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Patient Segmentation in ALS Studies

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Abstract

Does genotypic vs. phenotypic segmentation make sense in the context of an amyotrophic lateral sclerosis (ALS) study?

It's a provocative question, but one that warrants consideration. Research into the nature of ALS points to genetic mutations, often taking the form of miss aggregated or misfolded proteins. More than 20 genes have been causally linked to ALS [1]. Four genetic mutations are common; the others are relatively rare [2]. Certain genetic mutations appear to run in families but the same mutations can also be found in sporadic (i.e., non-familial) cases. These mutations have formed the basis for past patient segmentation efforts [3,4] but there is reason to question whether such segmentation efforts have any relevance to the presentation of ALS itself. Do these genetic mutations actually express different phenotypes? Do they play any role in the sign, symptoms, or trajectories of the disease? Conversely, do therapies targeting the identified genes-even if they could effectively correct the physiological consequences of a specific mutation-actually have an effect on ALS in the patient?

The recent history of drug development offers mixed answers to these questions. In the world of oncology, targeted, personalized genetic therapies are producing compelling outcomes [5-8]. Conversely, targeted therapeutic development for ALS has largely been ultimately unsuccessful, despite promising early phase clinical results [9]. It may be that targeted therapies in oncology have been successful because the underlying biology of different cancers is more fully understood. In contrast, much remains unknown about the underlying biology of ALS [1-3]. Further, treatment of a systemic disease such as ALS may inherently be more difficult than treatment of a disease with a specific target such as a solid tumor with a discreet set of genetic mutations as may be the case in oncology.

While many genetic mutations in ALS have been identified and studied, and while the phenotypic presentations of ALS are identifiable and agreed upon, the connections between genotype and phenotype remain incomplete. As efforts to find therapies targeting ALS continue down both paths, it becomes increasingly important for members of the clinical and research communities to develop a better understanding of where and how genotype and phenotype are linked, particularly because the links between phenotype and genotype can affect the design and conduct of ALS clinical trials in critical ways.

Keywords: Segmentation • Genetic mutations • Phenotypes • Therapy

Abbreviations

ALS: Amyotrophic Lateral Sclerosis; *C9ORF72*: Chromosome 9 Open Reading Frame 72; *SOD1*: Superoxide Dismutase 1; *TARDBP*: TAR DNA Binding Protein; FTD: Frontotemporal lobar Dementia; *FUS*: Fused in Sarcoma Protein.

Introduction

Genetic mutations and ALS

Some 5%-10% of patients with ALS have a familial or inherited form of the disease, while the approximately 90% of remaining ALS patients have a sporadic form of the disease with no known familial link [2]. Genetic mapping of patients with familial forms of ALS has enabled the identification of more than 20 genes [10] which may be involved in the pathogenesis of ALS, although mutation of many of these genes is extremely rare. Of the mutated genes currently identified, those most commonly mutated include *C9ORF72*, *SOD1*, *TARDBP* and *FUS*, which encode for the protein products superoxide dismutase (*SOD1*), TAR DNA Binding Protein 43 (TDP-43), chromosome 9 open reading frame 72 (*c9orf72*), and fused in sarcoma (*FUS*) respectively. (Table 1).

Mutation of these genes can also be found in some patients with sporadic ALS, indicating that common pathways may cause disease pathogenesis in both familial and sporadic forms of ALS. In some cases, mutated genes associated with familial ALS may also be implicated in sporadic ALS in their non-mutated forms. This is perhaps exemplified by *TARDBP* which is mutated in approximately 5%-10% of familial ALS cases, but which is found in mislocalized pathological inclusions in just about all post-mortem ALS assessments regardless of patient genotype and is estimated to play a role in approximately 97% of all ALS cases [12,13]. This indicates that regardless of patient genotype, disease pathways themselves may often be conserved.

