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Title: ALS Identified: 2-Year Findings From a Sponsored ALS Genetic Testing Program

Running head: ALS Identified: Sponsored Genetic Testing Program

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ABSTRACT

Objective: To report initial results from the Amyotrophic Lateral Sclerosis (ALS) Identified genetic testing (GT) program on characteristics of individuals tested and frequency of reported disease-causing variants.
Methods: ALS Identified used the Invitae Amyotrophic Lateral Sclerosis panel (Invitae, San Francisco, CA, USA) to assay 22 ALS-associated genes. Sponsored by Biogen (Cambridge, MA, USA), the program launched in June-2021 and was available at no charge to individuals ≥18 years in the United States and Puerto Rico with an ALS diagnosis or a known family history of ALS. Deidentified data were available to Biogen.
Results: As of 26-October-2023, 998 healthcare professionals ordered the panel at 681 unique care sites. Of 8,054 individuals examined, 7,483 (92.9%) were reported to have a clinical diagnosis of ALS while 571 (7.1%) were asymptomatic relatives. Of the individuals with a clinical ALS diagnosis, 57.7% were male (n=4,319), and 42.3% female (n=3,164). Mean (SD) age at diagnosis is 62 (13) years. Out of the 7,483 clinically diagnosed individuals, 1,810 (24.2%) showed genetic variations in ALS-associated genes. Among these, 865 individuals (47.8%) carried pathogenic variants, and 44 (2.4%) had likely pathogenic variants, totaling 12.1% of the clinically diagnosed population.
Interpretation: Since 2021 there has been robust uptake and sustained use of the ALS Identified program, one of the largest samples of people with ALS to date across the US, demonstrating the interest and need for genetic ALS testing.

Keywords: amyotrophic lateral sclerosis, genetic testing, *C9orf72*, *SOD1*

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rare, fatal neuromuscular disease characterized by signs and symptoms of upper and lower motor neuron degeneration resulting in progressive weakness of the bulbar, limb, thoracic, and abdominal muscles.[1, 2] Up to 15% of persons living with ALS develop dementia, typically frontotemporal dementia (FTD), and up to 50% of persons living with ALS develop some form of cognitive dysfunction.[1, 2] Death typically results from respiratory failure 2 to 5 years after diagnosis.[2-5]

Known risk factors include male sex, older age, environmental exposures, and a known family history, which can associate with specific gene variants.[1, 6-8] Since the identification of superoxide dismutase 1 (*SOD1*) in 1993, the first gene found to be associated with ALS, advances in gene mapping and DNA analysis have revealed additional genes associated with ALS.[3, 9-11] It is now well-established that individuals with, or without, a known family history of ALS (familial ALS [fALS] or sporadic ALS [sALS], respectively) can have an underlying genetic etiology associated with ALS.[3, 9-11] For example, the most frequent genetic cause of ALS in populations of European descent is a hexanucleotide repeat expansion of the *C9orf72* gene, which is present in up to 45% of people with fALS and in up to 10% of the much larger population considered to have sALS.[12-15]

An ALS molecular diagnosis informs family risk assessment and allows at-risk relatives to consider predictive genetic counseling and testing.[16] Furthermore, with the 2023 USA Food and Drug Administration (FDA) accelerated approval of tofersen, the first genetically targeted treatment for *SOD1*-ALS,[17] and the development of further gene-based therapies in the pipeline,[18] genetic testing is an increasingly critical component of routine clinical care to assess eligibility for gene-targeted therapies or clinical trials. Recently published guidelines also highlight the importance of offering genetic counseling and testing to all persons living with ALS, regardless of known family history.[19] While the potential benefits of genetic testing for ALS are clear, barriers such as cost, perceived lack of impact on a person's treatment plan, and limited

access to genetic counseling[20, 21] can lead to wide variations in practices regarding the use of genetic testing among individuals with or at risk for ALS, despite evidence indicating a genetic etiology in both groups.[20-22]. This may contribute to underdiagnosis of genetic ALS, which may negatively impact outcomes for persons living with ALS, and limit access to therapies in development.[20]

The ALS Identified genetic testing program, sponsored by Biogen (Cambridge, MA, USA) and offered through a clinical diagnostic laboratory (Invitae Corporation [Invitae]; San Francisco, CA, USA), facilitates genetic testing for ALS by eliminating the cost barrier of the testing. Here, we report interim results and accession data from the first two years of this genetic testing pilot program.

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MATERIALS AND METHODS

Program design and participants

ALS Identified was launched on 30 June 2021 in the United States. The panel was available at no charge for all individuals aged 18 years or older living in the United States, including Puerto Rico, with an ALS diagnosis and/or a known family history of ALS. Once the participant acknowledged the informed consent (Supplementary File 1), the licensed healthcare provider gave attestation of the consent and orders the test online or via a printed test requisition form (Supplementary File 2). The ordering clinician had the option to provide participant demographics and ALS disease characteristics. Post-test genetic counseling through Invitae was available to participants by request at no charge.

