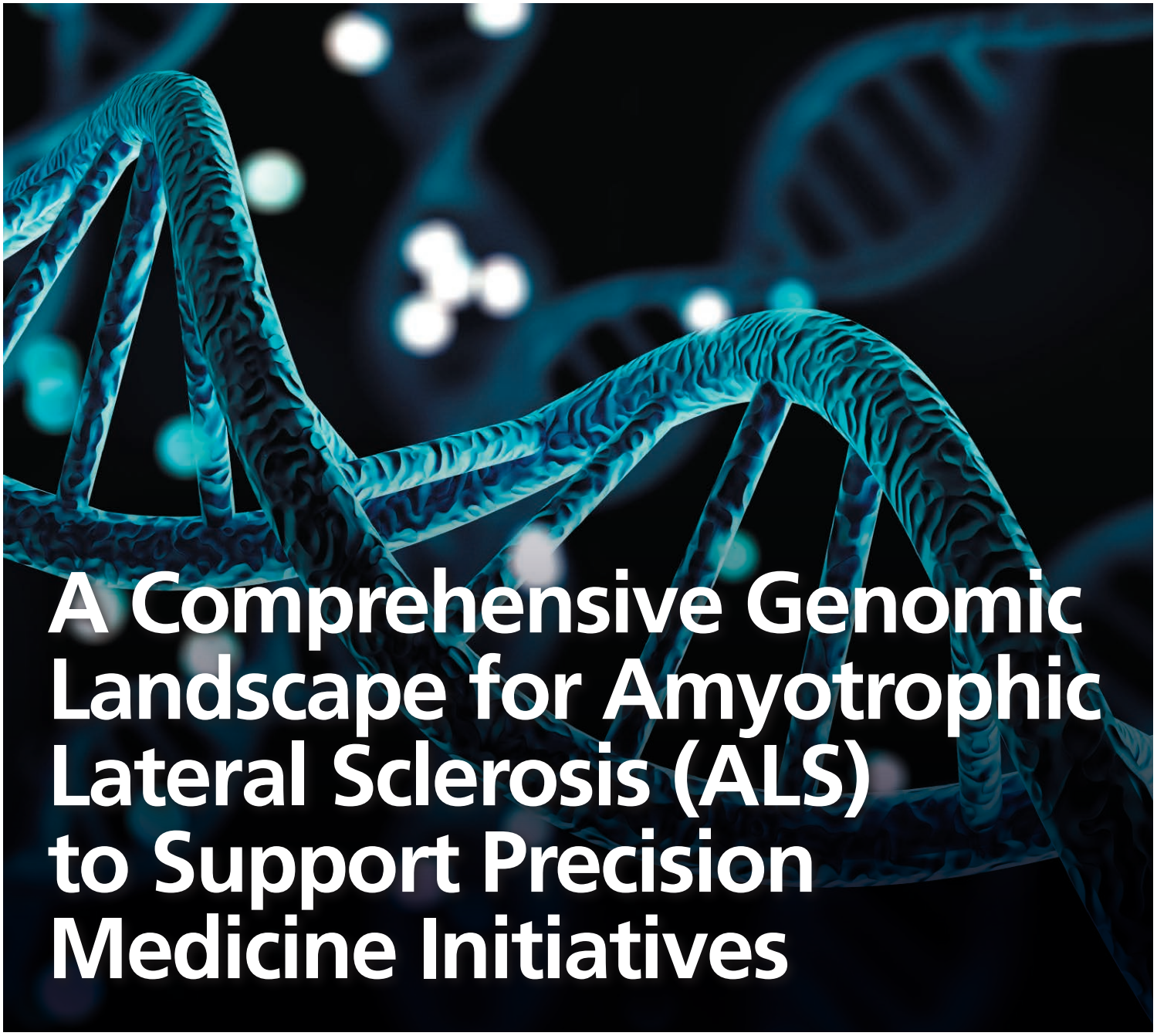


A Comprehensive Genomic Landscape for Amyotrophic Lateral Sclerosis (ALS) to Support Precision Medicine Initiatives

By **Lauren Chunn, Genomenon, Inc.**

The Clinical and Genetic Complexity of ALS and Impacts on Diagnosis and Genetic Testing

ALS is a neurodegenerative disease characterized by progressive loss of both upper and lower motor neurons, ultimately resulting in death due to respiratory paralysis. The clinical presentation of ALS is highly heterogeneous with respect to the populations of affected motor neurons, rate of disease progression, and the co-occurrence of other neurological/neurodegenerative conditions, particularly frontotemporal dementia (FTD).[1-2] This heterogeneity, combined with the nonspecific nature of the earliest symptoms, contributes to delayed clinical diagnosis – for most patients, approximately a year past symptom onset.[3-4] With a median survival of ~3 years past symptom onset, this delay is devastating and can preclude effective treatment, especially for patients with a more aggressive disease course.[5]

Efforts to reduce this delay in diagnosis are being undertaken and include improving the recognition of early symptoms of ALS, earlier, high-quality electromyography (EMG) studies, the use of biomarkers and neuroimaging studies, and the development of a refined set of diagnostic criteria for clinical use.[6-8] Current diagnostic methodologies, however, have not yet successfully included genetic testing for diagnosis, prognosis, or treatment.