The complexity of the disease and the role of genotype in dictating the disease may be further complicated by the intricate interplay that seems to exist between mutated proteins associated with ALS. For instance, downregulation of *c9orf72* protein levels has been shown to lead to TDP-43 inclusions, even in the absence of *TARDBP* genetic mutation [14]. Further, there may be multiple pathways through which an individual genetic mutation may be implicated in ALS. For example, mutation of *TARDBP* causes a gain of function through which the protein product is mislocalized and aggregated to form toxic inclusions, but mislocalization also confers a loss of canonical DNA/RNA binding function which may cause dysregulation of gene expression [15].

Literature Review

Do mutations map to distinct phenotypes?

ALS is a complex disease with a myriad of clinical presentations and phenotypes. Indeed, the vast heterogeneity of dysfunctions, both symptomatic and pathological, associated with ALS has led some to consider ALS as a syndrome rather than a disease [16]. This may be owed in part to the array of different genetic mutations implicated in the progression of ALS, which often correspond to phenotypic characteristics associated with the mutated gene in question [10].

The nature of a patient's genetic mutation may affect such attributes as rate of disease progression, survival time, site of disease onset (i.e., bulbar or limb onset), or the presence or absence of cognitive impairment. For example, *SOD1* mutations are associated with rapid disease progression, more frequent limb onset, and rare occurrences of cognitive impairment. In

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Table 1. *C9ORF72, SOD1, TARDBP*, and *FUS* are the most commonly mutated genes found in ALS. Note that proportion of ALS cases associated with each genetic mutation vary by population studied [2, 4, 26].

Gene	Nature of mutation	Protein	Associated phenotypes	Proportion of ALS cases with gene mutation	
				Familial	Sporadic
C9ORF72	Manifold hexanucleotide repeat	Chromosome 9 open reading frame 72 (*c9orf72*)	Bulbar onset and cognitive impairment more frequent, lower median survival compared to patients with *TARDBP* or *SOD1* mutations	20-50%	10%
SOD1	Missense mutations (over 185 mutations identified)	Superoxide dismutase 1 (*SOD1*)	Rapid disease progression, bulbar onset less frequent, cognitive impairment rare, phenotype variable based upon specific *SOD1* mutation	10-20%	2%
TARDBP	Point mutations	TAR DNA Binding Protein 43 (*TDP*-43)	Limb or bulbar onset, variable disease course, may involve frontotemporal lobar dementia (*FTD*)	5%	<1%
FUS	Point mutations	Fused in sarcoma protein (*FUS*)	Classical ALS phenotype often without cognitive impairment, diverse clinical courses	5%	<1%

contrast, *C9ORF72* mutation is associated with lower median survival time compared to patients with *SOD1* mutation, and more frequent bulbar onset and cognitive impairment [2].

It is important to note that even within patients sharing a similar genotype there may still be considerable phenotypic heterogeneity. For instance, patients with *TARDBP* mutation may have variable sites of disease onset or variable disease course, and patients with *FUS* mutations often have diverse clinical courses of the disease [2]. Further, over 185 disease-related mutations of *SOD1* have been identified and the rate of disease progression and overall patient survival can vary with the specific nature of the *SOD1* mutation [10]. For instance, patients with A4V *SOD1* mutations display a faster rate of functional decline and reduced median survival time compared to patients with other *SOD1* mutations [17].

Differential responses to therapy

To date, studies have insufficiently addressed whether genetic or phenotypic attributes predict differential response to treatment. Theoretically, genetically mediated differences in ALS phenotypes could predict response to therapy. This is most definitively illustrated by drug therapies that target a specific genetic mutation such as the antisense oligonucleotide therapy targeting mutated *SOD1* under development [18]. It is also reasonable to predict that patient genotype and phenotype may also be predictive of efficacy for drugs targeting specific cellular pathways that are dysregulated in ALS. For instance, edaravone is an antioxidant and free radical scavenger approved for treatment of ALS in the US, and it is plausible, although not proven, that patients with genetic mutations which would cause defects in oxidative stress pathways, such as *SOD1* mutations, may have enhanced response to therapy.