ALS panel and testing methods

The Invitae Amyotrophic Lateral Sclerosis Panel (Invitae, San Francisco, CA, USA) assayed 22 genes associated with ALS (*ALS2, ANG, ANXA11, C9orf72, CHCHD10, DCTN1, ERBB4, FUS, HEXA, KIF5A, OPTN, PFN1, SETX, SOD1, SPG11, SQSTM1, TARDBP, TBK1, TFG, UBQLN2, VAPB, VCP)* with the option to test 9 additional genes that did not currently have a definitive clinical association with ALS but may prove to be clinically significant in the future (*ATP13A2, CHMP2B, DDHD1, ERLIN1, FIG4, MATR3, NEFH, SIGMAR1, TIA1*). These genes were selected based on the available evidence at the time of program launch (30 June 2021) to provide a broad ALS evaluation,[16] allowing for an efficient evaluation of many potentially relevant genes for a single clinical indication. The genes in the primary panel were evaluated for all ALS Identified participants, as these genes are demonstrably associated with ALS.

The laboratory accepted saliva, buccal swabs, or blood samples for DNA extraction (Omega bio-tek [Norcross, GA, USA] Mag Bind Blood and Tissue with HDQ DNA extraction reagents). For sequencing, Invitae used Illumina (San Diego, CA, USA) HiSeq and NovaSeq instruments, which were both clinically validated. Sequence data were processed through a

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custom-built bioinformatics pipeline that included a combination of publicly available but specifically optimized toolkits, such as GATK Haplotype caller (Broad Institute, Cambridge, MA, USA) to call single nucleotide variants and small insertions and deletions, and Invitae's software for calling exonic copy number variants, variants embedded in homopolymeric regions, and specific types of rearrangements.[23, 24] The sequence analysis covered clinically relevant regions of each gene, including coding exons and 10 to 20 base pairs of adjacent intronic sequences on either side of the coding exons in the transcript listed in the test catalog, depending on the specific gene or test. Select non-coding variants are covered, as well. Based on validation study results, the assay achieved >99% analytical sensitivity and specificity for single nucleotide variants, insertions and deletions <15 bp in length, and exon-level deletions and duplications. Insertions and deletions >15 bp but less than a full exon were also detected, but sensitivity for these may be marginally reduced. Deletion/duplication analysis determined copy number at a single exon resolution at virtually all targeted exons. However, in rare situations, single-exon copy number events may not be analyzed due to inherent sequence properties or isolated reduction in data quality. The *C9orf72* hexanucleotide repeat expansion (HRE) was assessed using repeat-primed polymerase chain reaction, characterizing repeats with lengths ranging from 4 to greater than 30.[25] Repeat lengths greater than 30 may indicate considerably larger expansions, ranging from hundreds to thousands of repeats, but the assay did not distinguish among these.

It is important to emphasize that, within our dataset cohort, the *C9orf72* gene was not included in the request for testing for 11 of the participants; thus, results for these 11 individuals were reported only for the remaining 21 genes tested in the primary panel. While the reason for exclusion of *C9orf72* from these 11 test requisitions is unclear, it is possible that *C9orf72* testing was completed outside of the ALS Identified program given the established high prevalence of *C9orf72* HRE among persons living with ALS.[9, 26]

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Variant interpretation and categorization followed the American College of Medical Genetics and Genomics (ACMG) guidelines, as refined by Sherloc, Invitae's score-based system. [27, 28]

Furthermore, the pattern of inheritance of genetic variants within families can be used to facilitate accurate categorization:

- Positive: Indicates that an individual is likely to develop ALS due to the variant(s) they carry.
- Carrier: Denotes a heterozygous variant present at a recessive locus where a second pathogenic (P) variant is not identified. Therefore, the individual is classified as a carrier, but does not have a positive molecular diagnosis due to the absence of a second P variant.
- Uncertain: This category aligns with the interpretation of variants of unknown significance, where a definitive conclusion is not possible at the time of report release.

Details regarding zygosity, variant interpretation, and ClinVar classification, including novel genetic variations not previously reported on ClinVar or the Invitae database (approximately 14%) can be found in Supplementary File 3, which includes all variation types. Additionally, Table 1 includes an overview of variants classified as P or likely pathogenic (LP).**Data analytics**

Power BI, Excel (Microsoft Inc, Redmond, WA, USA), and RStudio (RStudio, PBC, Boston, MA, USA) were utilized for data handling and analyses, enabling a comprehensive integration and examination of clinical and genetic datasets, allowing valuable insights to be gleaned from anonymized ALS Identified data. For the geographical distribution analysis of the participant population, we utilized the United States Census Bureau's regional and divisional map. Tableau Public software (version 2021.4.2, Tableau Software, LLC, Seattle, WA) was also used to generate visualizations and analyze data, particularly for generating maps.

Age at accessioning was calculated using the participant's date of birth and the date Invitae accessioned the sample. It was capped at the age of 89 due to the small number of

participants above this age. Consequently, we have not included data on participants over the age of 89. In addition, to avoid conflicts among the different age metrics reported, the following rule was applied: the age of symptom onset should be less than or equal to the age of diagnosis, which, in turn, should be less than or equal to the age at accessioning.