ALS was confirmed to have a genetic component in 1993, when variants in the *SOD1* gene were implicated in a proportion of ALS cases with a

family history of the disease.[9] Since then, at least 25 genes have been reproducibly implicated in the development of ALS, the most common causes being a repeat expansion in *C9orf72* and variants in *SOD1*, *FUS*, and *TARDBP*.[1] Despite this rapid increase in understanding of the genetic background of ALS, the prospect of diagnosis based on genetic testing results is impeded by several factors, including reduced penetrance of certain variants, oligogenic inheritance, and the interplay between genetics and environmental risk factors.[10] These complexities, combined with the lack of a well-annotated and comprehensive database of variants associated with ALS, have severely minimized the clinical actionability of these tests.

As a result, the approach to genetic testing for ALS is both conservative and inconsistent, likely resulting in additional confusion for clinicians. There are no official genetic testing guidelines for ALS in the United States; however, recommendations are available based largely on whether the patient has a family history of the disease, which has historically been used to differentiate ALS into two types.[11] Patients are deemed to have *familial ALS* when there is a demonstrated family history and *sporadic ALS* when no such history is ascertained.

For a variety of reasons, such as reduced penetrance and a highly variable age at disease presentation, only about 10% of ALS patients at diagnosis have a living family member who is also diagnosed with ALS.[1] In the past, genetic testing has been offered almost exclusively to this group. In these circumstances, a genetic cause is determined in ~70% of cases. For the 90% of patients for whom there is no family history, ~15% of cases have been found to have a genetic cause.[12] Indeed, all genes that have been associated with familial ALS have also been associated with sporadic ALS, and the same biological mechanisms underlie the development of disease in both cases.[13] The more we learn about the genetics of ALS, the less clinically meaningful the distinction between familial and sporadic ALS becomes.

Limiting testing to familial ALS cases alone can significantly impact clinical care for sporadic patients. Particularly, it can prevent accurate risk assessment for a patient's family members and determination of eligibility for clinical trial enrollment as well as hamper understanding of the patient's potential disease course/prognosis. However, a dramatic shift is on the horizon regarding the collective approach to genetic testing for ALS patients. One reason for this shift is the anticipated availability of targeted therapies whose prescription would be predicated on the results of genetic testing.[14-15] In fact, this is indicative of a major trend in ALS treatment – from a one-size-fits-all to a precision medicine model.

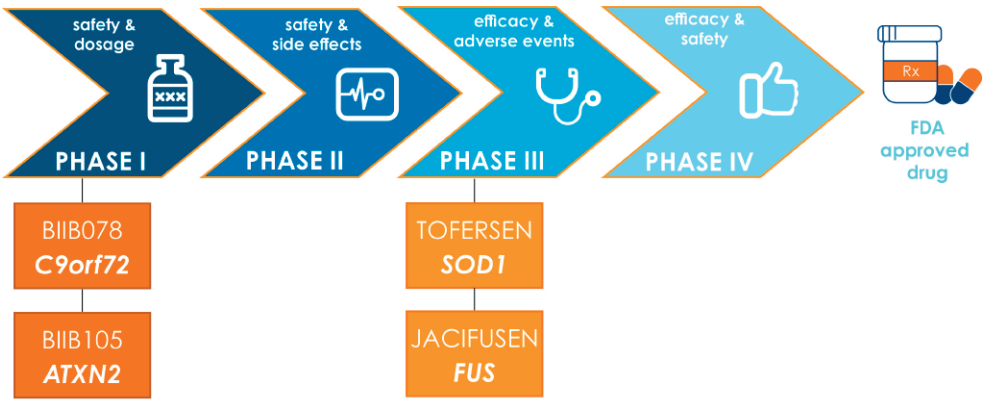
The Move Towards Precision Medicine in ALS and the Need for a Comprehensive Source of Genetic Variants

Currently, only two drugs are approved by the FDA to treat ALS – riluzole and edaravone – both of which have limited efficacy and only moderately slow the progression of the disease. Since riluzole's approval in 1995, more than 80 clinical trials have been performed, and all have ultimately failed to produce significant results.[16] These failures were caused by a multitude of factors, including the late onset of symptoms, the intrinsic heterogeneity of the disease and its underlying mechanisms, the difficulty of recruiting eligible patients due to the rarity of the disease, and limitations in clinical trial design such as the development of sensitive outcome measures and patient stratification methods.[16-17] Overall, the one-size-fits-all model that has been employed in clinical trials for many conditions has proven to be ineffective in ALS as well as other, similar diseases with extreme rarity, late-onset symptoms, and clinical heterogeneity. These circumstances have prompted pharmaceutical companies to explore a precision medicine avenue to tailor their therapies to specific subgroups of patients.

