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Incidence of pathogenic, likely pathogenic, and uncertain ALS variants in a clinic cohort

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Abstract

Objective

To determine the incidence of amyotrophic lateral sclerosis (ALS) genetic variants in a clinic-based population.

Methods

A prospective cohort of patients with definite or probable ALS was offered genetic testing using a testing algorithm based on family history and age at onset.

Results

The incidence of pathogenic (P) or likely pathogenic (LP) variants was 56.0% in familial ALS (fALS); 11.8% in patients with ALS with a family history of dementia, and 6.8% in sporadic ALS ($p < 0.001$). *C9orf72* expansions accounted for the majority (79%) of P or LP variants in fALS cases. Variants of uncertain significance were identified in 20.0% of fALS cases overall and in 35.7% of *C9orf72*-negative cases. P or LP variants were detected in 18.5% of early-onset cases (onset age <50 years); the incidence of P or LP variants was not significantly different between family history types in this group.

Conclusions

Our data suggest that the incidence of P and LP variants in genes other than *C9orf72* is lower than expected in Midwestern fALS cases compared with research cohorts and highlights the challenge of variant interpretation in ALS. An accurate understanding of the incidence of pathogenic variants in clinic-based ALS populations is necessary to prioritize targets for therapeutic intervention and inform clinical trial design.

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Glossary

ACMG = American College of Medical Genetics; **ALS** = amyotrophic lateral sclerosis; **dALS** = patients with ALS with a family history of dementia; **fALS** = familial ALS; **LP** = likely pathogenic; **P** = pathogenic; **sALS** = sporadic ALS; **VUS** = variant of uncertain significance.

Remarkable and rapid progress in the discovery of amyotrophic lateral sclerosis (ALS)-associated genes, and a growing appreciation of the genetic component of clinically sporadic ALS (sALS), has opened the door to an era of personalized, gene-targeted therapies for people with ALS. As gene-targeted therapies move through the preclinical and clinical trial pipeline, there is a pressing need to improve the practice of genetic testing and to determine the incidence of genetic forms of ALS in clinic-based populations. Despite the progress in ALS gene discovery, the offer of testing to people with ALS is not yet "standard of care," and the incidence of clinically meaningful genetic variants in clinic populations has not been studied.

A genetic etiology is reported in ~70% of familial ALS (fALS) and ~15% of sALS in North American research cohorts.[1] However, US guidelines for ALS management do not address the offer of genetic testing,[2] and European guidelines direct that testing should be offered only to patients with fALS or the *SOD1* D90A phenotype.[3] Lack of guidance with respect to genetic testing practices,[4] in addition to challenges with testing methods and result interpretation,[5,6] has likely limited the application of genetic testing in the clinic.

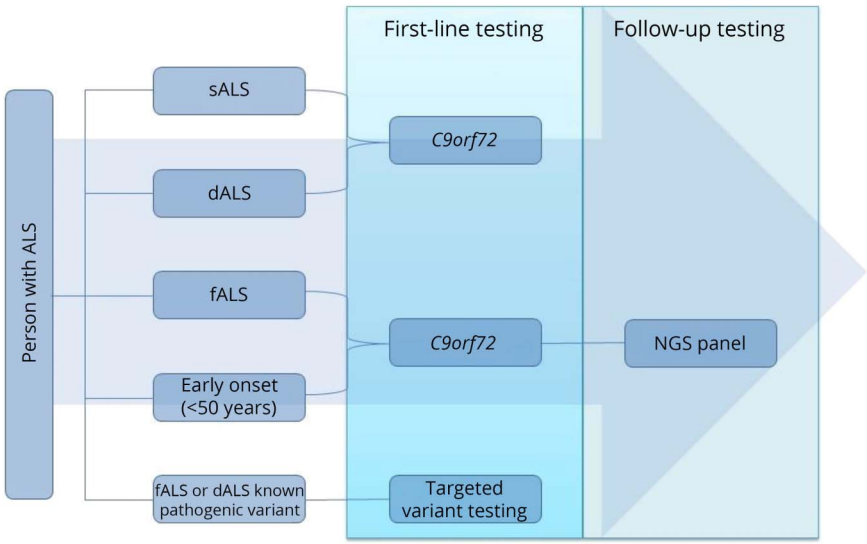
In this study, we determined the incidence of pathogenic (P) and likely pathogenic (LP) variants in a prospective clinic-based ALS cohort at an academic medical center, using a testing algorithm based on family history and age at onset. Second, we compared the incidence of P or LP variants in fALS, patients with ALS with a family history of dementia (dALS), sALS (having no known family history of ALS or dementia), and in early-onset cases (onset <50 years of age).