The ClinGen Classification for ALS-associated genes was sourced directly from the Clinical Genome Resource website using the Gene-Disease Validity tab (ClinGen Gene-Disease Validity Curations [clinicalgenome.org]). The ClinGen classification for all identified genetic variants was annotated through Varsome API (Supplementary File 3).

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RESULTS

Uptake of genetic testing program

From 30 June 2021 to 26 October 2023, 998 healthcare professionals ordered the Invitae Amyotrophic Lateral Sclerosis panel at 681 unique care sites. Monthly testing volume remained steady throughout the dataset period (whole month testing accrual shown in Fig. 1, US map of total testing accrual by state shown in Fig. S1), including through the FDA Advisory Committee Meeting in March 2023 and the FDA accelerated approval of tofersen in April 2023. A testing peak can be seen around May 2023, after FDA approval, followed by a return to a normative level in June 2023 (Fig. S1).

Participant characteristics

As of 26 October 2023, a total of 8,054 individuals were tested; 7,483 (92.9%) with symptoms (detailed below) and a clinical diagnosis of ALS and 571 (7.1%) asymptomatic family members of persons living with ALS; 7,277 (97.2%) reported no, missing, or unknown family history of ALS. The data below includes all participants who were symptomatic with a clinical diagnosis of ALS (referred to throughout as “diagnosed with ALS”. Participants who were asymptomatic (n = 571) were not included in this analysis due to heterogenous/unspecified screening data. Of participants with a clinical diagnosis of ALS (n = 7,483), most (75.8%) were White, 57.7% were male, and the mean (SD) age at diagnosis was 62 (13) years (Table 2). The highest frequency of tests was ordered in the South census region (37.7%), followed by the Midwest (28.6%), West (18.6%), and Northeast (14.7%) regions. Most ordering clinicians specialized in Neurology (70.6%), followed by Neuromuscular Medicine (12.8%) and Family Medicine/General Practice (5.5%). Ordering clinician specialties (reported using the MedPro Clinical Specialty Database and based on the unique National Provider Identifier) are detailed in Table S1. Less than 1% of all participants tested took advantage of the no charge posttest genetic counseling offered by the ALS Identified program (data not shown). While no data are available that elucidate rationale for the low rate of genetic counseling utilization, it is possible

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that providers ordering testing through the program had established methods for providing genetic counseling services within their institution.

Main clinical manifestations

Progressive muscle weakness (76.5%) and muscle fasciculations (61.3%) were the most commonly reported symptoms. Approximately 44.3% of participants were reported to show signs of increased muscle tone, deep tendon reflexes, and/or hyperreflexia. In contrast, psychiatric, behavioral, and progressive cognitive decline were reported for smaller groups (2.7%, 5.5%, and 8.4%, respectively) (Table 3). The region of symptom onset was reported to be limb-related for the majority of participants (72.0%), while 33.6% had a bulbar onset, 18.2% presented with respiratory function impairment, and 8.5% exhibited cognitive deficits at disease onset (Table 2) with 8.4% demonstrating persistent cognitive decline (Table 3).

Furthermore, participants who had a molecular diagnosis in a gene reported in more than 10 individuals within this dataset were selected for a thorough analysis of clinical symptoms. Detailed information is available in Tables S2 and S3. In summary, individuals with a positive *C9orf72* diagnosis predominantly displayed either bulbar- (42%) or limb-onset (66.61%) symptoms. Conversely, those with a positive molecular diagnosis in *SOD1, SQSTM1, TARDBP*, and *FUS* predominantly experienced limb-onset disease, with percentages ranging from 72.73% (*FUS*) to 87.76% (*SOD1*). A substantial proportion of participants with molecular diagnoses in *C9orf72, SOD1, SQSTM1, TARDBP,* and *FUS* presented with progressive muscle weakness and atrophy. Muscle fasciculations were notably observed in participants with *SOD1* (67.35%), *TARDBP* (64.52%), *C9orf72* (59.07%), and *SQSTM1* (48.65%) molecular diagnoses. Increased muscle tone, deep tendon reflexes, and hyperreflexia manifested more frequently in participants with *C9orf72, SQSTM1*, and *FUS* molecular diagnoses, while dysarthria and dysphagia were more common in participants with *C9orf72* and *SQSTM1*. Approximately one-quarter of participants with *C9orf72, SQSTM1*, and *FUS* molecular diagnoses also exhibited spasticity. Additionally, while pseudobulbar affect, progressive cognitive decline, and behavioral changes

were infrequently observed, they were most prevalent in those with a molecular diagnosis in *C9orf72* (Tables S2 and S3).