Drugs Currently in Clinical Trials

Precision medicine has traditionally been associated with the development of cancer therapeutics, particularly immunotherapy. In recent years, however, the reach of precision medicine has expanded to include a number of non-cancer indications. In 2016, a major breakthrough was achieved in this vein with the FDA approval of Spinraza® for the treatment of spinal muscular atrophy (SMA), another motor neuron disease similar to ALS. Spinraza is an antisense oligonucleotide (ASO) directed to the *SMN2* gene that induces the expression of a fully functional SMN protein, which produced remarkable results with 51% of infants achieving motor milestones and a significantly increased survival rate.[18] On the heels of this achievement, similar targeted therapies are in development for the treatment of ALS.

One of these developing therapies targets the *SOD1* gene, which is responsible for ~20% of familial ALS and ~2% of sporadic ALS (**Figure 1**).[19] Tofersen is an ASO that mediates degradation of *SOD1* mRNA, and Phase I/II clinical trial results indicated successful reduction of *SOD1* levels in the cerebrospinal fluid as well as a trend towards slower clinical decline.[20] Two Phase III trials to further characterize the efficacy of Tofersen are currently underway, for both symptomatic and pre-symptomatic adults with a confirmed *SOD1* variant.[21-22] As the disease process for ALS



Gene	Proportion of familial ALS*	Proportion of sporadic ALS*	Therapy	Clinical Trial ID	Clinical Trial Phase
SOD1	20%	2%	Tofersen	NCT02623699, NCT04856982	Phase III
FUS	10%	<1%	Jacifusen	NCT04768972	Phase III
C9orf72	25%	10%	BIIB078	NCT03626012	Phase I
ATXN2	-	-	BIIB105	NCT04494256	Phase I

Figure 1: Targeted Therapies for ALS

* Adapted from Taylor *et al.* 201619

Figure 2: Flattening the VUS Curve

The addition of literature evidence can reduce the number of Variants of Uncertain Significance (VUS) as well as increase the number of clinically actionable variants by providing sufficient evidence to satisfy pathogenicity criteria.

P – pathogenic, LP – likely pathogenic, B – benign, LB – likely benign

begins prior to symptom onset, the ability to treat pre-symptomatic patients would be revolutionary and could provide much higher efficacy in slowing or even inhibiting the development of ALS in these patients.[23]

Another similar therapy – BIIB078 – is in development for patients with the *C9orf72* expansion, which is responsible for ~25% of familial and ~10% of sporadic ALS cases (**Figure 1**).[19] This therapy is an ASO that selectively targets repeat-containing transcripts of *C9orf72* and has been shown to reduce RNA foci and toxic dipeptide repeat proteins in preclinical studies – two factors that are believed to play a role in the pathogenesis of *C9orf72*-related ALS.[24] A Phase I trial is currently underway.[25] One other therapy – Jacifusen – is in a Phase III trial for patients with variants in *FUS*, which is responsible for ~5% of familial and <1% of sporadic ALS cases, and is often associated with very aggressive, fast progressing forms of ALS (see Table in **Figure 1**).[19,26] Finally, another therapy – BIIB105 – is in development for patients with a poly-CAG expansion in the *ATXN2* gene, a known risk factor for ALS, and is currently in a Phase I trial.[19,27]

Hesitancy Concerning the Offer of Genetic Testing Hinges on Therapeutic Availability

As these targeted therapies progress through trials as well as if/when they receive FDA approval, two significant barriers to ensuring maximal treatment remain. The administration of these therapies is predicated on a patient having a confirmed, pathogenic variant in the targeted gene; thus, their eligibility for treatment hinges on:

1. Their clinician's willingness to order a genetic test.
2. The ability of clinical laboratories to interpret the results with the utmost accuracy.

Hesitation among clinicians concerning the offer of genetic testing is expected to diminish as therapeutic options expand – in a survey of 41 neurologists, 90.7% stated that their attitude towards genetic testing would change upon the availability of an effective therapy.[14] Unfortunately, the ability to interpret genetic testing results continues to be challenged by high rates of Variants of Uncertain Significance (VUS), which could effectively preclude the patient from treatment due to a lack of sufficient information to satisfy pathogenicity criteria. In a recent survey, the rate of VUS across four clinical laboratories offering genetic testing for ALS patients, including evaluation of multiple ALS-associated genes, was between 12-28%.[28] In addition, some patients may have multiple variants with varying levels of evidence, which can further complicate interpretation of the results. A recent study found that 13% of patients carried more than one variant in an ALS-associated gene and 24% of patients with a pathogenic variant additionally carried a VUS.[29]

In order to ensure that the largest number of patients possible receive appropriate treatment, we must reduce this burden of VUS, and the largest source of quality evidence to support a variant's pathogenicity is the medical literature (**Figure 2**). However, penetrating the medical literature to extract this evidence is extraordinarily difficult, particularly for well-studied diseases such as ALS where a simple search for the disease in PubMed returns nearly 30,000 articles. As a result, manual approaches to this evidence-gathering by an individual are simply too time-consuming and error-prone to provide

