis other motor nerve diseases, monitor disease progression, and assess therapeutic development and efficacy.

On the other hand, much less is known of MND diseases that mimic aspects of ALS [3]. Their resemblance to ALS dysfunction, nonetheless, is likely to reflect underlying similarities in molecular, cellular and even circuit mechanisms. Significantly, recent research has shown that molecular defects in spinal muscular atrophy (SMA) are shared with one of the three chief causative factors for ALS, the processing of gene transcripts [4]. These results are therefore consistent with the notion that diagnosis of non ALS MNDs would generally benefit from ALS findings through their potential to reveal overlapping causative and degenerative mechanisms. For example, such study can be expected to assist in populating marker matrices that would be useful for gauging the evolution of MND diseases [5].

Extant work indicates that most MNDs are due to aberrant genetic factors that induce a succession of failures in fundamental subcellular systems, which are uniquely expressed in motor nerves [1]; that is, predisposing risk alleles initiate molecular cascades that converge at common points of disease evolution. The characterization of their convergence, therefore, is likely to be mutually beneficial for monitoring disease progression and in gauging therapeutic development and interventional efficacy. This paper will review current studies bridging the genetic induction of these diseases to their ensuing cellular events, illustrating common and diverging mechanisms that both link and distinguish ALS and the non ALS MNDs.

Genetic Elements of ALS and the MND Mimics

Linking MNDs to the three major etiological categories are the discoveries of numerous alleles affecting key elements of their mechanisms. In total, some 25 different genes have been implicated as risk alleles for ALS [1] with most falling within these domains. The identification of risk alleles for other MNDs has also advanced, though not to the same degree [6]. These latter findings generally confirm the unique classification of most MNDs and in cases, like that of SMA, also reveal the close interaction of their protein products with those of ALS risk alleles.

ALS genetics

Cytoskeletal associated risk alleles

Three ALS genes are known to encode proteins that are important to maintaining normal cytoskeletal functions, which have been shown to involve microtubules and actin polymers. Affecting microtubule function are the microtubule associated proteins tubulin 4A and dynactin, whereas mutant versions of Profilin 1 affect actin filaments. Also implicated with actin function is the modifier gene *EPHA4* whose activity limits axon extension.

Microtubules comprise one of the major cytoskeletal filament systems of eukaryotic cells and function chiefly in maintaining axonal structure and transport. They serve as a platform along which secretory vesicles, organelles, and macromolecular assemblies are transported between the cell soma and synaptic terminals. Mutations in Tubulin 4A inhibit microtubule polymerization leading to a weakening of axonal structure and reduced transport [7]. Dynactin, a 23 member polymer required for the binding of transported elements to the retrograde axonal transport motor protein, dynein, is also associated with synapse formation. Dynactin mutants accumulate in synapses where they appear to induce the formation of abnormally wide neuromuscular junctions [8,9]. Mutations in dynactin thus impact both retrograde axonal transport and synaptic structure. The actin associated protein profilin functions in nerve growth cone formation, with profilin supporting filament assembly at its barbed ends through its binding to G-actin. Profilin mutations reduce the ability of the protein to shift its conformation between free and bound forms thus affecting actin homeostasis and growth cones and is likely to also affect the coordination between the actin and microtubule cytoskeletons [10]. Increased expression of ephrin 4 has been shown to slow the extension of motor axons.

RNA and protein processing alleles

An expanding group of ALS related alleles codes for RNA interacting proteins. Products of these alleles are involved with localizing transcribed sequences to their processing sites, their exchange between nucleus and cytoplasm, and RNA splicing events, among others [1]. A key RNA binding protein implicated in ALS is coded by the allele TDP-43. Through its involvement in the cutting and splicing of mRNA transcript molecules into different sequences, the TDP-43 protein controls the production of closely related protein variants.

Its mislocalization from the nucleus to the cytosol, and improper posttranslational processing, including cleavage, phosphorylation, and ubiquitination, have been observed in sporadic ALS. Significantly, TDP-43 is likely to be causative for frontotemporal dementia also, as well as other neurodegenerative diseases [11]. *FUS-TLS* encodes another RNA-binding protein that is homologous to TDP-43 and that in mutant forms is also causative for ALS. FUS/TLS binds directly to U1-snRNP and SMN complexes. Its mutations abnormally enhance interaction with SMN1 dysregulating its function through a reduced affinity for U1-snRNP, thereby affecting RNA splicing activity. Other proteins associated with RNA processing include, ATXN2, TAF15, EWSR1, hnRNPA1, hnRNPA2/B1, MATR3 and TIA1. Among the altered effects of these mutants, in addition to the aforementioned, are aberrant stress granule dynamics and an over propensity to aggregate [12].