Frequency of variants

Of the 7,483 individuals tested with a diagnosis of ALS, 1,810 (24.18%) had a molecular finding from the analysis of the primary 22-gene panel. A total of 2045 genetic variants, 44.6% P/LP and 55.4% variants of uncertain significance (VUS), were identified among the 1,810 ALS participants with a genetic finding (Table 4, Figure 2). Specifically, 1,595 ALS participants carried one variant, 198 carried two genetic variants, 14 carried three genetic variants, and 3 participants carried 4 variants. In total, 807 participants met criteria for a positive (797) or potentially positive (10) molecular diagnosis of ALS, indicating a strong association between their ALS diagnosis and an identifiable genetic cause. The remaining 106 symptomatic participants with P or LP variants were classified as carriers. For more details on variant interpretation and categorization, please refer to the Methods section and Supplementary File 3.

The occurrence of genetic variants classified as P or LP was most prevalent in the *C9orf72* gene (27.2%), followed by the *SOD1* gene (4.8%) (Table 4). All variants classified as P/LP were recurrent. The majority of P and LP variants were identified in five or fewer individuals, with the exception of the *C9orf72* HRE (n=557 occurrences), *SQSTM1* p.Pro392Leu variant (n = 32), *SOD1* p.Ala5Val (n = 23), and p.Ile114Thr (n = 19) variants, *ANXA11* p.Gly38Arg variant (n=8), and the *CHCHD10* p.Arg15Leu variant (n=7). There were 9 *C9orf72* HRE instances classified as VUS, with their repeat length spanning between 25 and 30 repeats. All other identified HRE were greater than 30 in size and ranged from hundreds to thousands of repeats.

In terms of genes with recessive inheritance and the co-occurrence of VUS with P/LP variants, participants with either *ALS2* or *HEXA* variants carried heterozygous P variations. Only one individual was found to carry a VUS (p.Ala496Gly) in the *HEXA* gene. One participant was identified with a homozygous P variation in the *SPG11* gene, while 3 participants had a P

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SPG11 variant and a VUS (pThr69Met, p.Leu2101Val, and p.Pro253Ser). Additionally, one individual carried an *OPTN* homozygous LP variant (splice acceptor), and another participant had an *OPTN* LP variant (gain [exons 7-10]) along with a VUS (p.Lys557Thr).

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DISCUSSION

We report the use of ALS Identified, a no-charge ALS genetic testing program, sponsored by Biogen and offered through Invitae. There are many potential barriers to testing, with cost identified as a leading factor.[22, 29] With the removal of the cost barrier in ALS Identified program, maintenance of robust uptake has been observed since the program's inception. These findings are consistent with the current literature suggesting persons living with ALS have high interest in genetic testing,[19] and that reducing cost-related barriers may result in broader and more equitable access Although a molecular finding does not constitute a diagnosis of ALS in isolation, genetic testing may help a person living with ALS understand the basis of their condition, and also may inform the risk assessment for family members.. Additionally, genetic testing informs assessment of clinical trial eligibility and can support individuals in making informed healthcare and life planning decisions, as well as long-term lifestyle choices.[9, 16, 29, 30] As the landscape of drug development undergoes a transformative shift toward the integration of precision medicine, genetic testing is poised to become standard-of-care in ALS. This is reinforced by evidence-based consensus guidelines published in 2023, recommending genetic testing for all persons living with ALS.[19]

Demographic characteristics of participants in the program with a diagnosis of ALS are comparable to those of the overall US ALS population reported in the 2016 National ALS Registry, with ALS more common among people with demographics of White ancestry, male gender, and persons aged 60–69 years at diagnosis.[31]

In general, progressive muscle weakness was a common finding among people with a diagnosis of ALS with P/LP genetic variants. Muscle fasciculations, particularly prevalent, were

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more frequently observed in participants with *SOD1* and *TARDBP* molecular diagnoses than in the cohort as a whole. Dysarthria and dysphagia were most common in participants with *C9orf72* and *SQSTM1* molecular diagnoses. And while cognitive deficits and behavioral changes were rarely observed across the examined cohort, they were most prevalent in participants with a molecular diagnosis in *C9orf72*.

P/LP variants in *C9orf72* and *SOD1* were the most commonly reported, consistent with evidence from published literature.[3] These were followed by variants in the *SQSTM1*, *TARDBP*, and *FUS* genes. Interestingly, the p.Pro392Leu variant in the *SQSTM1* gene was the second most common variant after the *C9orf72* HRE. This latter was followed by the p.Ala5Val and p.Ile114Thr *SOD1* variants, identified in 23 and 19 participants, respectively. All other variants were identified at lower frequencies. Differences in variant frequencies between the ALS Identified data and published literature are likely a result of the high variability in estimates due to the small number of individuals with variants in any gene other than *C9orf72* in ALS Identified.[9]

ALS Identified data suggest that up to 12.1% of all ALS diagnoses may have a genetic etiology, including 11.5% with P variants and 0.6% with LP variants. In ALS Identified participants, the proportion of *C9orf72* diagnoses among participants diagnosed with ALS and a genetic variant (30.7%) was comparable to estimates from a published meta-analysis in populations of European descent (33.7%) (refer to Table S4 for further details).[9] The observed frequency of participants with *C9orf72*, *SOD1, TARDBP, and FUS* P/LP variants aligned with findings from large-scale studies and meta-analyses (Table S4).[9,33]

Limitations of this program include a lack of standardization in reporting family history and information gaps as clinical history depends on the person completing the test requisition form (paper version or online). The sample of persons living with ALS examined is a convenience sample determined by healthcare professionals opting to have a patient or patients tested through the program. Ancestry, regional, and/or ordering physician type subgroup

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analysis were not planned for this dataset as these data were relatively sparse; future, larger studies would be helpful to analyze potential differences in subgroups of potential interest. As with all genetic testing panels, the ALS Identified panel did not capture all possible genetic causes as new associations will be made between ALS and other genes in the future. The panel also did not include ATXN2.