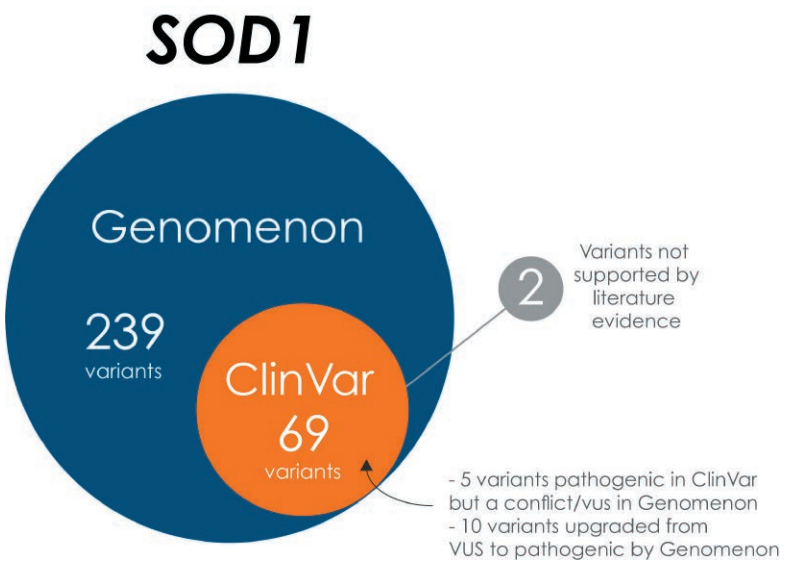


Figure 3: Comparison of variant yield for *SOD1* between Genomenon and ClinVar

Diagram contains all variants with a pathogenic or likely pathogenic designation in either of the Genomenon or ClinVar datasets

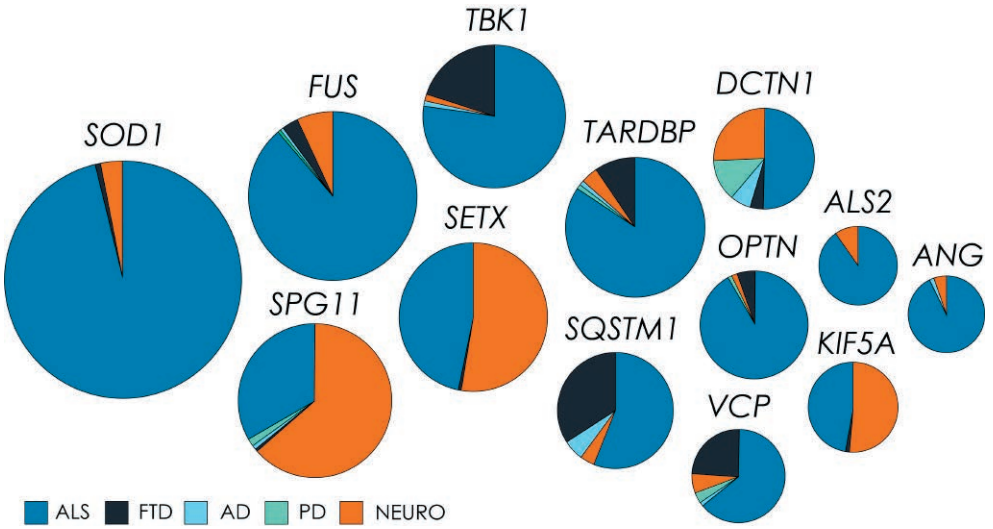


Figure 4: Disease presentations of patients with variants in 13 ALS-associated genes

The size of the pie chart is proportional to the total number of patients diagnosed with ALS or another neurodegenerative/neurological condition that have variants in the respective gene.

ALS – Amyotrophic Lateral Sclerosis, FTD – Frontotemporal Dementia, AD – Alzheimer Disease, PD – Parkinson Disease, NEURO – Other neurological condition

the sort of comprehensive depth that many VUS would require, as the recovery of a single article holds the potential to fundamentally change the actionability of such a variant.

Producing a Genomic Landscape for ALS

To address this need for a comprehensive view into the literature, Genomenon has used a combination of Artificial Intelligence (AI) and expert curation to produce a Genomic Landscape for ALS – a comprehensive collection of every published variant in 36 ALS-associated genes, interpreted according to the American College of Medical Genetics (ACMG) criteria for assessing variant pathogenicity.[30] The identification and accurate interpretation of any of these variants could conceivably aid in early diagnosis, perhaps even before symptom onset, and subsequent targeted treatment for ALS patients.

The Genomenon ALS Genomic Landscape currently contains over 7,000 variants across 36 ALS-associated genes. These variants and their ACMG interpretations are supported by extensive review of the literature by expert curators, with supporting evidence provided alongside detailed annotations of clinical characteristics and functional testing, as well as data from external population frequency databases (gnomAD) and prediction algorithms (PolyPhen, SIFT, and Mutation Taster). The combination of these data sources provides a significant foundation for variant interpretation that significantly increases the yield of pathogenic variants without sacrificing precision or accuracy.

