

Variable reporting of *C9orf72* and a high rate of uncertain results in ALS genetic testing

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Ten years ago, commercial ALS genetic testing was limited to *SOD1* sequencing. Commercial laboratories now offer a variety of multigene ALS panels, assays for the *C9orf72* hexanucleotide expansion, and whole exome sequencing. The utility of genetic testing as part of ALS clinical management is valued by people with ALS¹ and ALS clinicians.² However, US care guidelines do not address the offer of genetic testing, and European guidelines specify that ALS genetic testing should be offered only to patients with familial ALS or the *SOD1* D90A phenotype.³ To understand the current state of ALS genetic testing, we surveyed certified commercial laboratories to gather data on test methods, outcomes, and reporting.

Methods

Eight commercial US laboratories were identified using laboratory registries (GTR.org and Genetests.org), which listed ALS genetic testing options. A 13-question survey was emailed to the laboratory directors or genetic counselors in July 2017, with 2 reminder emails at 1-month intervals thereafter. Two laboratories were excluded; one offered only 1 minor ALS gene (*VCP*) and the other did not offer testing specifically for ALS.

Results

Responses were received from 5/6 eligible laboratories (83.3%). All 5 responding laboratories (designated as Labs A-E) offered multigene ALS panels (ranging from 19 to 49 genes); 4 also offered *C9orf72* repeat expansion assays. *C9orf72* assays included repeat-primed PCR and/or fluorescent fragment-length assays. Laboratory-specific test methods and outcomes for *C9orf72* assays and multigene panels are shown in the table.

Discussion

Our survey data confirm that commercial ALS genetic testing options have increased in number and complexity in recent years and highlight potential limitations and challenges associated with the use of this technology. Concerns have been raised regarding the accuracy of PCR-based *C9orf72* assays. In a blinded study of commercial laboratories using PCR-based techniques,⁴ only 5/14 laboratories reported *C9orf72* results in complete concordance with the reference Southern blot result, and both false negative and false positive results were identified. A 10 base-pair deletion adjacent to the repeat has been shown to interfere with detection of the expansion using PCR-based assays.⁵ Despite the ensuing recommendation that Southern blot be used for clinical *C9orf72* testing, no surveyed laboratory offered this; only 1 laboratory performs a 2-step protocol combining both

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Table Commercial ALS genetic testing methodology, reporting, and outcomes

<i>C9orf72</i> assay methodologies and reporting					
Methods	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
Southern blot	—	—	—	N/O ^a	—
Repeat-primed PCR	+	+	+	N/O ^a	+
Fluorescent fragment-length assay	—	+	—	N/O ^a	—
Normal allele cutoff/repeat size reported	≤23/+	≤20/+	≤22/+	N/O ^a	≤24/+
Intermediate allele cutoff/repeat size reported	24–29/+	21–29/+	23–29/+	N/O ^a	25–59/+
Expanded allele cutoff/repeat size reported	≥30/–	≥30/–	≥30/–	N/O ^a	≥60/+ ^c
<i>C9orf72</i> assay test outcomes (%)					
Outcome	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
Positive	18.8	21.0	18.5	N/O ^a	N/R ^b
Negative	80.7	77.6	80.8	N/O ^a	N/R ^b
Intermediate/indeterminate	0.5	1.4	0.7	N/O ^a	N/R ^b
ALS multigene panel outcomes (%)					
Outcome	Laboratory A	Laboratory B	Laboratory C	Laboratory D	Laboratory E
Positive	26.4	20.7	14.4	16.3	N/R ^b
Negative	53.7	67.0	57.5	53.8	N/R ^b
VUS	19.6	12.3	28.1	29.8	N/R ^b

^a N/O, *C9orf72* assay not offered by laboratory.

^b N/R, data not reported by laboratory.

^c Repeat sizes of up to 145 reported.

a fluorescent PCR and repeat-primed PCR that increases sensitivity and specificity in detecting expansions. Furthermore, laboratory cutoffs for normal, intermediate, and expanded alleles varied, indicating that intermediate or small expansions, although rare, could be resulted differently at different laboratories. There is currently no validated cutoff that differentiates between pathologic and nonpathologic alleles, but most patients with pathogenic expansions have hundreds to thousands of repeats. The identification of intermediate alleles in patients with ALS may be incidental. In addition, expansion sizes in blood may differ from those in relevant neural tissues, further complicating result interpretation. Test reports should ideally emphasize the clinico-pathological variability and age-dependent penetrance of pathologic expansions and include a statement that the pathogenicity of repeat sizes between 20 and 100 is unknown but likely increases with the size of the repeat.

Variant of uncertain significant (VUS) rates on multigene panel testing ranged from 12% to 30%, suggesting that many patients with ALS have received a VUS result. VUS outcomes

are often frustrating for clinicians and patients, and it is not known what patients are told or understand about such results. Although rare variant burden may play a role in the etiology of ALS,⁶ VUS must be approached with caution in the clinical setting. VUS interpretation is particularly challenging in ALS, in part because affected family members are often not available for segregation analysis.⁷ Further data are needed regarding test methods and outcomes, including accuracy of current *C9orf72* assays, as well as VUS rates and interpretation. False positive or negative results could have profound implications for patients and family members.

One limitation of this study is that data were self-reported by a small number of US laboratories. Test method and interpretation data were checked against technical specification pages of laboratory websites whenever possible, but some data, such as test outcomes, were not possible to confirm. Nonetheless, the use of genetic testing is likely to grow with the advent of gene-targeted therapies for ALS. Although this will identify appropriate candidates for new therapies, this will also result in an increased need for clinician and patient education regarding all aspects of the testing process. ALS

genetic testing guidelines, addressing test indication, technical methodology, result interpretation and reporting, as well as genetic counseling, may assist clinicians in navigating the challenges of this technology.

Author contributions

H. Klepek: designed, piloted, and administered the survey; analyzed the data; and drafted the manuscript for intellectual content. S.A. Goutman: study conceptualization, survey design, and edited the manuscript. A.Quick: survey design and edited the manuscript. S.J. Kolb: study conceptualization, survey design, and edited the manuscript. J. Roggenbuck: study conceptualization, designed and piloted the survey; analyzed the data; and drafted the manuscript for intellectual content.

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