The ALS Identified program provides the opportunity for standardized genetic testing across the United States, using a panel of 22 ALS-associated genes, providing one of the largest samples of persons living with ALS to date. This ongoing program aims to deepen our understanding of the genetic landscape of ALS and emphasizes the significance of genetic testing and counseling, as they facilitate the provision of molecular diagnoses to ALS patients and their family members. Additionally, this program may contribute to the design and development of targeted therapies by identifying the most impacted genes within the ALS population.

In summary, gaining insights into an individual's genetic makeup holds immense potential across various facets of the medical field. Not only does it pave the way for targeted therapies tailored to specific genetic profiles, it facilitates the identification of individuals at increased risk for certain conditions. The significant engagement of persons with ALS and their relatives, as evidenced by their participation in our free genetic testing program, underscores a tangible and urgent need to offer comprehensive genetic testing and counseling services to both those currently affected by the disease and those at risk. This proactive approach not only empowers persons living with ALS with knowledge about their genetic predispositions but also opens avenues for early intervention, personalized treatment plans, and a deeper understanding of familial risk factors. Ultimately, integrating genetic testing into ALS care contributes to a more precise and patient-centered approach, addressing the unique genetic landscape of each individual for improved health outcomes.

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DISCLOSURES

SAG: no disclosures relevant to the publication. NAG: no disclosures relevant to the publication. KP: nothing to disclose. VK and MLK: employee of Biogen at the time of development. CP-R, AAM, and TEF: employees of and may hold stock in Biogen.

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Table 1: Novel P and LP variants identified in participants diagnosed with ALS

Gene	Protein effect	HGVS*	Zygosity	Interpretation	Diagnostic_Category
ALS2	Splice donor	NM_020919.3:c.3248+1G>C	heterozygous	Likely Pathogenic	carrier
FUS	p.Lys510Glu	NM_004960.3:c.1528A>G	heterozygous	Likely Pathogenic	positive
KIF5A	p.Lys167Argfs*17	NM_004984.2:c.500del	heterozygous	Pathogenic	positive
OPTN	Partial Deletion (Exons 3-6)	NM_021980.4:c.246_779+1041del	heterozygous	Likely Pathogenic	carrier
OPTN	Gain (Exons 7-10)	NM_021980.4:c.780-?_1242+?dup	copy number = 3	Likely Pathogenic	potentially_positive
OPTN	Deletion (Exons 3-6)	NM_021980.4:c.167-?_779+?del	heterozygous	Pathogenic	carrier
OPTN	p.Glu81*	NM_021980.4:c.241G>T	heterozygous	Pathogenic	carrier
OPTN	p.Glu175Lysfs*18	NM_021980.4:c.523del	heterozygous	Pathogenic	carrier
OPTN	Deletion (Exons 7-10)	NM_021980.4:c.780-?_1242+?del	heterozygous	Pathogenic	carrier
OPTN	p.Gln151Thrfs*13	NM_021980.4:c.450dup	homozygous	Pathogenic	positive
SETX	p.Val129Thrfs*31	NM_015046.5:c.385_388del	heterozygous	Pathogenic	carrier
SETX	p.Val1674Phefs*15	NM_015046.5:c.5019del	heterozygous	Pathogenic	carrier
SETX	Deletion (Exons 7-10)	NM_015046.5:c.719-?_5274+?del	heterozygous	Pathogenic	carrier
SPG11	Splice acceptor	NM_025137.3:c.3687-2A>G	heterozygous	Likely Pathogenic	carrier
SPG11	p.Asn557fs	NM_025137.3:c.1668_1669ins?	heterozygous	Pathogenic	carrier
SPG11	Deletion (Exons 16-20)	NM_025137.3:c.2835-?_3520+?del	heterozygous	Pathogenic	carrier
SPG11	p.Glu1023*	NM_025137.3:c.3067G>T	heterozygous	Pathogenic	carrier
SPG11	p.Cys1734Metfs*3	NM_025137.3:c.5199dup	heterozygous	Pathogenic	carrier
SPG11	Deletion (Exon 34)	NM_025137.3:c.6344-?_6477+?del	heterozygous	Pathogenic	carrier
TBK1	Splice acceptor	NM_013254.3:c.1863-1G>A	heterozygous	Likely Pathogenic	positive
TBK1	Splice acceptor	NM_013254.3:c.1960-2A>T	heterozygous	Likely Pathogenic	positive
TBK1	Deletion (Entire coding sequence)	NM_013254.3:c.-31-?_*738+?del	heterozygous	Pathogenic	positive
TBK1	p.Asn652Lysfs*22	NM_013254.3:c.1956del	heterozygous	Pathogenic	positive
TBK1	p.Gln250*	NM_013254.3:c.748C>T	heterozygous	Pathogenic	positive