The Genomenon ALS Genomic Landscape Supplements Current Sequencing Efforts

Roughly 26% of the variants in the ALS Genomic Landscape had a demonstrated connection to ALS, and close to 18% were deemed pathogenic or likely pathogenic according to the ACMG standards. For the *SOD1* gene specifically, we uncovered three times more pathogenic and likely pathogenic variants than identified in ClinVar, a widely used database of clinical variants from user submissions (**Figure 3**). This demonstrates the additional value that a comprehensive reach into the medical literature can provide to supplement current sequencing and data gathering efforts, including ClinVar and others such as Project MinE.[31]

In addition, we developed a comprehensive patient-level database for the *C9orf72* expansion, which contains over 2,500 individuals with detailed annotations of their demographics, phenotype, family history, and genetic profile (including variants found in genes other than *C9orf72*). This database serves as a retrospective natural history study, and represents a 23-times increase compared to the ALSoD database, which has a total of 108 patients with this expansion.[32]

Association of ALS Genes with Clinical Presentations

In order to characterize the clinical presentation associated with each of the 36 ALS-associated genes, a representative sample of case studies was extracted for every gene besides *C9orf72*, which was characterized separately, as described above. This amounted to a total of 2,283 patients that were diagnosed with ALS or another neurological condition across 13 genes that had more than 50 patients in the sample – *SOD1, FUS, SPG11, SETX, TBK1, TARDBP, SQSTM1, OPTN, DCTN1, VCP, KIF5A, ALS2,* and *ANG*.

Every one of the 13 genes analyzed were associated with diagnoses other than ALS, including frontotemporal dementia (FTD), Alzheimer disease (AD), Parkinson disease (PD), and other neurological conditions (**Figure 4**). The percentage of cases associated with ALS per gene varied from 33% (*SPG11*) to 96% (*SOD1*), showcasing the extreme heterogeneity that exists across ALS-associated genes. Additionally, 36 patients (1.57%) had more than

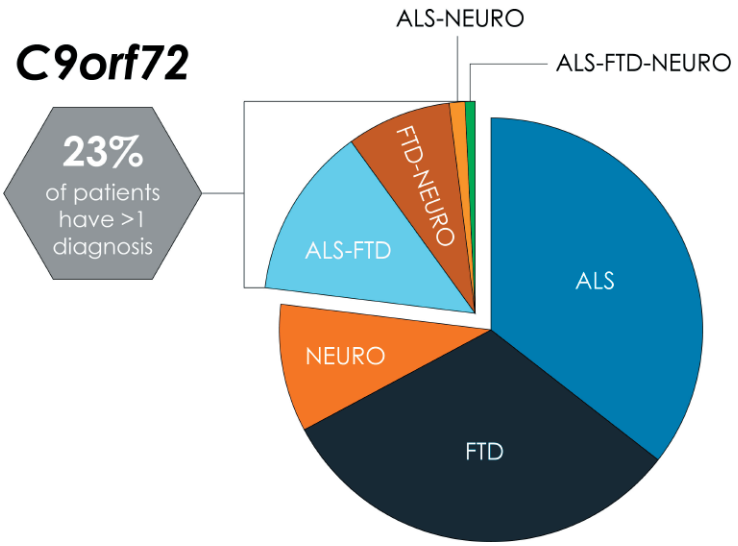


Figure 5: Disease presentations of patients with variants in *C9orf72*

n = 2270

ALS – Amyotrophic Lateral Sclerosis, FTD – Frontotemporal Dementia, NEURO – Other neurological condition

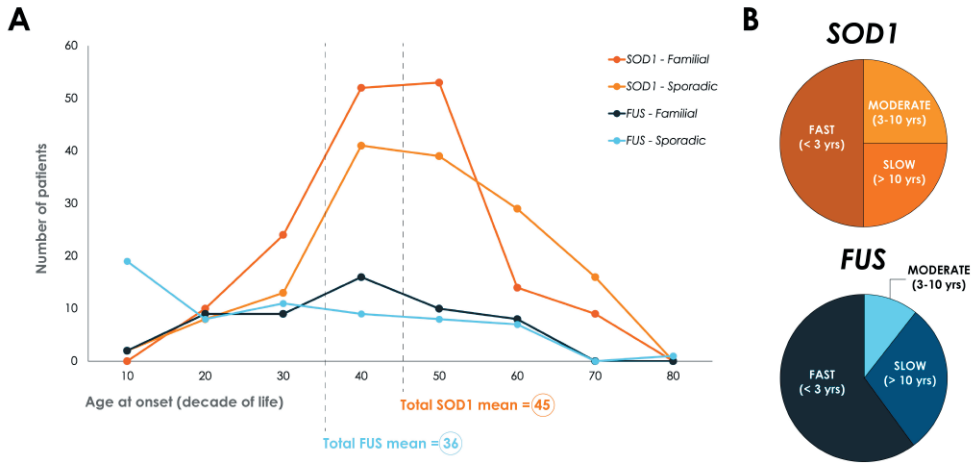


Figure 6: Age at onset and speed of disease progression for patients with variants in *FUS* and *SOD1*

A: Age at onset of ALS patients with variants in *SOD1* and *FUS*; *SOD1*-age at onset, n = 310; *FUS*-age at onset, n = 161

B: Speed of progression of ALS patients with variants in *SOD1* and *FUS*; *SOD1*-speed of progression, n = 131; *FUS*-speed of progression, n = 75

one diagnosis, which is a further indication that neurodegenerative diagnoses are often highly interrelated and can even co-occur within the same patient.

