CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

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

Holly Klepek, MS, Stephen A. Goutman, MD, Adam Quick, MD, Stephen J. Kolb, MD, PhD, and Jennifer Roggenbuck, MS, CGC

Correspondence
Ms. Roggenbuck
jennifer.roggenbuck@osumc.edu

Neurol Genet 2019;5:e301. doi:10.1212/NXG.0000000000000301

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 ALS[1] 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

From the Department of Neurology (H.K.), Indiana University Medical Center, Indianapolis; Department of Neurology (S.A.G.), University of Michigan Medical Center, Ann Arbor; Department of Neurology (A.Q., S.J.K., J.R.) and Department of Biological Chemistry & Pharmacy (S.J.K.), The Ohio State University Wexner Medical Center, Columbus.

Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

The Article Processing Charge was funded by The Ohio State University Wexner Medical Center, Department of Neurology.

Table Commercial ALS genetic testing methodology, reporting, and outcomes

C9orf72 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]

C9orf72 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.

Study funding

No targeted funding reported.

Disclosure

H. Klepek reports no disclosures. S. Goutman has received commercial research support from Cytokinetics; has received governmental research support from the NIH/National Institute of Environmental Health Sciences and the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry; and has received foundation/society research support from Target ALS and the ALS Association. A. Quick reports no disclosures. S.J. Kolb has served on the scientific advisory board of Genentech and has received governmental research support from the NIH, the NINDS, and the National Institute of Child Health and Human Development. J. Roggenbuck has served on the advisory board of Invitae. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Publication history

Received by *Neurology: Genetics* June 27, 2018. Accepted in final form November 2, 2018.

References

1. Wagner KN, Nagaraja HN, Allain DC, Quick A, Kolb SJ, Roggenbuck J. Patients with sporadic and familial amyotrophic lateral sclerosis found value in genetic testing. Mol Genet Genomic Med 2018;6:224–229.
2. Vajda A, McLaughlin RL, Heverin M, et al. Genetic testing in ALS: a survey of current practices. Neurology 2017;88:991–999.
3. Andersen PM, Abrahams S, Borasio GD, et al. EFNS guidelines on the clinical management of amyotrophic lateral sclerosis (MALS)–revised report of an EFNS task force. EFNS Task Force on Diagnosis and Management of Amyotrophic Lateral Sclerosis. Eur J Neurol 2012;19:360–375.
4. Akimoto C, Volk AE, van Blitterswijk M, et al. A blinded international study on the reliability of genetic testing for GGGGCC-repeat expansions in C9orf72 reveals marked differences in results among 14 laboratories. J Med Genet 2014;51:419–424.
5. Rollinson R, Bennison Callister J, Young K, et al. A small deletion in C9orf72 hides a proportion of expansion carriers in FTLD. Neurobiol Aging 2015;36:1601.e1–1601.e5.
6. Cady J, Allred P, Bali T, et al. Amyotrophic lateral sclerosis onset is influenced by the burden of rare variants in known amyotrophic lateral sclerosis genes. Ann Neurol 2014;77:100–113.
7. Roggenbuck J, Quick A, Kolb SJ. Genetic testing and genetic counseling for amyotrophic lateral sclerosis: an update for clinicians. Genet Med 2017;19:267–274.