

Title: Defining pre-symptomatic amyotrophic lateral sclerosis

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Summary

Successful treatment of neurodegenerative disease may hinge on early therapeutic intervention. This requires an understanding of early/pre-symptomatic disease, a need that is underscored by advances in antisense oligonucleotide and viral-vector-based gene therapies. In ALS, the study of pre-symptomatic disease ~~has been hampered, in part, by the absence of both a clear,~~requires a cohesive conceptual framework ~~and a well-defined lexicon~~ for describing this phase of disease. Informed by the literature in other neurodegenerative diseases and ~~our own~~ extensive personal experience, ~~we propose~~ a model is proposed that distinguishes ALS as a clinical syndrome from ALS as a disease, and characterizes pre-symptomatic ALS as having two identifiable stages: pre-manifest and prodromal. ~~We articulate~~ the unique and critical importance of biomarker development is articulated ~~s~~ and ~~offer~~ an operational definition of phenoconversion is ~~provided~~. ~~We are~~ It is hoped ful that this framework will accelerate ~~our~~ collective efforts to study pre-symptomatic ALS, and aid in the design and implementation of an early intervention- or disease-prevention trial.

Key words:

Pre-symptomatic

Pre-manifest

Prodromal

Biomarkers

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Introduction

Central to the vision of primary prevention for amyotrophic lateral sclerosis (ALS), an invariably fatal neurodegenerative disease, are answers to the questions “When does ALS begin?” and “What is the duration of the pre-symptomatic phase of disease?”. These questions have been the subject of considerable discussion¹⁻³, but a paucity of data has precluded a definitive answer⁴⁻¹⁰. Limitations of the available data reflect, in large part, the manifold challenges that are inherent to studying the onset and early progression of a relatively rare, adult-onset condition with few known risk factors¹¹⁻¹⁸. Additional complexity has emerged from the discovery that the motor-predominant neurodegenerative syndrome of ALS has clinical, genetic and pathological overlap with frontotemporal dementia, typically the behavioral variant¹⁹; and that the first clinical manifestations of disease may be motor or cognitive/behavioral, especially among those with particular genetic mutations, notably a *C9orf72* hexanucleotide repeat expansion²⁰.

An additional and perhaps under-recognized challenge, however, is the confusing array of terms (e.g. asymptomatic, pre-symptomatic) that has been used to describe the period of disease preceding the emergence of overt motor, cognitive or behavioral manifestations that would permit a clinical diagnosis. Since ~~our~~ choice of ~~words (language) terms~~ often shapes ~~our thinking~~ ~~(concepts)~~, which in turn influences research approaches and paradigms, it is essential to clarify the ~~lexicon-language~~ that surrounds the description and study of this aspect of the natural history of ALS. Here ~~we present~~ a conceptual framework ~~is presented~~ (Figure), informed by ~~our~~ ~~personal decade-long~~ experience studying individuals at genetic risk for ALS¹¹, and by relevant publications from the Huntington’s disease, Alzheimer’s disease and Parkinson’s disease literature²¹⁻²⁵. ~~Our~~ The hope is that this framework will propel the field forward in our

collective understanding of early disease - and perhaps even hasten the prospect of an early intervention or a disease prevention trial.

Pre-symptomatic ALS: A Conceptual Model

The terms ‘asymptomatic’ and ‘pre-symptomatic’ have been both used to describe the phase of disease prior to the emergence of clinically manifest disease – and both terms are reflected in common medical parlance. In infectious diseases, for example, we recognize an ‘incubation period’ between exposure to a microbial agent and the appearance of symptoms of disease – and people in this subclinical stage of disease are said to be asymptomatic ²⁶. By contrast, in the genetic arena, we typically speak of predictive or pre-symptomatic genetic testing among people with a family history of disease, but who have no symptoms or other manifestations of disease at the time of testing ²⁷. Since the study of this stage of disease in ALS has really only been possible in the population at genetic risk for disease, ~~we have a proclivity for~~ the term ‘pre-symptomatic’ is commonly used, which is similarly reflected in the literature relevant to the study of other neurodegenerative disorders ²⁸⁻³⁰ and encapsulates the idea that unaffected people with an ALS associated gene mutation have a high likelihood of developing ALS in the future.

ALS: Disease vs. Clinical Syndrome

The clinical syndrome of ALS is easily recognized based on a history of progressive (yet painless) weakness, along with evidence of both upper and lower motor neuron signs in the same body region. But the underlying disease, by which ~~we-is meant~~ the presence of some underlying biological (e.g. molecular, cellular, or systems-level) process that eventually leads to the clinical syndrome, ~~likely-almost certainly~~ begins before the clinical syndrome emerges. Such a paradigm is well recognized in cancer, infectious disease and even other neurodegenerative diseases such