*HGVS: Nucleotide variation following the Human Genome Variation Society's nomenclature

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Table 2. Demographics and baseline characteristics

Characteristic		Diagnosed with ALS N = 7,483
Sex, n (%)	Female	3,164 (42.28%)
	Male	4,319 (57.72%)
Ancestry, n (%)	White	5,668 (75.75%)
	Black/African American	419 (5.60%)
	Hispanic	518 (6.92%)
	Native American/Pacific Islander	36 (0.48%)
	Asian	190 (2.54%)
	Unknown	604 (8.07%)
	Others	48 (0.64%)
Age at diagnosis, years	Nonmissing, n (%)	7,414 (99.08%)
	Unknown/missing, n (%)	69 (0.92%)
	Mean±SD (range)	62±13 (16-97)
	Median (IQR)	63 (55,71)
	<18	4 (0.05%)
	18-29	92 (1.23%)
	30-39	316 (4.22%)
	40-49	672 (8.98%)
	50-59	1,646 (22.00%)
	60-69	2,513 (33.58%)
	70-79	1,808 (24.16%)
	80+	363 (4.85%)
Age at symptom onset, years	Nonmissing, n (%)	7138 (95.39%)
	Unknown/missing, n (%)	345 (4.61%)
	Mean±SD (range)	60.49±12.4 (16-89)
	Median (IQR)	62 (16)
	<18	7 (0.09%)
	18-29	121 (1.62%)
	30-39	352 (4.70%)
	40-49	726 (9.70%)
	50-59	1751 (23.40%)
	60-69	2407 (32.17%)
	70-79	1536 (20.53%)
	80+	238 (3.18%)
Age of accessioning, years	Nonmissing, n (%)	7483 (100%)
	Mean±SD (range)	63±11.9 (18,89)
	Median (IQR)	64 (16)
	<18	0 (0%)
	18-29	67 (0.90%)
	30-39	271 (3.62%)
	40-49	610 (8.15%)
	50-59	1,607 (21.48%)
	60-69	2,573 (34.38%)
	70-79	1,926 (25.74%)
	80+	429 (5.73%)
Family history of ALS, n (%)	Yes	206 (2.75%)
	No	180 (2.41%)
	Unknown	4 (0.05%)
	Missing	7,093 (94.79%)
Family history of FTD, n (%)	Yes	36 (0.48%)
	No	158 (2.11%)
	Unknown	4 (0.05%)
	Missing	7,285 (97.35%)
Family history: other/unspecified dementia, n (%)	Yes	182 (2.43%)
	No	98 (1.31%)

	Unknown	0 (0%)
	Missing	7,203 (96.26%)
Accession year	2021	1,530 (20.45%)
	2022	3,304 (44.15%)
	2023	2,649 (35.40%)
Genetic testing panel, n (%)	Invitae panel only (Primary panel)	3,285 (43.90%)
	Add-on panel	4,042 (54.02%)
	Others	156 (2.08%)
Ordering clinician specialty, n (%)	Neurology	5,284 (70.61%)
	Neuromuscular Medicine	961 (12.84%)
	Family Medicine/Internal Medicine/General Practice	411 (5.49%)
	Genetic Counselor	216 (2.89%)
	Medical Genetics	92 (1.23%)
	Psychiatry/Psychology	15 (0.20%)
	Other	145 (1.94%)
	Missing	359 (4.80%)
Geography (by institution state), n (%)	Midwest	2,142 (28.62%)
	Northeast (including Washington DC)	1,096 (14.65%)
	Puerto Rico	37 (0.49%)
	South	2,820 (37.69%)
	West	1,388 (18.55%)
Bulbar onset, n (%)	Unknown	270 (3.61%)
	Missing	2,262 (30.23%)
	No	2,439 (32.59%)
	Yes	2,512 (33.57%)
Limb onset, n (%)	Unknown	124 (1.66%)
	Missing	1,332 (17.80%)
	No	637 (8.51%)
	Yes	5,390 (72.03%)
Respiratory onset, n (%)	Unknown	281 (3.76%)
	Missing	2,759 (36.87%)
	No	3,078 (41.13%)
	Yes	1,365 (18.24%)
Cognitive onset, n (%)	Unknown	325 (4.24%)
	Missing	2,845 (38.02%)
	No	3,677 (49.14%)
	Yes	636 (8.50%)

Percentages are shown out of total column N except for age of diagnosis, symptom onset, and accession, which are based on nonmissing values. Age of accession refers to the participant's age at the time the test is performed and is calculated based on participant's date of birth.