Besides alleles that modify RNA transcripts, a number are also involved with the processing of protein products and homeostasis, i.e. stages in protein processing subsequent to RNA mediated specialization. These include defects in cellular pathways for protein degrada-

tion and adapter proteins involved in protein maintenance and degradation. The alleles code for valosin-containing protein (VCP) and the proteins optineurin (OPTN), TANK-binding kinase 1 (TBK1), and sequestosome 1 (SQSTM1/p62). Interestingly, the TBK1-OPTN protein has also been linked to Parkinson's disease gene via the gene *PINK1.*

One of the most frequently observed mutations in ALS occurs in the C9ORF72 gene [13]. Various studies of this allele suggest that a hexanucleotide repeat expansion in the first intron of the gene is the most common cause of familial FTD and ALS pathology. In normal individuals the hexanucleotide is repeated up to approximately 30 times, whereas it is found in hundreds or even thousands of times in ALS and frontotemporal dementia individuals [14]. The C9ORF72 protein is thought to function as a guanine nucleotide exchange factor (GEF) that acts to regulate specific Rab GTPases, a role that appears to be critical for lysosome biogenesis, vesicular trafficking, autophagy, and rapamycin complex1 (mTORC1) signaling.

Neuroinflammatory alleles

The superoxide dismutase (SOD1) mutation has been shown to strongly induce neuroinflammation [15]. Microglia and reactive astrocytes accumulate in the spinal cord of rats expressing the (ALS)-linked SOD1 mutation where they surround motor neurons, generating a localized environment that is highly toxic. In mouse models, moreover, elevated neuron-specific expression of SOD1 induces motor neuron degeneration and paralysis. Correspondingly, spinal cords of patients with ALS also exhibit astrocytosis in both the ventral and dorsal horns. The SOD1 protein is known to bind copper and zinc ions and is one of three superoxide dismutases responsible for destroying free superoxide radicals in the body. The encoded isozyme acts as a homodimer to convert naturally occurring, but harmful, superoxide radicals to molecular oxygen and hydrogen peroxide. Interestingly, astrocytosis occurs in both cortical grey matter and subcortical white matter in the brain and is not restricted to the motor cortex, suggesting a more general effect of the mutation on nerve tissue. Consistent with a less specific impact, degeneration is variable as are symptoms, time of onset, and life expectancy. A majority of the observations of SOD1 are based on post-mortem tissue and so represent the final stages of the disease [16]. Accordingly, rodent models have been used to study disease progression, which show that microglial cells become activated before motor neurons disappear from the lumbar spinal cord at about 90 days of age, and so well before clinical disease onset. Astrocytic activation, moreover, parallels the decrease in neuronal cell bodies, which occurs initially and prominently in the ventral horn. The numbers of activated microglia and astrocytes increase further until the end stage of disease. Taken together, the data suggest that activation of both classes of cells leads to an ongoing and concomitant degeneration of motor nerve cells, particularly within the ventral horn.

The genetics of ALS motor nerve disease mimics

Considerably less is known of the genetics of non ALS-MNDs. Moreover, the contribution of genetics to the etiopathogenesis of these MNDs varies considerably as does the mode of inheritance, with examples of autosomal dominant (AD), autosomal recessive (AR), or X-linked kindreds. However, because MNDs can be distinguished by their anatomical targets - those with lower motor neuron involvement - spinal muscular atrophy, progressive muscular atrophy, postpolio syndrome and spinal bulbar muscular atrophy also known as Kennedy's disease; those with upper motor neuron involvement - primary lateral sclerosis (PLS)[1,2] and the spastic paraplegias; and those with combined upper and lower motor neuron involvement - amyotrophic lateral sclerosis (ALS) - regionally specific alleles are likely to exhibit comparable modes of action in both ALS and its respective mimics. These common alleles are discussed below.