as Alzheimer's³¹⁻³⁴, Parkinson's³⁵⁻³⁷ and Huntington's disease^{38, 39}. As such, we-it is possible to recognize both a pre-symptomatic and a symptomatic phase of disease in ALS (~~which we will~~ elaborated below). ~~The differentiation~~ A consequence of recognizing the distinction between ALS as a disease process (i.e. the pathobiology that underlies the degeneration of upper and lower motor neurons) vs. and ALS as a clinical syndrome (i.e. the overt clinical syndrome characterized by progressive painless weakness with both upper motor neuron and lower motor neuron signs on physical examination), it becomes necessary to itates differentiate a distinction between the onset of the underlying disease and from the onset of symptoms. The El Escorial criteria (original and revised^{40, 41}), ~~for example, provide a definition of~~ onset (without explicitly distinguishing 'disease' from 'clinical syndrome') as the "time of first subjective symptom noticed by the patient which later is confirmed by examination"; ~~imply~~ the language used by the authors of these criteria implicitly referencing the emergence of the clinical syndrome, but are silent on the question of when the disease process begins. Operationally Similarly, most clinical trials operationally specify the initial symptom to be weakness (excluding 'softer' symptoms such as fasciculations). By contrast, there has been relatively little discussion in the literature about an operational definition of the onset of disease^{1, 2, 42}

Risk for ALS: Genetic and Environmental Factors

In at least some individuals the pre-symptomatic phase of disease may be preceded by a period during which the individual is at an elevated risk of disease. For those with a genetic risk factor (e.g. an *SOD1* mutation or a *C9orf72* repeat expansion), for instance, this period of elevated risk may begin very early in life. For those in whom disease is caused by some environmental exposure(s)¹³⁻¹⁸, for example, the period of increased risk of disease begins only after the

relevant exposure. This model would also accommodate the possibility that multiple risk factors (e.g. genetic risk(s) combined with some environmental exposure(s)) are required in order to develop disease ^{43, 44}. In such a scenario, one's baseline risk might be elevated due to genetic susceptibility, then further increased at some point in one's lifetime by environmental exposure(s). ~~We-It is recognized~~, ~~of course~~, that the causal relationship between environmental exposures and ALS is not yet well-defined, but ~~we discuss~~ the example of elevated risk for disease based on some environmental (non-genetic) factor is used primarily to clarify that the conceptual model ~~we proposed~~ is, at least in theory, relevant ~~not only to~~ beyond genetic forms of ALS.

Phases of Disease: Pre-Symptomatic vs. Symptomatic

As noted above, we view the disease *ALS* as being characterized by two phases – a *pre-symptomatic* phase, followed by a *symptomatic* phase. The transition from the former to the latter is marked by the emergence of subjective symptoms, and/or objective motor (clinical or electromyographic) or cognitive/behavioral signs that a trained evaluator would reasonably interpret as unequivocal evidence of overt disease. This operational definition of the onset of clinically manifest disease builds upon that proposed by the El Escorial criteria (“time of first subjective symptom noticed by the patient which later is confirmed by examination”), but the distinction between symptoms and signs is ~~we purposefully blurred the distinction between symptoms and signs. Our reasons:~~ Either may come first, depending on a complex interplay of factors—for example, the degree to which an individual is physically self-aware and observant; whether the person may be in denial (i.e. neglecting or ignoring symptoms, even if significant); whether the person is enrolled in a pre-symptomatic research study (in which case subtle clinical

signs might be observed before the study participant notices any symptom); whether the earliest manifestations of disease are motor or cognitive/behavioral (e.g. if the earliest symptoms include loss of insight, then reporting may be delayed especially in the absence of a reliable informant); etc. It is important to recognize that someone who develops ALS and then FTD may be ‘symptomatic’ for ALS but ‘pre-symptomatic’ for FTD, and vice versa for someone who develops FTD and then ALS.

Stages of Pre-Symptomatic Disease: Pre-Manifest vs. Prodromal

Within the *symptomatic* phase of disease, [different clinical stages based on progression and severity](#) ~~are~~ already recognized ~~the different clinical stages based on progression and severity~~^{45, 46}. (These should not be conflated with the more speculative concept of *pathological* staging, which is currently necessarily based on *post mortem* histology⁴⁷.) Similarly, for the pre-symptomatic phase, we find it helpful to recognize two stages – a *pre-manifest* stage, followed by a *prodromal* stage.

~~We operationally define~~ the *pre-manifest* stage [is defined](#) as the period from disease onset, ~~however defined~~, to the emergence of the earliest clinical manifestation of disease--namely, the first appearance of possible (or non-specific) symptoms/signs. As explained below, this stage of disease can, by definition, be observed only through biomarker evidence of disease. Such biomarkers may reflect underlying molecular or cellular perturbations or relatively successful compensation, for example at a systems-level.