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Table 3. Main clinical manifestations assessed by corresponding physicians in ALS Identified

Clinical features	Diagnosed with ALS (n = 7,483) n, %
Spasticity	
Yes	2,010 (26.86)
No	2,642 (35.31)
Unknown	604 (8.07)
Missing	2,227 (29.76)
Dysphagia	
Yes	2,816 (37.63)
No	2,607 (34.84)
Unknown	159 (2.12)
Missing	1,901 (25.40)
Increased muscle tone/increased extremity/deep tendon reflexes/hyperreflexia	
Yes	3,313 (44.27)
No	1,501 (20.06)
Unknown	576 (7.70)
Missing	2,093 (27.97)
Dysarthria	
Yes	3,167 (42.32)
No	2,420 (32.34)
Unknown	119 (1.59)
Missing	1,777 (23.75)
Progressive muscle weakness/atrophy	
Yes	5,722 (76.47)
No	421 (5.63)
Unknown	67 (0.90)
Missing	1,273 (17.01)
Muscle fasciculations	
Yes	4,587 (61.30)
No	986 (13.18)
Unknown	238 (3.18)
Missing	1,672 (22.34)
Hyporeflexia	
Yes	1,410 (18.84)
No	2,959 (39.54)
Unknown	673 (8.99)
Missing	2,441 (32.62)

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Pseudobulbar affect	
Yes	981 (13.11)
No	3,776 (50.46)
Unknown	421 (5.63)
Missing	2,305 (30.80)
Progressive cognitive decline	
Yes	628 (8.39)
No	4,211 (56.27)
Unknown	397 (5.31)
Missing	2,247 (30.03)
Behavioral changes	
Yes	409 (5.47)
No	4,359 (58.25)
Unknown	409 (5.47)
Missing	2,306 (30.82)
Psychiatric illness	
Yes	201 (2.69)
No	4,406 (58.88)
Unknown	479 (6.40)
Missing	2,397 (32.03)

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Table 4. Variant frequency across the primary panel for participants diagnosed with ALS

Genes	*Variant Interpretation*	*Variant Frequency (variant count=2,045)*	*Variant Frequency (participant count=7,483)*
ALS2	P	2 (0.10%)	2 (0.03%)
	LP	2 (0.10%)	2 (0.03%)
	VUS	55 (2.68%)	54 (0.72%)
ANG	P	0	0
	LP	0	0
	VUS	11 (0.54%)	11 (0.15%)
ANXA11	P	10 (0.49%)	10 (0.13%)
	LP	0	0
	VUS	70 (3.42%)	70 (0.94%)
C9ORF72	P	557 (27.23%)	557 (7.44%)
	LP	0	0
	VUS	7 (0.34%)	7 (0.09%)
CHCHD10	P	7 (0.34%)	7 (0.09%)
	LP	2 (0.10%)	2 (0.03%)
	VUS	21 (1.02%)	21 (0.28%)
DCTN1	P	0	0
	LP	2 (0.10%)	2 (0.03%)
	VUS	101 (4.94%)	100 (1.34%)
ERBB4	P	0	0
	LP	0	0
	VUS	92 (4.49%)	90 (1.20%)
FUS	P	10 (0.48%)	10 (0.13%)
	LP	1 (0.05%)	1 (0.01%)
	VUS	62 (3.03%)	62 (0.83%)
HEXA	P	27 (1.32%)	26 (0.35%)
	LP	0	0
	VUS	20 (0.98%)	20 (0.27%)
KIF5A	P	2 (0.10%)	2 (0.03%)
	LP	2 (0.10%)	2 (0.03%)
	VUS	31 (1.51%)	31 (0.41%)
OPTN	P	30 (1.46%)	30 (0.40%)
	LP	7 (0.34%)	7 (0.09%)
	VUS	77 (3.76%)	77 (1.03%)
PFN1	P	1 (0.05%)	1 (0.01%)
	LP	0	0
	VUS	20 (0.98%)	20 (0.27%)
SETX	P	7 (0.34%)	7 (0.09%)
	LP	1 (0.05%)	1 (0.01%)
	VUS	88 (4.23%)	85 (1.14%)
SOD1	P	81 (3.96%)	81 (1.08%)
	LP	17 (0.83%)	17 (0.23%)
	VUS	48 (2.34%)	47 (0.63%)
SPG11	P	36 (1.76%)	36 (0.48%)
	LP	5 (0.24%)	5 (0.07%)

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	VUS	132 (6.45%)	131 (1.75%)
SQSTM1	P	37 (1.81%)	37 (0.49%)
	LP	0	0
	VUS	83 (4.06%)	81 (1.08%)
TARDBP	P	29 (1.42%)	29 (0.39%)
	LP	2 (0.10%)	2 (0.03%)
	VUS	36 (1.76%)	36 (0.48%)
TBK1	P	20 (0.98%)	20 (0.27%)
	LP	3 (0.15%)	3 (0.04%)
	VUS	92 (4.50%)	92 (1.23%)
TFG	P	2 (0.10%)	2 (0.03%)
	LP	0	0
	VUS	20 (0.98%)	20 (0.27%)
UBQLN2	P	1 (0.05%)	1 (0.01%)
	LP	0	0
	VUS	27 (1.32%)	27 (0.36%)
VAPB	P	5 (0.24%)	5 (0.07%)
	LP	0	0
	VUS	20 (0.98%)	20 (0.27%)
VCP	P	5 (0.24%)	5 (0.07%)
	LP	0	0
	VUS	19 (0.93%)	19 (0.25%)