For the *C9orf72* gene, the heterogeneity of disease presentations was even more extreme, with 49% of patients having a diagnosis not involving ALS, and 23% having some combination of ALS, FTD, and other neurological conditions (**Figure 5**). These additional neurological conditions were highly variable as well, with 27 alternative diagnoses (excluding FTD, PD, and AD) being found in these patients. These results are consistent with the more recent proposal that ALS and FTD actually form a spectrum of disease with converging mechanisms and may suggest that this spectrum can become intertwined with other neurodegenerative/neurological conditions.[33]

The Genomic Landscape also revealed heterogeneity in age at onset as well as rate of disease progression between certain ALS-associated genes. For example, ALS patients with *FUS* variants had a mean age at onset almost a decade earlier compared to ALS patients with variants in *SOD1* (36 vs. 45; **Figure 6**). In addition, 60% of patients with *FUS* variants displayed a rapidly progressing disease course (£ 3 years from age at onset to death) compared to 50% of patients with variants in *SOD1* (**Figure 6**). This is consistent with reports in the literature that *FUS* variants are associated with an early onset, aggressive disease course. Variants in *FUS* have been found to account for ~35% of familial cases under 40 years old, and more than 60% of familial patients with the *FUS* variant show disease onset before 45 years – some even presenting in their late teens and early 20s.[34]

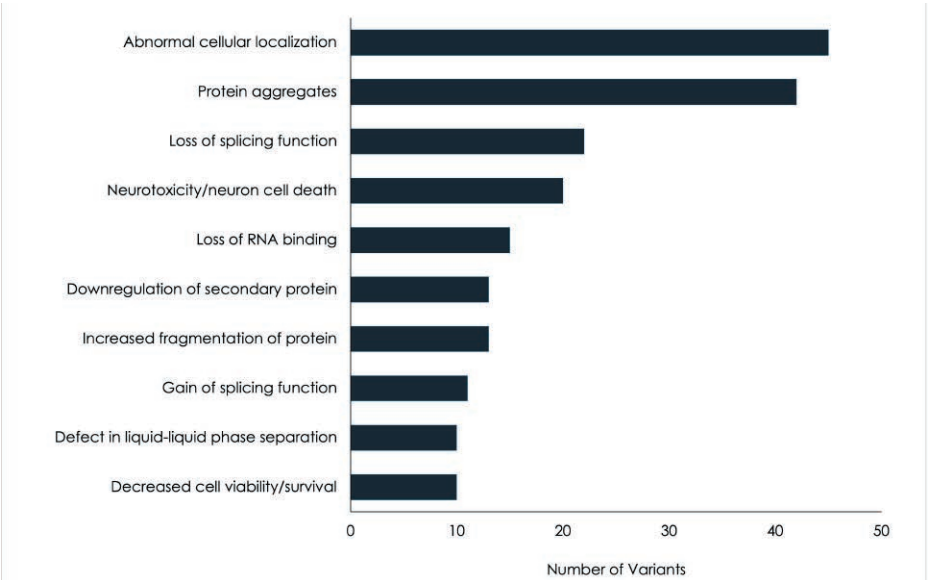


Figure 7: Most common functional consequences for variants in *TARDBP*

(n=10)

Variants may have multiple functional consequences and appear in this chart multiple times.

Association of ALS Genes with Disease Mechanisms

Beyond clinical characteristics, the ALS Genomic Landscape also provides comprehensive insight into functional consequences at both a wider gene level and a more granular variant level. For example, *TARDBP* (also known as TDP-43) is the pathological hallmark of most ALS cases, with about 97% showing ubiquitinated, aggregated inclusions of the *TARDBP* protein. Variants in the *TARDBP* gene itself, however, are also responsible for ~5% of familial ALS and <1% of sporadic ALS cases.[10,19]

The ALS Genomic Landscape contains 324 variants in *TARDBP*, 116 of which had at least one corresponding functional study. The most common functional consequences of these variants, involving protein aggregation, abnormal cellular localization, and effects on RNA binding/processing/splicing, were consistent with the overall function of *TARDBP*, as well as the protein aggregates frequently seen in ALS cases (**Figure 7**).

At a more granular level, our analysis of these individual variants also revealed a cluster of variants within the first and second RNA recognition motifs (RRM1 and RRM2) of the *TARDBP* protein, as well as within the nuclear export sequence (NES) and the nuclear localization sequence (NLS), which is consistent with defects in localization and RNA binding (**Figure 8**). This is an interesting addition to what is seen in ClinVar and known by consensus that most variants in *TARDBP* reside towards the C-terminal of the protein.[10] Knowledge of these unexpected *TARDBP* variants can assist in providing more accurate interpretations of sequencing results as well as further understanding of the underlying disease mechanism in ALS.