Lower MNDs

SMA

Most cases of SMA are due to mutations (deletions, duplications, point mutations) in the survival motor neuron-1 (SMN1) gene (5q-SMA) [17] involving exons 7 and 8. Mutations in the SMN1 gene reduce the amount of SMN1 protein, which is essential for the assembly

of spliceosomal ribonucleoproteins [18] that function in processing RNA transcripts. Depending on the age at onset and the severity, four types of SMA (SMA 1 - 4) are distinguished, each with a distinct genotype, depending on the number of SMN2 genes producing residual amounts of fully functional SMN protein or a truncated protein (SMND7). SMA types 1-4 are usually caused by a homozygous deletion of exons 7 and 8 in the SMN1 gene [19] whereas an isolated deletion of exon 8 of the SMN1 gene is rare.

SMA-phenotype and disease severity are modulated by several genes near SMN1 in the 15q13 region, including SMN2, NAIF, GTF2H2, and H4F5, the best known of which is SMN2, which provides a small amount of stable SMN1 protein [20]. In humans SMN2 is nearly identical to SMN1 and SMN1 deletions are compensated by SMN2. All patients have been observed to retain one or more copies of the SMN2 gene, which modulates disease severity [20], but no patients have been reported lacking both genes. SMA develops only if SMN2 is unable to compensate for the SMN1 absence, which occurs when the loss of exon 7 results in a truncated SMN2. There is an inverse relation between disease severity and the copy number of SMN2.

Besides SMN1, causative genes for SMA include a number of others whose products also participate in RNA processing machinery. Among these are Exosome Component 8 (EXOSC8) [21], the Heat Shock Protein Family B (HSPB1), Thyroid Hormone Receptor Interactor 4 (TRIP4) and Activating Signal Cointegrator 1 Complex 1 (ASCC1) [22], which are components of the RNAP II/U1 snRNP machinery. Mutations in HSPB1 have also been identified in patients with another neuropathy, Charcot-Marie-Tooth disease [23]. Although none of these alleles are causative for ALS, their protein products have been shown to interact directly with products of ALS risk alleles that are associated with RNA processing. Such interaction is revealing for the multimeric nature of subcellular functions, and the likely siting of shared allele targets that can lead to either disease.

Finally, mutations in the motor adaptor BICD2 gene yield an SMA-phenotype characterized by proximal onset in early childhood with predominant involvement of the lower extremities, and very slow progression [24]. BICD2 mutations do not affect RNA modifying functions but instead represent another member of a group of alleles, which affects retrograde axonal transport. The mutation causes increased dynein-binding, with the accumulation of BICD2 in the microtubule-organizing complex and Golgi-fragmentation.

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a rare, primarily lower motor neuron X-linked recessive disease occurring in individuals aged 18 - 64 years. Given the genetic component, females with the gene are considered carriers and exhibit no symptoms. BSMA is caused by a CAG-triplet repeat expansion in the androgen receptor (AR) gene [25]. Mutation products of the AR gene appear to target FUS and the calcitonin gene related peptide a, which is involved in reducing blood calcium levels. Suppression of CGRP1 suppresses neurodegeneration in mice and overexpression has been shown to be cytotoxic.

Upper motor nerve diseases

Primary lateral sclerosis

Low frequency mutant variants of the FIG4 allele in ALS patients (about 2%), are also observed in primary lateral sclerosis (PLS), where heterozygosity for the mutant allele of FIG4 appears to be a risk factor for both diseases. FIG4 codes for a phosphoinositide 5-phosphatase that regulates the cellular abundance of $PI(3,5)P_2$, a signaling lipid located on the cytosolic surface of membranes of the late endosomal compartment [26]. $PI(3,5)P_2$ mediates retrograde trafficking of endosomal vesicles to the trans-Golgi network. While mutations manifest primarily in the upper motor neurons, inactivation of the phosphatase in mutant alleles of mice has been shown to result in widespread degeneration of neurons in sensory and autonomic ganglia, motor cortex, striatum, and other regions of the central nervous system, with extensive vacuolization of neurons. Within the sciatic-nerve-conduction velocity is also reduced. Mutations of the human FIG4 gene on chromosome 6q21 are also responsible for the recessively inherited disorder CMT4J (MIM 611228), a severe form of Charcot-Marie-Tooth disease (CMT) that affects both sensory and motor neurons.