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3 Immediately following the *pre-manifest* stage, the *prodromal* stage begins with the possible
4 symptoms/signs and ends with the emergence of definite symptoms/signs that reflect
5 unequivocal development of symptomatic disease and may reflect a stage of decompensation for
6 underlying molecular, cellular or network perturbations. The motivation for differentiating these
7 two stages of pre-symptomatic disease derives from ~~our~~ experience of following a large cohort of
8 individuals at genetic-risk for ALS and observing phenoconversion in over a dozen individuals
9 so far: Sometimes the earliest possible clinical manifestations of disease (e.g. isolated
10 denervation of thoracic paraspinal muscles) are non-specific and difficult to interpret; and it is
11 not until later when unequivocal weakness appears, that the emergence of clinically manifest
12 disease can be declared with confidence. As a result, simple use of the term '*pre-symptomatic*'
13 seems inadequate for characterizing these individuals, as it does not adequately distinguish those
14 in whom there are no clinical symptoms or signs whatsoever (i.e. pre-manifest) and those in
15 whom there are subtle clinical symptoms or signs, but which are insufficiently specific to permit
16 a confident conclusion that manifest disease has emerged (i.e. prodromal).
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18 ~~We~~ It is recognized that these two phases of pre-symptomatic disease are most easily recognized
19 and characterized in the population at genetic risk for disease. Although a similar model likely
20 applies to sporadic forms of ALS, it is far from clear that people without genetic susceptibility
21 should be classified as having pre-symptomatic disease based solely on the presence of non-
22 specific symptoms or signs such as fasciculation.

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49 **Phenoconversion and Phenoconverter**

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51 ~~We operationally define~~ *P*phenoconversion is operationally defined here as the first emergence
52 of definite clinical signs or symptoms of disease. ~~Phenoconversion~~ It is not synonymous with
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diagnosis; the latter typically requires the documentation of clinical evidence of disease through an in-person assessment with physical examination and EMG as needed, whereas the former may retrospectively be attributed, after subsequent diagnosis, to the time that symptoms were initially reported. Importantly, ~~we~~ it is recommended that the term *phenoconverter* be reserved to describe those individuals who have been studied both before and after the transition from the pre-symptomatic to the symptomatic phases of disease. Implicit in this definition is that “study” includes the performance of careful clinical (both motor and cognitive/behavioral) as well as EMG evaluations at every visit to determine the presence or absence of clinical manifestation of disease at time of visit.

The Role of Biomarkers

The study of pre-symptomatic disease critically depends on biomarkers. As implied by the term *pre-symptomatic* and as described above, there are only subtle or non-specific symptoms/signs of disease during the prodromal stage, and no clinical evidence of disease during the pre-manifest stage. Biomarkers are, therefore, not only valuable but essential for the study and detection of pre-symptomatic disease because they may: (1) provide early biological evidence of an active disease process, reflecting, for example, molecular, cellular or systems-level perturbations or, importantly, the compensatory responses; (2) serve as markers of pre-symptomatic neuronal injury (e.g. axonal loss, as evidence by an increase in serum or CSF neurofilament levels); or (3) predict the likely timing of the emergence of symptomatic disease.

Recently published longitudinal data from a large cohort of pre-symptomatic individuals, some of whom were followed through phenoconversion to clinically manifest disease, provide clear evidence that an elevation in serum and cerebrospinal fluid levels of neurofilament light (NfL) is

apparent at least as far back as a year prior to the emergence of any clinical manifestations of disease⁴⁸. Such biomarker data is critically important to the design of a disease prevention trial insofar as they may serve to identify the population at greatest short-term risk of developing overt clinical manifestations of ALS – the very population that would need to be enrolled in a clinical trial to determine whether early therapeutic intervention can delay or prevent phenoconversion to clinically manifest disease. Importantly, longitudinal measurement of biomarkers is necessary to demonstrate pre-symptomatic disease progression⁴⁸, since cross-sectional differences as compared to controls may simply reflect the difference in susceptibility to disease, rather than the onset and progression of disease^{5, 49-52}.

Actual Transitions vs. Observable Milestones

While, in theory, the transitions between the various phases and stages have clear definitions (e.g. the emergence of definite symptoms/signs), there are practical limitations to what is we are actually able-possible to observe—and when. The *earliest biomarker evidence of disease* serves as a milestone to mark that, by then, disease onset has occurred, and the individual has transitioned into the pre-manifest stage of pre-symptomatic disease. Observing such biomarker evidence is obviously critically dependent on the sensitivity of the biomarker to detect the disease characteristics that it aims to quantify. The development of increasingly sensitive biomarkers, therefore, will likely enable us to define the emergence of pre-symptomatic disease at an even earlier point in time, with the necessary proviso that thresholds for defining abnormal levels of these biomarkers have been established. Moreover, since biomarker measurement is usually not performed continuously (e.g. not done every day, week, or even month), our ability to observe the emergence of disease also depends on the timing of biomarker measurement. The

actual transition to pre-manifest disease in an individual, therefore, will most likely have occurred prior to when the biomarker was measured (i.e. when the milestone was observed).