A Comprehensive Genomic Landscape Provides an Evidence-Based Foundation for Precision Medicine

A Comprehensive Genomic Landscape is aptly named for its ability to offer a wide-reaching picture of the "landscape" of a particular disease, from the genes involved and the correlation between genotype and phenotype to the underlying disease mechanisms. That broad picture is also

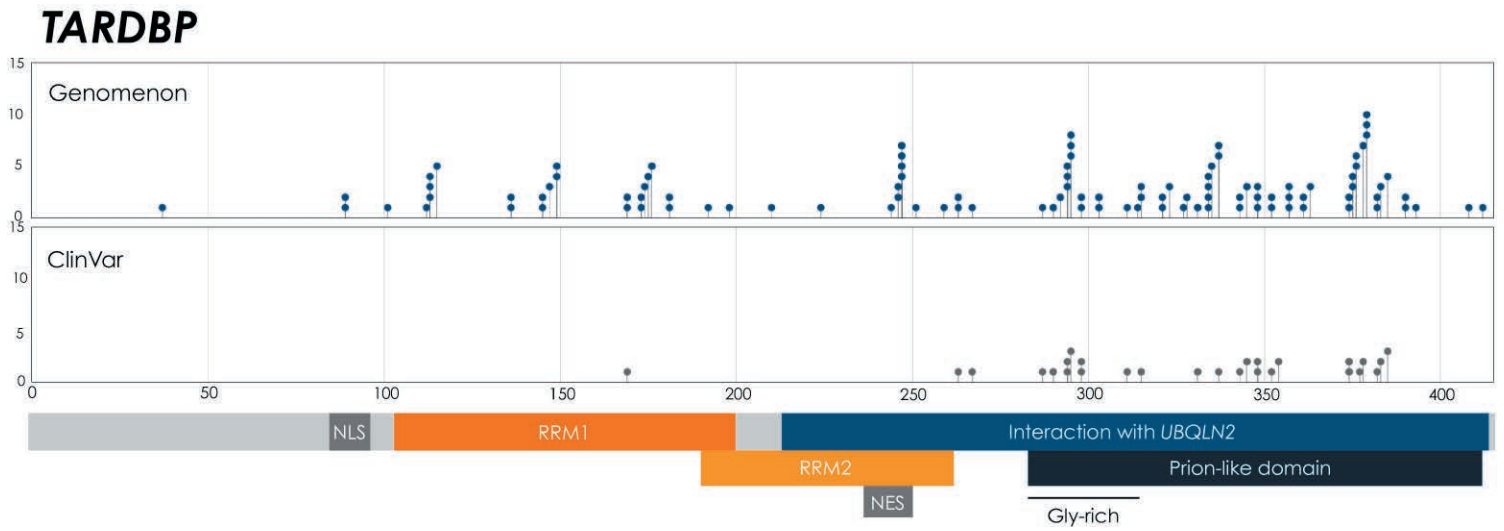


Figure 8: Variants in TARDBP by protein position

supported by an immense amount of detailed, categorized evidence that allows for rapid, yet meticulous analysis of selected genes and variants. This dual function places a Genomic Landscape in the perfect position to serve as a foundation for precision medicine initiatives, from early research and discovery to clinical trials and on to actively treating patients in the clinic.

Pharmaceutical and Biotech Applications

For pharmaceutical companies involved in the development of targeted therapies, the value of the Genomic Landscape is four-fold and extends through the entire development process (**Figure 9**):

1. Identification of target genes
2. Identification of candidate variants for disease models
3. Companion diagnostics for clinical trial enrollment
4. Support of natural history studies

As noted for the clinical case, drug discovery and development processes in pharma and biotech have historically been undertaken through manual and time-consuming searches of the medical literature that may not allow for a sufficiently comprehensive review. When screening potential drug targets or variants to be used in disease models, this lack of sensitivity may result in insufficient supporting evidence for a decision, and consequentially, a higher likelihood that early drug candidates fail. On the other hand, drug targets with clear genetic support are more than twice as likely to be approved compared to drugs without such evidence, and as such, having a comprehensive understanding of the genetic landscape for a given indication is crucial to success in clinical trials.[35,36]

Beyond the target identification stage, a comprehensive set of variants interpreted according to standard pathogenicity criteria is also crucial to enrolling a sufficient number of patients, and perhaps more importantly, for enrolling the *most appropriate* patients for a targeted gene therapy. As any given patient would be required to possess a pathogenic variant in the target gene, having the largest set of pathogenic variants can maximize the number of eligible patients. The Genomic Landscape, combined with large-scale sequencing efforts and other databases such as ClinVar, can provide the greatest chance of success for these therapies in clinical trials. Finally, the annotation of clinical characteristics in the Genomic Landscape can support natural history studies, which are utilized throughout the entire clinical trial process to ensure adequate understanding of the disease/patient population, to develop outcome assessments and endpoints, to inform overall design of the clinical trial, and ultimately, to aid in interpretation of results and lend support to the FDA approval process.

For ALS, there are currently three targeted gene therapies in clinical trials and many in pre-clinical development. As time goes on, the number of therapies entering the pipelines of pharmaceutical companies is expected to increase as clinical trials chip away at the myriad causes of ALS. To get these therapies into the hands of the patients who need them, a solid foundation of evidence will be necessary to support these trials, especially for gene targets that are less understood and/or affect fewer patients.

Clinical Genetic Testing Applications

In addition to providing value to pharmaceutical companies and the clinical trial process, the Genomic Landscape also benefits the clinical diagnostics laboratories that interpret genetic testing results as well as the clinicians and genetic counselors that are tasked with applying those results.