Hereditary spastic paraplegia

Hereditary spastic paraplegia (HSP), also known as familial spastic paraplegia, is a heterogeneous group of conditions that clinically presents as an upper motor neuron disorder. HSP is distinctive for the axonal degeneration of the caudal ends of the corticospinal tracts and posterior columns. Members of the group variously include autosomal dominant and recessive, and X-linked, forms and are designated SPG, for spastic gait.

To date, five genes have been implicated in HSP, none of which fall within the three principal subcellular domains of ALS risk alleles. In X-linked HSP, SPG1 mutations of L1 cellular adhesion molecule (L1CAM) are strong risk alleles [27]. L1CAM is involved in neuronal guidance and the establishment of contact with other neurons, glia or muscle during development or injury. Mutations of L1CAM lead to a range of overlapping diseases, including CRASH (corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus), and MASA (mental retardation, aphasia, shuffling gait (with spasticity), and adducted thumbs). Another X-linked HSP is the group of SPG2 mutations that affect the myelin proteolipid protein (PLP), which is a component of CNS myelin.

Missense mutations in the gene SPG3A affect coding for the protein atlastin that binds guanylate and is GTPase active. Expression of atlastin occurs predominantly in human brain compared to other adult human tissues. Interestingly, the GTPase regulatory protein, alsin, appears to be causative in both ALS and PLS, implicating GTPase activity impairment as a causative mechanism in both diseases. Finally, SPG4 mutations of the spastin gene have been discovered, a medically large population where nearly 50% of all autosomal dominant HSP patients display mutations of the gene.

Converging Mechanisms in MNDs: Overlapping Influences in Disease Progression

The most extensive findings revealing a close overlap between the causative mechanisms of ALS and those of another MND involve the lower motor nerve disease SMA [28]. Mutant alleles in both diseases have been shown to affect a class of proteins associated with the post transcriptional processing of RNA gene transcripts.

ALS and SMA targeting of RNAP II/U1 snRNP machinery

In both diseases protein products of these alleles have been found to associate with the RNAP II/U1 snRNP complex, which splices pre mRNA transcripts and prevents the premature termination of the modified mRNA. Their siting within a relatively circumscribed, yet fundamental, cellular mechanism suggests that the proteins lie in close proximity where they mutually influence one another's function. How they interact with this machinery, and so with one another, however, appears to be distinct, with mutant proteins yielding unique but nonetheless converging mechanistic profiles eventually leading to motor nerve degeneration.

ALS and SMA proteins have distinct roles in the RNAP II/U1 snRNP machinery

All of the affected ALS proteins co-immuno precipitate with RNAP II following RNAse digestion, but not with the U1snRNP component, revealing that their chief association is with RNAP II [28] primarily through protein protein interactions; that is, neither U1 snRNP nor RNAs mediate binding between the ALS proteins and the RNAP II/U1 snRNP machinery. The use of gene knockouts in these studies showed that the complex was generally intact, indicating that individual knockouts did not affect the overall integrity of the complex when one or another member was removed and that the proteins generally interacted with unique components within the complex. In the ALS causative protein FUS KO, the majority of proteins that dissociate are splicing factors, in the EWSR1 KO, components of the tRNA ligase complex, in the TAF15 KO transcription factors, and in the MATR3 KO components of the ASC-1 complex. Together, these data indicate that, despite having a similar subcellular target, the proteins play largely distinct roles within the RNAP II/U1 snRNP machinery. However, the discovery that all four KOs also dissociated from the ASC-1 complex of the machinery indicated that they share a common

binding locus in this complex, which is likely to be the site of their common effects on degeneration. Members of the ASC-1 hub in ALS were identified as the proteins FUS, TAF15, EWSR1, MATR3, TIA1, Valosin Containing Protein (VCP), TARDBP, Heterogeneous Nuclear Ribonucleoprotein A1 (HNRNPA1), and Heterogeneous Nuclear Ribonucleoprotein A2/B1 (HNRNPA2B1), and in SMA, SMN1, EXOSC8, HSPB1, TRIP4 and ASCC1.