LP = likely pathogenic; P = pathogenic; VUS = variant of uncertain significance.
The third column displays the total variant counts and percentages (n=2,045) identified in participants with an ALS diagnosis and genetic variation (n =1,810). The last column illustrates the percentage of variant carriers, calculated against the overall population of symptomatic carriers (n=7,483).

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Figure 1. Order accrual per month

Data for the full months of June 2021 and October 2023 are not available. ALS Identified launched 30 June 2021, and the data cutoff used for this analysis was 26 October 2023.

Figure 2. Distribution of (A) participant and (B) variant counts according to variant classification. Figure 2A considers co-occurrences of participants with various variant types, providing an accurate count of participants associated with specific variant types. Figure 2B shows classification of the total number of genetic variants by variant interpretation.

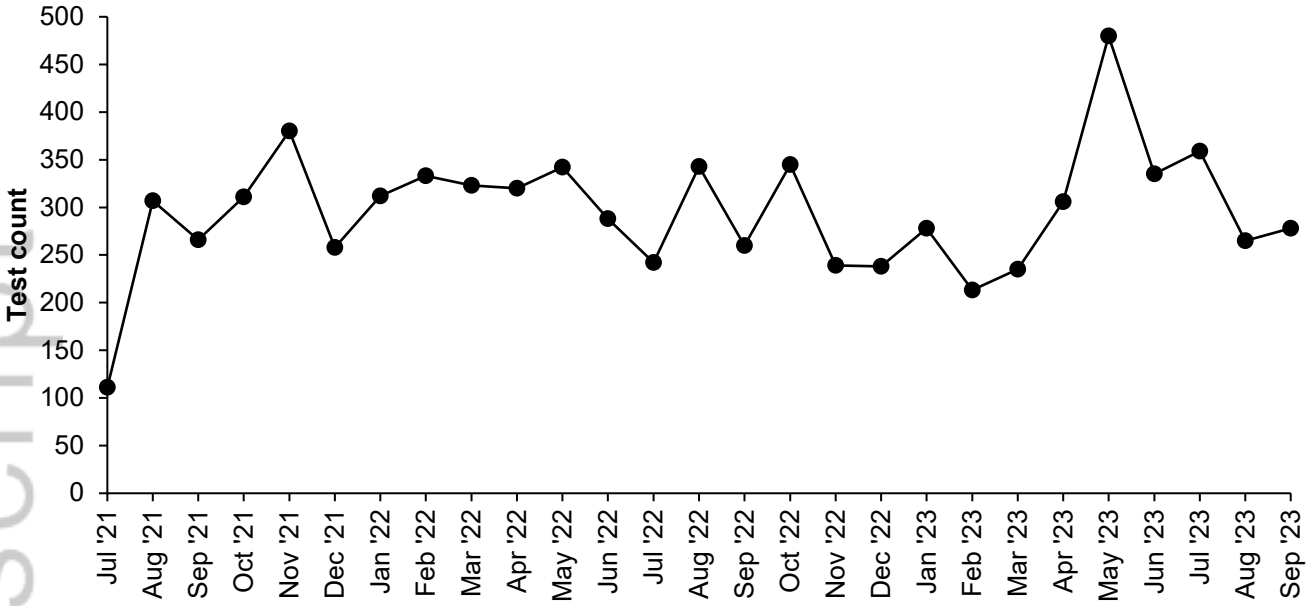
VUS = variant of unknown significance.

Supplementary File 1. Invitae Participant Consent Form

Supplementary File 2. ALS Identified requisition form

Supplementary File 3. ALS Identified Primary Panel Variants

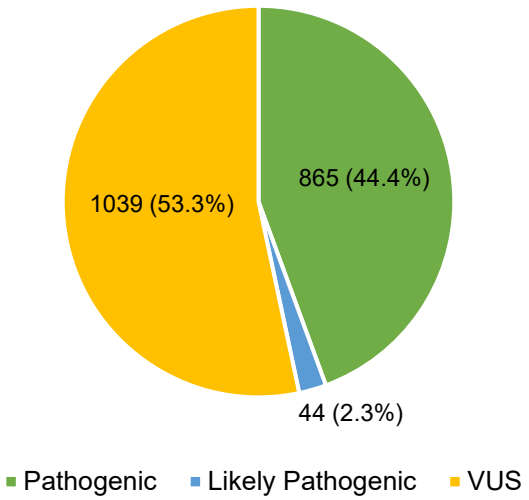
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A



B