As targeted therapies become available, a patient's eligibility will hinge on diagnostic testing and interpretation of results. Importantly, this testing and interpretation process is dependent on the availability of a comprehensive database of known variants, supported by evidence, to interpret the results both consistently and accurately. Due to the lethality of the disease, it is of the utmost importance that this evidence is readily available to allow for the earliest possible therapeutic intervention. The Genomic Landscape for ALS provides the necessary evidence in a comprehensive and organized form to both expedite and ensure accuracy in variant interpretation.

In addition, the quality and breadth of evidence provided by these laboratories for a patient's variant(s) can be critical for clinicians and genetic counselors to counsel the patient properly on the expected disease course as well as the risk to family members. Due to the detailed annotations of clinical characteristics that are available in the Genomic Landscape such as age at onset and speed of progression, counseling of patients can become even more evidence-based through close evaluation of other known cases.

Finally, as the medical literature continues to expand, any variants that are currently of undetermined significance can become categorized as pathogenic in the near future as new evidence is validated, making re-analysis of a patient's variant(s) an important and necessary task. Fortunately, the Genomic Landscape is continually updated alongside the medical literature to expedite this process.

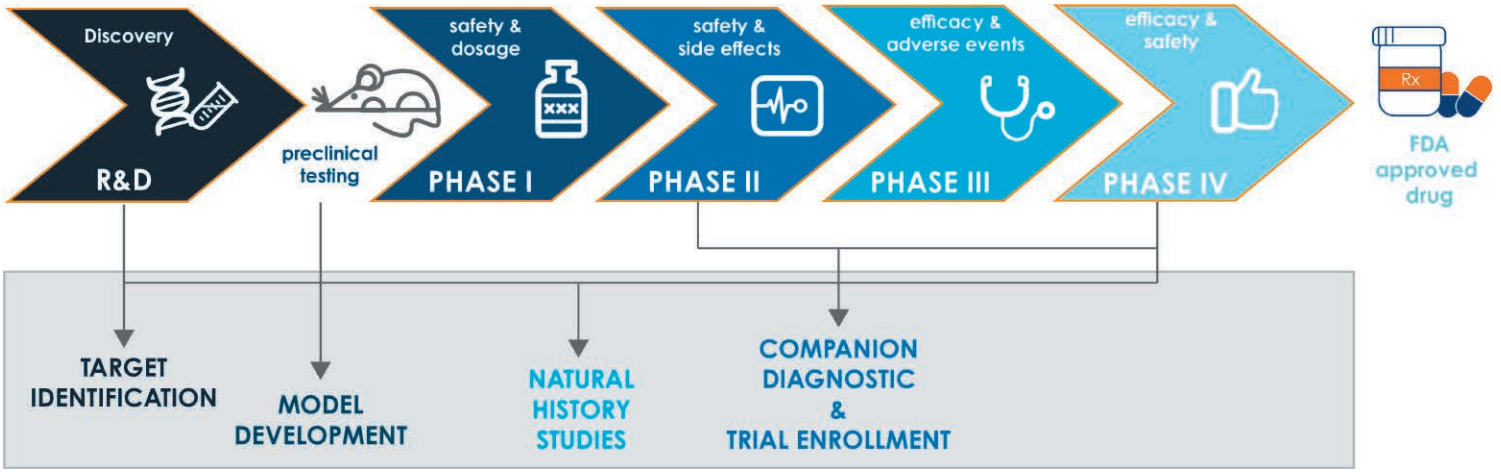


Figure 9: Needs throughout the clinical trial process that can benefit from a comprehensive genomic landscape

Taken together, the Genomic Landscape for ALS can provide an evidence-based and actionable foundation for the entire precision medicine process, from early drug development all the way to determining eligibility for and administering therapy through clinical laboratory testing. Combined with the multitude of efforts being undertaken by the ALS research community to further understand the disease and its genetics (from annotation of the existing literature to novel sequencing studies) we stand to improve the value of genetic testing as well as to ensure that patients are appropriately diagnosed and treated.

As precision medicine continues to advance and more of these targeted therapies are developed, an evidence-based foundation will be even more valuable to ensuring success in the mission to make debilitating diseases like ALS treatable.

Summary of The Genomenon ALS Genomic Landscape

- Contains over 7,000 variants across 36 ALS-associated genes with an additional patient-focused database for *C9orf72*, which contains over 2,500 cases
- Increases the number of clinically informative variants, which both supports and augments the value of current large-scale sequencing/data-gathering efforts
- Exposes substantial clinical heterogeneity across ALS-associated genes, involving diagnoses, age at onset, and speed of progression
- Reveals novel associations between variants and their position across the protein with functional consequences
- Supports and accelerates the drug development/clinical trial process as well as clinical genetic testing ToPM

Lauren Chunn

Lauren Chunn, Genomenon, Inc., is the data science and bioinformatics manager at Genomenon, where she ensures the speed and accuracy of variant interpretation and patient database production. She holds a Bachelor of Science degree in Cell and Molecular Biology and Philosophy from Grand Valley State University and is a passionate advocate for rare disease awareness.

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