Methods

Patients with a diagnosis of definite or probable ALS per El-Escorial criteria were offered ALS genetic testing using a testing algorithm based on family history and age at onset (figure) over a 4-year period. A 3-generation pedigree was recorded for each patient. No attempt was made to document reported family history information via review of medical records of affected relatives. Patients who provided limited or incomplete family histories were classified as sALS, unless a family history of ALS or dementia was reported.

All patients who accepted testing (n = 182) underwent *C9orf72* repeat expansion testing as a first step. A validated assay consisting of amplicon-length analysis and repeat-primed PCR was used to detect the presence or absence of a pathogenic GGGGCC hexanucleotide repeat expansion (>30 repeats).[7] The amplicon-length assay was previously described.[8] Two repeat-primed PCR assays, 3′ and 5′ from the hexanucleotide repeat region, were used as previously described.[9]

Figure ALS genetic testing algorithm



sALS, sporadic ALS with no family history of ALS or dementia; dALS, ALS with a family history of dementia but no ALS; fALS, ALS with a family history of ALS; early onset, onset of ALS symptoms <50 years of age. ALS = amyotrophic lateral sclerosis; NGS = next generation sequencing.

Patients with fALS and/or onset of symptoms before age 50 years, who tested negative for *C9orf72*, were offered multigene panel testing as a second step. Multigene panel testing included, at minimum, sequencing of 13 genes (*ALS2, CHMP2B, DCTN1, FUS, OPTN, PFN1, SETX, SIGMAR1, SOD1, TARDBP, UBQLN2, VAPB,* and *VCP*). Additional genes were tested in most patients (including TBK1 in 20/33, 60.6%), reflecting gene discovery and validation during the 4-year study period (see table e-1, links.lww.com/NXG/A212 for detailed panel data). Sequencing was performed using Illumina technology. For cases tested during the first year of the study, 96% of targeted regions were covered at a depth of 20×. During the remainder of the study period, all targeted regions were sequenced with ≥50× depth or supplemented with additional analysis. Reads were aligned to a reference sequence (GRCh37), and sequence changes were identified and interpreted in the context of a single clinically relevant transcript. Enrichment and analysis focused on the coding sequence and 10bp of flanking intronic sequence. All clinically significant observations were confirmed by orthogonal technologies, including Sanger sequencing, Pacific Biosciences Single Molecule, Real-time sequencing, Multiplex Ligation-dependent Probe Amplification, Multiplex Ligation-dependent Probe Amplification-seq, and Array Comparitive Genomic Hybridization.

Descriptive statistics were used to summarize demographic and clinical characteristics, as well as incidence of P and LP variants, overall and by family history classification (fALS, dALS, or sALS). Comparisons between groups were made using a χ^2 or Fisher exact test, where appropriate. Analyses were performed in SAS 9.4 (SAS Institute, Cary, NC).

Data availability

Study data are available on request.

Standard protocol approvals, registrations, and patient consents

The Office of Responsible Research Practices at The Ohio State University Medical Center considers this project exempt from review.

Results

Of 167 persons with ALS who completed the testing algorithm, the majority reported Caucasian ancestry (93%), followed by African American (3%), and Asian (1%); 4% reported other or mixed ancestry. The median age at onset of motor neuron disease symptoms was 61 years (range 20–83 years); 16.2% were classified as early onset with onset <50 years. Fifteen percent (25/167) were classified as fALS (having a positive family history of ALS in a 1st-, 2nd-, or 3rd-degree relative), 41% (68/167) as dALS (having a positive family history of dementia of any type in a 1st- or 2nd-degree relative), and 44.0% (74/167) as sALS (table 1).

Variants classified as P or LP were identified in 56.0% of fALS cases; the majority being *C9orf72* expansions (11/25, 44.0%), followed by P or LP variants in *SOD1* (2/25, 8.0%), and *FUS* (1/25, 4.0%). Variants of uncertain significance (VUS) were identified in 20.0% of fALS cases overall and in 35.7% of *C9orf72*-negative cases. *C9orf72* expansions were detected in 8/68 (11.8%) dALS cases. Among sALS cases, P or LP variants were identified in 5/74 (6.8%), including 3 in *C9orf72* (4.0%), 1 in *SOD1* (1.4%), and 1 in *FUS* (1.4%). The overall incidence of P or LP variants was significantly different among fALS, dALS, and sALS cases (56.0%, 11.8%, and 6.8%, respectively; $p < 0.001$) (table 2).