Moreover, defining the timing of the first appearance of *possible symptoms/signs and definite symptoms/signs of disease (i.e. phenoconversion)* is similarly challenging. For *symptoms*, [we clinicians](#) must rely on subjective report, which are prone to the potential vagaries of [someone's](#) memory if the symptoms were not reported right away. Observation of clinical *signs* depends on the timing of careful clinical assessment which, like biomarker measurement, can only realistically be performed at discrete time points rather than in an ongoing or continuous manner. Notwithstanding these inherent, real-world limitations in how one marks the milestones of progression in an individual--from pre-manifest to prodromal stage, or from pre-symptomatic to symptomatic phase, [the conceptual framework proposed nonetheless applies.](#)

Conclusion

[Our goal here is to propose a clearer](#) framework to define the pre-symptomatic phase of ALS [is proposed.](#) It is hoped that greater conceptual clarity will help to stimulate further research into the pre-symptomatic phase of ALS; facilitate thoughtful study and experimental designs for natural history studies, biomarker development, and early therapeutic intervention or even disease prevention trials; shed light on the potential use of established ALS therapeutics such as Riluzole and Edaravone (which cannot yet be recommended in the pre-symptomatic phase); and improve the clarity of communication and dissemination of research findings.

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Literature Review

References were identified by searching PubMed with no date restrictions. We used the disease topic text words "Amyotrophic Lateral Sclerosis" "Alzheimer Disease" "Parkinson Disease" and "Huntington Disease" combined with categorical text words "Preclinical" "Pre-symptomatic" "Asymptomatic" "Pre-manifest" "Prodromal" and "Pre-disease". We restricted search results to include human studies by adding the terms "NOT mouse" and "NOT mice" in the search text (selecting the "Human" exclusion criteria did not eliminate these results). Results were further refined to include only English language articles. When results were abundant, the criterion "[TIAB]" was added in order to generate articles that include the search terms in the title and/or abstract verbatim. The final reference list was generated on the basis of relevance to the topic covered in this review.

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Declaration of Interest

The authors report no conflicts of interest.

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Role of the Funding Source

None

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Figure Legend

Phases and Stages of ALS: A Conceptual Model. (A) The natural history of ALS: From (heightened) susceptibility to disease onset; from the pre-symptomatic to the symptomatic phase of disease; and from the pre-manifest to the prodromal stage within the pre-symptomatic phase. Color gradient reflects the likelihood that these phases and stages exist along a continuum. (Note that the figure is not drawn to scale, as the relative duration of each phase and stage is largely unknown and may vary between individuals.) The vertical dotted line demarcates the earliest biomarker evidence of disease (e.g., neurofilament light concentration based on currently published data), but development of more sensitive biomarkers might permit even earlier detection of pre-symptomatic disease. (B) The increase in serum and cerebrospinal fluid levels of neurofilament proteins (red), as has been observed during the pre-symptomatic and early symptomatic phases of disease, indicating underlying axonal degeneration. The red arrow marks the earliest evidence of disease as evidenced by an increase in neurofilament. (C) Hypothesized biomarker(s) (blue) indicative of a molecular, cellular, or network phenotype that *begins* prior to axonal loss. The blue arrow marks the earliest detectable increase in the novel biomarker(s). The period of this novel biomarker increase without evidence of axonal loss (and prior to clinical manifestations) may be regarded as a “compensated” state; the emergence of axonal degeneration in the pre-symptomatic phase indicates a “decompensating” state; and the emergence of clinical manifestations along with axonal degeneration reflect a “decompensated” state.

Title: Defining pre-symptomatic amyotrophic lateral sclerosis

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Summary

Successful treatment of neurodegenerative disease may hinge on early therapeutic intervention. This requires an understanding of early/pre-symptomatic disease, a need that is underscored by advances in antisense oligonucleotide and viral-vector-based gene therapies. In ALS, the study of pre-symptomatic disease requires a cohesive conceptual framework for describing this phase of disease. Informed by the literature in other neurodegenerative diseases and extensive personal experience, a model is proposed that distinguishes ALS as a clinical syndrome from ALS as a disease, and characterizes pre-symptomatic ALS as having two identifiable stages: pre-manifest and prodromal. The unique and critical importance of biomarker development is articulated and an operational definition of phenoconversion is provided. It is hoped that this framework will accelerate collective efforts to study pre-symptomatic ALS, and aid in the design and implementation of an early intervention- or disease-prevention trial.

Key words:

Pre-symptomatic

Pre-manifest

Prodromal

Biomarkers

Introduction

Central to the vision of primary prevention for amyotrophic lateral sclerosis (ALS), an invariably fatal neurodegenerative disease, are answers to the questions “When does ALS begin?” and “What is the duration of the pre-symptomatic phase of disease?”. These questions have been the subject of considerable discussion¹⁻³, but a paucity of data has precluded a definitive answer⁴⁻¹⁰. Limitations of the available data reflect, in large part, the manifold challenges that are inherent to studying the onset and early progression of a relatively rare, adult-onset condition with few known risk factors¹¹⁻¹⁸. Additional complexity has emerged from the discovery that the motor-predominant neurodegenerative syndrome of ALS has clinical, genetic and pathological overlap with frontotemporal dementia, typically the behavioral variant¹⁹; and that the first clinical manifestations of disease may be motor or cognitive/behavioral, especially among those with particular genetic mutations, notably a *C9orf72* hexanucleotide repeat expansion²⁰.