Biomarkers for ALS and non ALS motor nerve diseases

The presence of MND risk alleles is customarily taken as an indication of risk for a respective MND and occasionally is regarded as confirmatory (as in the case of SMN1 in SMA). Variants of certain specific alleles, moreover, provide insight into disease prognosis. For example, long before identification of the SMA causative gene, clinicians recognized a continuum of severity in SMA patients, which eventually resulted in a disease classification into types 1 - 4, with descending order corresponding to an increased severity and earlier onset of disease symptoms. The order of classification was shown to be correlated with *SMN2* copy number, which thus provided a molecular basis for the classification of the different SMA subtypes. As such the SMA2 allele copy number has provided a useful marker of SMA identity and prognostic outcome.

Most MND alleles, however, are relatively uninformative with respect to prognosis. For most of the chief reasons cited for developing of biomarkers, therefore - a deepening of disease understanding through exploration of pathophysiological mechanisms that highlights targets for novel therapies; a differentiation of the disease population into sub-groups, to characterize prognostic outcome; and assessment of therapeutic efficacy - risk alleles of MNDs are unlikely to be useful. Accordingly, recent research has focused extensive effort on identifying biomarkers that are more closed associated with disease progression.

Among these, biomarkers that detect points of convergence between MNDs are likely to offer greater insight into the temporal dynamics of common etiopathological mechanisms. For example, while there is striking heterogeneity in the genetic causes of familial ALS, both familial and sporadic ALS display similarities in their pathological, as well as in their clinical features, suggesting that the cellular and molecular events that lead to motor neuron degeneration follow similar pathways. These points of convergence thus also define therapeutic targets that could be identified with biomarkers. Limiting discovery, however, are a number of pragmatic concerns, including the development of biomarkers that are minimally invasive to obtain, simple to undertake, and time efficient, e.g., those derived from biofluids, such as blood, or CSF.

Common Biomarkers for Lower Motor Nerve Disease

ALS and SMA

Phosphorylated neurofilament heavy and neurofilament light chains.

As neuron-specific structural components of motor axons, neurofilaments are of interest as potential biomarkers. The neurofilament proteins, pNfH and NfL, are readily detected by antibody-based immunoassays and can be obtained from the CSF. Several studies have demonstrated their specificity for ALS and SMA [29]. In ALS they are released into CSF and peripheral blood in a broad range of pathological conditions. Because some studies reveal that the half-life of NF within axons may be up to 8 months, however, the presence of pNF-H in plasma could reflect release that occurred weeks or months earlier. The lack of a close temporal correlation with motor neuron degeneration means, therefore, that the impact of therapeutic intervention on disease progression can only be loosely guaged.

In SMA, plasma pNF-H levels are elevated in infants, with levels inversely correlating with age. Nusinersen treatment results in a significant decline in pNF-H levels and relative stabilization of motor neuron loss. Together these data suggest plasma pNF-H is a promising marker of disease activity/treatment response in infants with SMA. Nonetheless, as in ALS, the slow time course of release of neurofila-

ment proteins is likely to make determining its immediate effect difficult. Moreover, in both diseases effects on neurofilaments appear to occur much later than mRNA splicing and termination, which appears to be the causative mechanism shared by the two diseases.

RNA Related biomarkers in ALS and SMA

Recently, it has been demonstrated that aberrant miRNA expression contributes to disease pathophysiology in ALS and may represent a target for pharmacological intervention [28]. Increasing evidence suggests, moreover, that specific miRNAs determine the selective vulnerability of motor neurons in SMA also [30]. Two miRNAs, miR-9 and miR206, are associated with cytoskeletal dynamics, including intracellular transport and growth cone formation.

A practical advantage of miR-9 is its abundance in the CNS, which is useful for correlating its presence with functional differentiation. For example, miR-9 has been demonstrated to regulate axonal development in mammals through the inhibition of microtubule-associated protein 1B (MAP1B) [30] and to be reduced in the early phases of nerve injury in order to facilitate cell regeneration. MiR-206, on the other hand, has a neuro- protective role and is involved in the regeneration of neuromuscular junctions (NMJs). Silencing in ALS mouse models, for instance, accelerates disease progression, in which its serum level is increased, possibly in a compensatory manner.