Table 1 Demographic and disease characteristics of the tested cohort

Characteristic	fALS (n = 25)	dALS (n = 68)	sALS (n = 74)	Total (n = 167)
Ethnicity				
African	1 (4%)	2 (3%)	2 (3%)	5 (3%)
Asian/Native American	0 (0%)	0 (0%)	1 (1%)	1 (1%)
European	24 (96%)	64 (94%)	67 (91%)	155 (93%)
Mixed/unknown/other	0 (0%)	2 (3%)	4 (5%)	6 (4%)
Age at onset				
Median [IQR] (min, max)	60 [50, 61] (34, 70)	63 [54, 67] (20, 80)	61.5 [52, 70] (21, 83)	61 [52, 69] (20, 83)
Age at testing				
Median [IQR] (min, max)	61 [53, 64] (37, 71)	65 [56, 70] (24, 80)	65 [55, 71] (31, 84)	64 [55, 70] (24, 84)
Onset <50 y				
No	19 (76%)	59 (87%)	62 (84%)	140 (84%)
Yes	6 (24%)	9 (13%)	12 (16%)	27 (16%)

Abbreviations: dALS = patients with ALS with a family history of dementia; fALS = familial ALS; IQR = interquartile range; sALS = sporadic ALS.

Table 2 Summary outcomes of the ALS genetic testing algorithm

ALS family history	Test type	Positive result	Negative result	VUS/intermediate result	Positive (%)
fALS (n = 25)	C9	11	0	0	14 (56.0%)
	C9 + panel	3	6	5	
dALS (n = 68)	C9	8	52	0	8 (11.8%)
	C9 + panel	0	6	2	
sALS (n = 74)	C9	3	59	1	5 (6.8%)
	C9 + panel	2	9	0	
Total (n = 167)	C9	22	111	1	27 (16.2%)
	C9 + panel	5	21	7	

Abbreviations: ALS = amyotrophic lateral sclerosis; dALS = patients with ALS with a family history of dementia; fALS = familial ALS; sALS = sporadic ALS; VUS = variant of uncertain significance.

The subgroup of early-onset cases was examined separately; 6 (22.2%) were classified as fALS, 9 (33.3%) as dALS, and 12 (44.4%) as sALS. P or LP variants were detected in 18.5% of early-onset cases, with an LP *SOD1* variant in 1/6 (16.7%) fALS cases, a P *FUS* variant in 1/9 (11.1%) dALS cases, and an LP *SOD1* variant, a P *FUS* variant, and *C9orf72* expansion in 3/12 (25.0%) sALS cases, respectively. The rate of P or LP variant detection was not substantially different between family history types in the early-onset group (fALS: 16.7%, dALS: 11.1%, sALS: 25%; $p = 0.823$) (table 3).

Discussion

Test outcome data from our clinic population suggest that the incidence of P or LP variants in Midwestern fALS cases may be lower than that reported in research cohorts. In particular, the lower incidence of pathogenic variants in *SOD1* and genes other than *C9orf72* is notable. Possible explanations for this observation include the following: (1) the standards used to guide the interpretation of genetic variants identified in research may differ from those applied in clinical testing; (2) research cohorts may be enriched for fALS cases from "high penetrance" families; and (3) the incidence of pathogenic variants in specific genes varies by geographic ancestry. In favor of the first possibility, VUS were identified in 35.7% of our *C9orf72*-negative fALS cases; such variants may be considered causative in research testing but may not reach the American College of Medical Genetics (ACMG) evidentiary standards for LP or P classification in clinical testing. ACMG criteria for variant pathogenicity rely on several lines of published evidence, including well-established functional studies demonstrating a deleterious effect, and cosegregation with disease in multiple affected individuals.[10] Functional data are not available for many ALS genes, and segregation data are limited because affected relatives are not often available for testing. Efforts are underway to revise ACMG criteria for ALS variant interpretation using disease-specific and gene-specific data.[11]

Table 3 Outcomes of the ALS genetic testing algorithm for individuals with early onset (<50 y)

ALS family history	Test type	Positive result	Negative result	VUS/intermediate result	Positive (%)
fALS (n = 6)	C9	0	0	0	1 (16.7%)
	C9 + panel	1	3	2	
dALS (n = 9)	C9	1	0	0	1 (11.1%)
	C9 + panel	0	6	2	
sALS (n = 12)	C9	1	0	0	3 (25.0%)
	C9 + panel	2	9	0	
Total (n = 27)	C9	2	0	0	5 (18.5%)
	C9 + panel	3	18	4	

Abbreviations: ALS = amyotrophic lateral sclerosis; dALS = patients with ALS with a family history of dementia; fALS = familial ALS; sALS = sporadic ALS; VUS = variant of uncertain significance.