An additional and perhaps under-recognized challenge, however, is the confusing array of terms (e.g. asymptomatic, pre-symptomatic) that has been used to describe the period of disease preceding the emergence of overt motor, cognitive or behavioral manifestations that would permit a clinical diagnosis. Since choice of terms shape concepts, which in turn influences research approaches and paradigms, it is essential to clarify the language that surrounds the description and study of this aspect of the natural history of ALS. Here a conceptual framework is presented (Figure), informed by personal experience studying individuals at genetic risk for ALS¹¹, and by relevant publications from the Huntington’s disease, Alzheimer’s disease and Parkinson’s disease literature²¹⁻²⁵. The hope is that this framework will propel the field forward

in our collective understanding of early disease - and perhaps even hasten the prospect of an early intervention or a disease prevention trial.

Pre-symptomatic ALS: A Conceptual Model

The terms ‘asymptomatic’ and ‘pre-symptomatic’ have been both used to describe the phase of disease prior to the emergence of clinically manifest disease – and both terms are reflected in common medical parlance. In infectious diseases, for example, we recognize an ‘incubation period’ between exposure to a microbial agent and the appearance of symptoms of disease – and people in this subclinical stage of disease are said to be asymptomatic ²⁶. By contrast, in the genetic arena, we typically speak of predictive or pre-symptomatic genetic testing among people with a family history of disease, but who have no symptoms or other manifestations of disease at the time of testing ²⁷. Since the study of this stage of disease in ALS has really only been possible in the population at genetic risk for disease, the term ‘pre-symptomatic’ is commonly used, which is similarly reflected in the literature relevant to the study of other neurodegenerative disorders ²⁸⁻³⁰ and encapsulates the idea that unaffected people with an ALS associated gene mutation have a high likelihood of developing ALS in the future.

ALS: Disease vs. Clinical Syndrome

The clinical syndrome of ALS is easily recognized based on a history of progressive (yet painless) weakness, along with evidence of both upper and lower motor neuron signs in the same body region. But the underlying disease, by which is meant the presence of some underlying biological (e.g. molecular, cellular, or systems-level) process that eventually leads to the clinical syndrome, almost certainly begins before the clinical syndrome emerges. Such a paradigm is well recognized in cancer, infectious disease and even other neurodegenerative diseases such as

Alzheimer's³¹⁻³⁴, Parkinson's³⁵⁻³⁷ and Huntington's disease^{38,39}. As such, it is possible to recognize both a pre-symptomatic and a symptomatic phase of disease in ALS (elaborated below). A consequence of recognizing the distinction between ALS as a disease process (i.e. the pathobiology that underlies the degeneration of upper and lower motor neurons) and ALS as a clinical syndrome (i.e. the overt clinical syndrome characterized by progressive painless weakness with both upper motor neuron and lower motor neuron signs on physical examination), it becomes necessary to differentiate the onset of the underlying disease from the onset of symptoms. The El Escorial criteria (original and revised^{40,41}) provide a definition of onset (without explicitly distinguishing 'disease' from 'clinical syndrome') as the "time of first subjective symptom noticed by the patient which later is confirmed by examination"; the language used by the authors of these criteria implicitly reference the emergence of the clinical syndrome, but are silent on the question of when the disease process begins. Similarly, most clinical trials operationally specify the initial symptom to be weakness (excluding 'softer' symptoms such as fasciculations). By contrast, there has been relatively little discussion in the literature about an operational definition of the onset of disease^{1,2,42}

Risk for ALS: Genetic and Environmental Factors

In at least some individuals the pre-symptomatic phase of disease may be preceded by a period during which the individual is at an elevated risk of disease. For those with a genetic risk factor (e.g. an *SOD1* mutation or a *C9orf72* repeat expansion), for instance, this period of elevated risk may begin very early in life. For those in whom disease is caused by some environmental exposure(s)¹³⁻¹⁸, for example, the period of increased risk of disease begins only after the relevant exposure. This model would also accommodate the possibility that multiple risk factors

(e.g. genetic risk(s) combined with some environmental exposure(s)) are required in order to develop disease^{43, 44}. In such a scenario, one’s baseline risk might be elevated due to genetic susceptibility, then further increased at some point in one’s lifetime by environmental exposure(s). It is recognized that the causal relationship between environmental exposures and ALS is not yet well-defined, but the example of elevated risk for disease based on some environmental (non-genetic) factor is used primarily to clarify that the conceptual model proposed is, at least in theory, relevant beyond genetic forms of ALS.