The theme of differential response to therapy is perhaps best illustrated by the multitude of ALS clinical trials that have failed over the past 30 years. While some have displayed favorable results within subpopulations of subjects, these subpopulations were not statistically large enough to prove efficacy in the subpopulation [19]. This would argue for more specified inclusion criteria in clinical ALS trials to target subpopulations of patients with the greatest likelihood to therapeutically benefit from a given class of agent.

A number of covariates have been identified in the literature which affect ALS patient survival, and thus contribute to the heterogeneity of ALS progression. These include time from symptom onset to diagnosis, age of onset, site of onset, weight loss and BMI at diagnosis, genetic status, forced vital capacity, and rate of disease progression as measured by the ALSFRS-R [20]. Given the potential effects of these covariates on end-point heterogeneity, selection of the appropriate ALS patient population is important for demonstration of efficacy within trials. However, this must be balanced against the need for a trial to be generalizable to the ALS patient population as a whole [21].

Patient segmentation

Patient segmentation within clinical trials is a frequent methodologic maneuver utilized to make differences between treatment groups easier to detect in a longitudinal study. Patient segmentation may be achieved in a number of ways, including pre-specified sub-groups, stratification, or patient eligibility criteria. Generally, these methods are employed to homogenize an otherwise heterogenus population for clinical study. Segmentation in a trial can result in a need for fewer participants, given increased chances of signal detection through reduction of statistical variability. It may also be used to validate unique product attributes that target a distinct mechanism of action within a specific subset of a larger population. In oncology studies, for example, patient segmentation based on specific genetic mutations may allow researchers to study the effects of highly targeted therapies whose mechanism of action is dependent upon the presence of the mutation [8,22,23].

With the advent of more sophisticated therapeutic agents and greater precision in tests that can identify specific genetic markers, these segmentation techniques are becoming increasingly popular in indications outside of the oncology space. This is especially the case in early proof of concept investigations where clinical trial efficiency (costs and timelines) need to coincide with maximal signal detection. The ALS clinical development space is no different, and the application of patient segmentation is being increasingly utilized with the goal of enhanced signal detection and shorter timelines. [3,4]

One such example is the use of *SOD1* mutations as a means to segment the ALS population into “slowly progressive ALS” and “rapidly progressive ALS.” Indeed, this strategy was used as part of the arimoclomol program, wherein a 12-month placebo-controlled trial was completed using the key eligibility criterion of rapidly progressive *SOD1* ALS defined by demonstrable mutation in the *SOD1* gene that is reported to be associated with a rapid rate of disease progression [9,24]. Additionally, this study conducted a further refined analysis based on patient segmentation in the form of a pre-specified sub-group based on the presence of A4V *SOD1* mutation carriers, as a known population of patients with rapid disease progression.

Discussion and Conclusion

Does genotypic *vs*. phenotypic segmentation make sense in the context of an ALS study?: Current strategies employed in ALS trials for patient segmentation are often based upon disease stage or disease progression. These are largely phenotypic characteristics, though they may be influenced, at least in part, by genotype [3]. Whether or not patient segmentation based upon genotype is relevant in the context of an ALS interventional study depends heavily on the nature of the study drug and the predicted population of patients in which a reasonable expectation of therapeutic benefit may be derived. In a trial studying the efficacy of a therapy targeting a particular genetic mutation, regardless of whether that therapy targets the mutation on a protein, mRNA, or DNA level, genotypic patient segmentation would clearly be critical.

Genotype by itself, though, will never be entirely sufficient. While genotype dictates, at least in part, the phenotype of the patient, phenotypic presentations within individuals with the same genetic mutation may differ dramatically. Moreover, it is clear from the studies conducted to date that no single genetic

mutation accounts for all the phenotypic heterogeneity found in ALS. For these reasons segregation of these two elements entirely may be difficult. Indeed, a prognostic model of survival encompassing eight predefined factors related to demographic variables, clinical phenotype, and genotype has been validated [25]. Its use has been suggested as a potential patient segmentation factor, highlighting the importance of both phenotype and genotype in disease progression and in patient segmentation [3,21].