We believe that our initial test outcome data generally support the use of the genetic testing algorithm shown in the figure. This approach includes the offer of *C9orf72* testing to all patients. As noted above, current US care guidelines do not address genetic testing, and European recommendations specify that genetic testing be offered only to patients with a family history of ALS or the *SOD1* D90A phenotype. However, our data suggest that limiting the offer of *C9orf72* testing to patients with a positive family history of ALS would lead to approximately half of *C9orf72* carriers being missed. In our data set, 11/22 (50.0%) *C9orf72*-positive cases had a family history of ALS. Similarly, in a published clinic-based research cohort, 60% of C9orf72-positive cases had a family history of ALS.[12] We believe that the offer of *C9orf72* to all patients with ALS is therefore justified, particularly given the availability of *C9orf72*-targeted therapeutic trials and relatively low cost of testing.

A potential limitation of the algorithm is that it would miss cases that are positive for the *C9orf72* repeat expansion in addition to a second pathogenic variant, which has been reported.[12] More data are needed regarding potential oligogenic inheritance in ALS, which may affect the approach to testing. In the meantime, clinicians may wish to consider both *C9orf72* repeat expansion testing in addition to multigene panel testing in fALS cases and should certainly do so if there is a family history of ALS in more than 1 branch of the family. Finally, emerging data suggest that *ATXN2* expansions (known to cause spinocerebellar ataxia type 2) also cause ALS and may be as common in ALS cohorts as pathogenic variants in *TARDBP*.[13] Therefore, clinicians should consider adding this assay for fALS and early-onset cases in whom no genetic etiology is found after *C9orf72* repeat expansion and multigene panel testing.

We propose the designation of dALS to denote ALS patients whose family history is negative for ALS but positive for dementia. Although FTD is the only type of dementia known to share a genetic etiology with ALS, the specific dementia type reported in family members cannot be reliably determined from family history information, or even necessarily from review of medical records. We believe that our data warrant this inclusive classification, given the higher rate of *C9orf72* expansions identified in this group (11.8%) compared with sporadic cases with no family history of ALS or dementia (6.8%). The importance of a positive family history of any dementia in identifying potential cases of *C9orf72* in clinically sALS was shown in another clinic cohort study in which 60% of sALS cases who tested positive for *C9orf72* had a positive family history of dementia. Finally, our data support the offer of testing to patients with early onset of ALS symptoms, irrespective of family history, given that the positive yield of testing in this group (18.5%) was second only to fALS (56.0%) among the clinical categories examined. These data, if replicated, could also be useful in counseling patients with ALS who are considering genetic testing.

The majority of individuals in this study reported European ancestry, which limits the applicability of our data to other populations. The variable ethnogeographic incidence of specific pathogenic variants (e.g., the *C9orf72* expansion)[14] may warrant consideration of population-specific testing approaches. Our current understanding of the familial clustering and genetic basis of ALS is primarily derived from the study of Caucasian individuals and reflects a significant disparity in ALS research and care. Further study is needed regarding the genetic basis of ALS in ethnically and geographically diverse populations. An accurate understanding of the incidence of clinically meaningful variants in different ALS populations is necessary to prioritize targets for therapeutic intervention and inform clinical trial design.

We advocate the offer of *C9orf72* testing to all persons with ALS and multigene testing as a second step for those with fALS or onset of symptoms before age 50 years. However, the incidence of P or LP variants in genes other than *C9orf72* (including *SOD1*) may be lower than expected from published research cohorts, and many cases of fALS may remain unsolved with current testing and interpretation standards. Consistent, equitable genetic testing practices, and an accurate understanding of the incidence of clinically meaningful variants in clinic-based, geographically diverse ALS populations, are necessary as the community of ALS patients and clinicians prepares for the clinical trials and approved therapies of the future.

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Disclosure

Disclosures available: Neurology.org/NG.

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Appendix Authors

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Jennifer Roggenbuck, MS, CGC	The Ohio State University Wexner Medical Center	Author	Designed and conceptualized the study; data collection and analysis; and drafted the manuscript for intellectual content
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Leah Vicini, BS	The Ohio State University Wexner Medical Center	Author	Data collection
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Continued

Appendix *(continued)*

Name	Location	Role	Contribution
Adam Quick, MD	The Ohio State University Wexner Medical Center	Author	Drafting and revision for intellectual content
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