Phases of Disease: Pre-Symptomatic vs. Symptomatic

As noted above, we view the disease *ALS* as being characterized by two phases – a *pre-symptomatic* phase, followed by a *symptomatic* phase. The transition from the former to the latter is marked by the emergence of subjective symptoms, and/or objective motor (clinical or electromyographic) or cognitive/behavioral signs that a trained evaluator would reasonably interpret as unequivocal evidence of overt disease. This operational definition of the onset of clinically manifest disease builds upon that proposed by the El Escorial criteria (“time of first subjective symptom noticed by the patient which later is confirmed by examination”), but the distinction between symptoms and signs is purposefully blurred. Either may come first, depending on a complex interplay of factors—for example, the degree to which an individual is physically self-aware and observant; whether the person may be in denial (i.e. neglecting or ignoring symptoms, even if significant); whether the person is enrolled in a pre-symptomatic research study (in which case subtle clinical signs might be observed before the study participant notices any symptom); whether the earliest manifestations of disease are motor or cognitive/behavioral (e.g. if the earliest symptoms include loss of insight, then reporting may be

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3 delayed especially in the absence of a reliable informant); etc. It is important to recognize that
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5 someone who develops ALS and then FTD may be ‘symptomatic’ for ALS but ‘pre-
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7 symptomatic’ for FTD, and vice versa for someone who develops FTD and then ALS.
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10 11 12 13 14 15 Stages of Pre-Symptomatic Disease: Pre-Manifest vs. Prodromal

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17 Within the *symptomatic* phase of disease, different *clinical* stages based on progression and
18
19 severity are already recognized^{45, 46}. These should not be conflated with the more speculative
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21 concept of *pathological* staging, which is currently necessarily based on *post mortem* histology
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23⁴⁷. Similarly, for the pre-symptomatic phase, we find it helpful to recognize two stages – a *pre-*
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25 *manifest* stage, followed by a *prodromal* stage.
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30 The *pre-manifest* stage is defined as the period from disease onset to the emergence of the
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32 earliest clinical manifestation of disease--namely, the first appearance of possible (or non-
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34 specific) symptoms/signs. As explained below, this stage of disease can, by definition, be
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36 observed only through biomarker evidence of disease. Such biomarkers may reflect underlying
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38 molecular or cellular perturbations or relatively successful compensation, for example at a
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40 systems-level.
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46 Immediately following the *pre-manifest* stage, the *prodromal* stage begins with the possible
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48 symptoms/signs and ends with the emergence of definite symptoms/signs that reflect
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50 unequivocal development of symptomatic disease and may reflect a stage of decompensation for
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52 underlying molecular, cellular or network perturbations. The motivation for differentiating these
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two stages of pre-symptomatic disease derives from experience of following a large cohort of individuals at genetic-risk for ALS and observing phenoconversion in over a dozen individuals so far: Sometimes the earliest possible clinical manifestations of disease (e.g. isolated denervation of thoracic paraspinal muscles) are non-specific and difficult to interpret; and it is not until later when unequivocal weakness appears, that the emergence of clinically manifest disease can be declared with confidence. As a result, simple use of the term ‘*pre-symptomatic*’ seems inadequate for characterizing these individuals, as it does not adequately distinguish those in whom there are no clinical symptoms or signs whatsoever (i.e. pre-manifest) and those in whom there are subtle clinical symptoms or signs, but which are insufficiently specific to permit a confident conclusion that manifest disease has emerged (i.e. prodromal). It is recognized that these two phases of pre-symptomatic disease are most easily recognized and characterized in the population at genetic risk for disease. Although a similar model likely applies to sporadic forms of ALS, it is far from clear that people without genetic susceptibility should be classified as having pre-symptomatic disease based solely on the presence of non-specific symptoms or signs such as fasciculation.

Phenoconversion and Phenoconverter

Phenoconversion is operationally defined here as the first emergence of definite clinical signs or symptoms of disease. It is not synonymous with diagnosis; the latter typically requires the documentation of clinical evidence of disease through an in-person assessment with physical examination and EMG as needed, whereas the former may retrospectively be attributed, after subsequent diagnosis, to the time that symptoms were initially reported. Importantly, it is recommended that the term *phenoconverter* be reserved to describe those individuals who have

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3 been studied both before and after the transition from the pre-symptomatic to the symptomatic
4 phases of disease. Implicit in this definition is that “study” includes the performance of careful
5 clinical (both motor and cognitive/behavioral) as well as EMG evaluations at every visit to
6 determine the presence or absence of clinical manifestation of disease at time of visit.
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14 **The Role of Biomarkers**

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16 The study of pre-symptomatic disease critically depends on biomarkers. As implied by the term
17 *pre-symptomatic* and as described above, there are only subtle or non-specific symptoms/signs of
18 disease during the prodromal stage, and no clinical evidence of disease during the pre-manifest
19 stage. Biomarkers are, therefore, not only valuable but essential for the study and detection of
20 pre-symptomatic disease because they may: (1) provide early biological evidence of an active
21 disease process, reflecting, for example, molecular, cellular or systems-level perturbations or,
22 importantly, the compensatory responses; (2) serve as markers of pre-symptomatic neuronal
23 injury (e.g. axonal loss, as evidence by an increase in serum or CSF neurofilament levels); or (3)
24 predict the likely timing of the emergence of symptomatic disease. Recently published
25 longitudinal data from a large cohort of pre-symptomatic individuals, some of whom were
26 followed through phenoconversion to clinically manifest disease, provide clear evidence that an
27 elevation in serum and cerebrospinal fluid levels of neurofilament light (NfL) is apparent at least
28 as far back as a year prior to the emergence of any clinical manifestations of disease⁴⁸. Such
29 biomarker data is critically important to the design of a disease prevention trial insofar as they
30 may serve to identify the population at greatest short-term risk of developing overt clinical
31 manifestations of ALS – the very population that would need to be enrolled in a clinical trial to
32 determine whether early therapeutic intervention can delay or prevent phenoconversion to
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clinically manifest disease. Importantly, longitudinal measurement of biomarkers is necessary to demonstrate pre-symptomatic disease progression, since cross-sectional differences as compared to controls may simply reflect the difference in susceptibility to disease, rather than the onset and progression of disease ^{5, 49-52}.