From the perspective of a clinical trial, though, the question of genotypic *vs*. phenotypic segmentation does make a difference when it comes down to how one runs a study.

- Given the rarity of many genetic mutations in ALS, recruitment of patients based on genotype may be difficult, thus pointing to the importance of site selection in the recruitment of rare patient populations. Further, some genetic mutations are more frequently found in certain geographical regions [10], and a study targeting one of these mutations should carefully consider what countries will afford the best rate of patient recruitment.
- Genotypic segmentation will require access to labs that can run the requisite assays. Some assays are more common than others and can be conducted by a broad range of labs; others are much less common and may require engagement with specific labs, greater expense, and longer turnaround times [11].
- Phenotypic segmentation will require that study personnel be able to capture and access granular historical data that details the progression of ALS over the course of at least the previous 6 to 12 months. A study incorporating phenotypic segmentation would also need to construct a user friendly database that would facilitate capture of the details of phenotypic change during the course of the study.
- Genotypic and phenotypic segmentation both raise myriad questions about whether innovative trial designs can (or should) be used. A delayed start trial design might be viable for a study involving a gene-based therapy targeting a specific genetic mutation (a scenario in which genotypic segmentation would be warranted). However, innovative trial designs can introduce their own operational and bio statistical challenges (arising from participant attrition over time, for example, or the need to accommodate the statistical comparisons of multiple datasets) and should be considered in advance [26,27].
- Genotypic and phenotypic segmentation also raise different questions with regard to the need for engagement with a biosafety review committee (above and beyond the engagement with an IRB). If the approval of a biosafety review committee is required, this must be factored into the planning of the trial.
- Studies involving genotypic and phenotypic segmentation may need to incorporate different plans for follow-up with patients after completion of the trial. Any trials involving gene-based therapeutics, for example, may need a strategy (and a database) for tracking inter current events in patients over a long period of time [28]. While patients with ALS typically have an expected term of life of only two to three years after symptom onset [4], and the brevity of that period would normally obviate the need to develop a multi-year data capture plan, any sponsor whose therapy successfully changes that rate of decline will in fact require a longer-term strategy for data collection.

Finally, any pivotal trial that employs genotypic or phenotypic segmentation and shows positive results will need a commercialization strategy that takes that segmentation into consideration. Genotypic or phenotypic segmentation within the context of a trial will translate into genotypic or phenotypic segmentation in the marketplace. Regulators will likely approve the product for use among a subset of ALS patients who match into the genotype or phenotype of those participating in the trial. That in turn, will require sponsor to develop highly targeted publication and professional communications plans; it will also require sponsors to consider how best to enable individuals with ALS to validate that they are prospective candidates for receipt of this therapy. That may involve working with diagnostics developers and assay labs to expand access to specific tests. It may involve active engagement with advocacy groups to promote the existence of the treatment for specific groups of patients.

Naturally, the planning that would inform these commercialization efforts should not be put off till the successful conclusion of a phase 3 trial. While the day-to-day execution of any commercialization plans will of necessity depend on regulatory approval of the therapy, the seeds of these efforts should be planted early in the process so that trial designers and researchers can be planning for and capturing the details that will later inform these commercialization efforts.

Ultimately, most, if not all, clinical efficacy studies in ALS will encounter some number of complex questions when it comes to patient segmentation. Regardless of the phenotypic or genotypic nature of the segmentation, an operational solution will be required. Depending on the nature of patient segmentation, the operational challenges will affect each phase of a trial in different ways. How great a burden those challenges place on the trial largely depends on the experience of the parties coming together to run the trial and the degree to which sponsors and supporting agencies can strategize, early on in the process, about how best to accommodate the demands posed by phenotypic or genotypic segmentation.

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