Common biomarkers for upper motor nerve diseases

ALS and primary lateral sclerosis

Attempts to identify early stage biomarkers in upper motor nerve diseases have focused on electrophysiological indicators of subclinical effects. Comparisons of patients with PLS, who displayed a selective destruction of motor cortical layer V pyramidal neurons and degeneration of the corticospinal tract, but without involvement of anterior horn cells were found deficient in beta-band coherence unlike controls or PMA patients [31], who had normal corticospinal tracts but degeneration in the ventral horn. This finding has been taken to indicate that intermuscular coherence in the 15 - 30 Hz range of the EEG depends upon an intact corticospinal tract but not upon an unimpaired anterior horn and has therefore been proposed as a biomarker that can quantitatively assess subclinical upper motor neuron involvement in both diseases. Another physiological candidate for both diseases is the motor evoked potential (MEP) from the tongue and anterior tibialis muscles. In PLS patients [31] the central motor conduction time (CMCT), amplitude of MEPs, and duration of controlateral silent period (CSP) all showed statistically significant changes in PLS and upper motor nerve variants of ALS indicating the potential of these parameters for marking the progression of upper nerve degeneration.

Discussion

Research into the causes and outcomes of ALS is yielding an abundance of data about the underlying mechanisms leading to selective degeneration of motor neurons, information that is also revealing details of the close relationship between ALS and other MNDs. Evolving technologies for gene mapping and DNA analysis have aided the identification of multiple ALS genes that cluster in three subcellular categories, cytoskeletal dynamics, protein and RNA processing, homeostasis, and trafficking, and neuroinflammation. Downstream, dysfunctions in each category lead to distinct patterns of cellular abnormalities, such as the deposition of intranuclear and cytosolic protein and RNA aggregates, perturbed protein degradative mechanisms, mitochondrial dysfunction, defective nucleocytoplasmic trafficking, altered neuronal excitability, altered axonal transport, and endoplasmic reticulum stress [1]. In some cases, such as endoplasmic reticulum stress, these events also activate and recruit nonneuronal cells that amplify motor nerve destruction (astrocytes and microglia). A working hypothesis of ALS, therefore, implicates a genetic origin from multiple key genes, which independently or in concert result in a cascade of specific cellular abnormalities eventually leading to degeneration of the motor nerve.

Strikingly, MNDs can be distinguished according to their siting within the nervous system. While most MNDs recapitulate various features of ALS, these by and large selectively affect either upper or lower motor neuron groups. It is significant, moreover, that ALS is

multi-causal and that the prevalence of non ALS MNDs is a much smaller fraction of cases than ALS, less than 20% in total combined. Together these observations suggest that ALS is constituted from an umbrella of factors, which may be selectively or disproportionately perturbed in its various mimics. Current findings indicate that at least in the case of SMA there are overlapping mechanistic functions that are defective in both ALS and SMA. While shared mechanisms have yet to be observed in other MNDs, the selective degeneration of motor neurons suggests that unique but fundamental features distinguish this class of cells from other neurons and upper from lower motor neurons, features that are likely to be commonly affected by the MNDs and could be interrogated with the findings from ALS. The discovery of common risk alleles, like FIG, which is found both in PLS and ALS, and prospective biomarkers, represent examples that can be expected to facilitate this process of discovery.

One implication of the working view for diagnosis is that the sorts of inferences that may be made with respect to the level of diagnostic information is dependent upon the class of information that is obtained. The identification of risk alleles, for instance, is typically indicative of the presence of a particular MND, yet provides little information with respect to the downstream events that ensue. While this has incentivized the search for biomarkers, leading to the discovery of numerous candidates, the inability to easily monitor the direct gene products has meant that the use of these potential biomarker candidates will reflect effects that are both temporally and physically removed from the primary causes of the diseases. Hence, most current candidates do not monitor points of convergence early in the downstream cascade initiated by the mutant alleles.

Conclusion

Nonetheless, improvements in neuroimaging, metabolomics, and proteomics comprise a now large arsenal of diagnostic methods that has considerably expanded the prospects for assessing progression and prognosis. While the current diagnostic portrait for MND prognosis remains an approximate one, the discovery of numerous ALS specific alleles, the elucidation of their effects on subcellular targets, and the discoveries of shared etiopathological influences are likely to expedite the construction of a comprehensive view into ALS MND mimics as well.

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