Actual Transitions vs. Observable Milestones

While, in theory, the transitions between the various phases and stages have clear definitions (e.g. the emergence of definite symptoms/signs), there are practical limitations to what is actually possible to observe—and when. The *earliest biomarker evidence of disease* serves as a milestone to mark that, by then, disease onset has occurred, and the individual has transitioned into the pre-manifest stage of pre-symptomatic disease. Observing such biomarker evidence is obviously critically dependent on the sensitivity of the biomarker to detect the disease characteristics that it aims to quantify. The development of increasingly sensitive biomarkers, therefore, will likely enable us to define the emergence of pre-symptomatic disease at an even earlier point in time, with the necessary proviso that thresholds for defining abnormal levels of these biomarkers have been established. Moreover, since biomarker measurement is usually not performed continuously (e.g. not done every day, week, or even month), our ability to observe the emergence of disease also depends on the timing of biomarker measurement. The actual transition to pre-manifest disease in an individual, therefore, will most likely have occurred prior to when the biomarker was measured (i.e. when the milestone was observed).

Moreover, defining the timing of the first appearance of *possible symptoms/signs and definite symptoms/signs of disease (i.e. phenoconversion)* is similarly challenging. For *symptoms*,

clinicians must rely on subjective report, which are prone to the potential vagaries of memory if the symptoms were not reported right away. Observation of clinical *signs* depends on the timing of careful clinical assessment which, like biomarker measurement, can only realistically be performed at discrete time points rather than in an ongoing or continuous manner.

Notwithstanding these inherent, real-world limitations in how one marks the milestones of progression in an individual--from pre-manifest to prodromal stage, or from pre-symptomatic to symptomatic phase, the conceptual framework proposed nonetheless applies.

Conclusion

A framework to define the pre-symptomatic phase of ALS is proposed. It is hoped that greater conceptual clarity will help to stimulate further research into the pre-symptomatic phase of ALS; facilitate thoughtful study and experimental designs for natural history studies, biomarker development, and early therapeutic intervention or even disease prevention trials; shed light on the potential use of established ALS therapeutics such as Riluzole and Edaravone (which cannot yet be recommended in the pre-symptomatic phase); and improve the clarity of communication and dissemination of research findings.

Literature Review

References were identified by searching PubMed with no date restrictions. We used the disease topic text words "Amyotrophic Lateral Sclerosis" "Alzheimer Disease" "Parkinson Disease" and "Huntington Disease" combined with categorical text words "Preclinical" "Pre-symptomatic" "Asymptomatic" "Pre-manifest" "Prodromal" and "Pre-disease". We restricted search results to include human studies by adding the terms "NOT mouse" and "NOT mice" in the search text (selecting the "Human" exclusion criteria did not eliminate these results). Results were further refined to include only English language articles. When results were abundant, the criterion "[TIAB]" was added in order to generate articles that include the search terms in the title and/or abstract verbatim. The final reference list was generated on the basis of relevance to the topic covered in this review.

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Declaration of Interest

The authors report no conflicts of interest.

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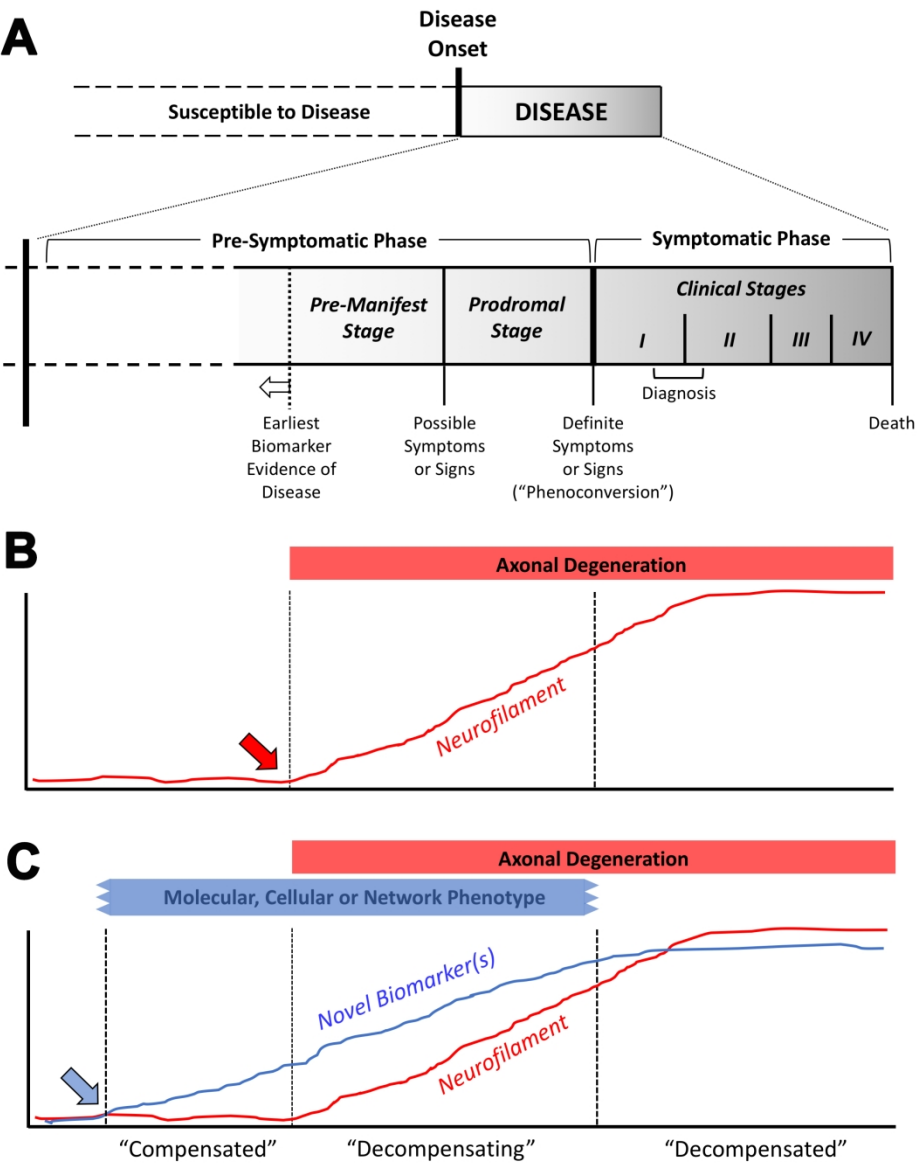
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Figure Legend

Phases and Stages of ALS: A Conceptual Model. (A) The natural history of ALS: From (heightened) susceptibility to disease onset; from the pre-symptomatic to the symptomatic phase of disease; and from the pre-manifest to the prodromal stage within the pre-symptomatic phase. Color gradient reflects the likelihood that these phases and stages exist along a continuum. (Note that the figure is not drawn to scale, as the relative duration of each phase and stage is largely unknown and may vary between individuals.) The vertical dotted line demarcates the earliest biomarker evidence of disease (e.g., neurofilament light concentration based on currently published data), but development of more sensitive biomarkers might permit even earlier detection of pre-symptomatic disease. (B) The increase in serum and cerebrospinal fluid levels of neurofilament proteins (red), as has been observed during the pre-symptomatic and early symptomatic phases of disease, indicating underlying axonal degeneration. The red arrow marks the earliest evidence of disease as evidenced by an increase in neurofilament. (C) Hypothesized biomarker(s) (blue) indicative of a molecular, cellular, or network phenotype that *begins* prior to axonal loss. The blue arrow marks the earliest detectable increase in the novel biomarker(s). The period of this novel biomarker increase without evidence of axonal loss (and prior to clinical manifestations) may be regarded as a “compensated” state; the emergence of axonal degeneration in the pre-symptomatic phase indicates a “decompensating” state; and the emergence of clinical manifestations along with axonal degeneration reflect a “decompensated” state.



Phases and Stages of ALS: A Conceptual Model. (A) The natural history of ALS: From (heightened) susceptibility to disease onset; from the pre-symptomatic to the symptomatic phase of disease; and from the pre-manifest to the prodromal stage within the pre-symptomatic phase. Color gradient reflects the likelihood that these phases and stages exist along a continuum. (Note that the figure is not drawn to scale, as the relative duration of each phase and stage is largely unknown and may vary between individuals.) The vertical dotted line demarcates the earliest biomarker evidence of disease (e.g., neurofilament light concentration based on currently published data), but development of more sensitive biomarkers might permit even earlier detection of pre-symptomatic disease. (B) The increase in serum and cerebrospinal fluid levels of neurofilament proteins (red), as has been observed during the pre-symptomatic and early symptomatic phases of disease, indicating underlying axonal degeneration. The red arrow marks the earliest evidence of disease as evidenced by an increase in neurofilament. (C) Hypothesized biomarker(s) (blue) indicative of a molecular, cellular, or network phenotype that begins prior to axonal loss. The blue arrow marks the earliest detectable increase in the novel biomarker(s). The period of this novel biomarker increase without evidence of axonal loss (and prior to clinical

manifestations) may be regarded as a "compensated" state; the emergence of axonal degeneration in the pre-symptomatic phase indicates a "decompensating" state; and the emergence of clinical manifestations along with axonal degeneration reflect a "decompensated" state.