The value of testing for ATXN2 intermediate repeat expansions in routine clinical practice for amyotrophic lateral sclerosis

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European Journal of Human Genetics (2022) 30:1205–1207; https://doi.org/10.1038/s41431-022-01146-2

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is genetically diverse, with numerous variants associated with either causing or increasing risk of developing the condition [1]. The increase in genetically-targeted therapies in development, combined with a greater appreciation for the role genetics plays in ALS, has led to a push for more widespread genetic testing as part of routine clinical care [2].

Ataxin-2, a polyglutamine (polyQ) protein, is a modifier of TDP-43 toxicity in ALS models, and intermediate polyQ repeats in the ATXN2 gene have been associated with increased susceptibility for ALS [3–4]. An antisense oligonucleotide targeting ATXN2 is currently in a Phase 1 clinical trial for people living with ALS, both with and without polyQ expansions (NCT04494256). To date, studies report varying incidence of ATXN2 intermediate repeats in ALS patients [5]. With ATXN2 repeat expansion testing now available on several commercial ALS testing panels, implementation of ATXN2 genetic testing provides an opportunity to identify suitable participants for clinical trials.

Here, we outline our clinic’s early experience with implementing ATXN2 genetic testing as part of routine clinical practice.

IMPLEMENTING ATXN2 GENETIC TESTING IN A CLINICAL SETTING

The ALS clinic at the Montreal Neurological Institute-Hospital (The Neuro) has been offering genetic testing to all newly diagnosed ALS patients since 2015. A tiered testing approach is used—for patients with a family history of ALS, FTD, related disorders, or an atypical presentation (such as a young age of onset), a comprehensive panel of ALS genes is ordered; and, for isolated ALS, only those genes for which there are targeted, ongoing clinical trials are ordered. With this testing algorithm, both SOD1 and C9orf72 testing have been consistently offered to all patients for several years. Initiation of a clinical trial targeting ATXN2 intermediate repeat expansions prompted an evolution of the clinic’s genetic testing practices to include offering ATXN2 testing to all patients.

Clinical genetic testing at The Neuro’s ALS clinic is performed by PreventionGenetics, who added ATXN2 testing to their offering in October 2020. Subsequently, all patients newly diagnosed with ALS would be offered ATXN2 repeat expansion testing as part of the clinic’s standard practice. To date, 61 newly diagnosed ALS patients have had ATXN2 genetic testing.

SCREENING FOR ATXN2 INTERMEDIATE REPEAT EXPANSIONS

For previously diagnosed patients, for whom genetic testing was completed prior to October 2020, repeat testing would consume considerable clinical and financial resources. However, patients at The Neuro’s ALS clinic are given the opportunity to consent to the Clinical Biospecimen Imaging and Genetic (C-BIG) Repository, where their DNA is banked for future analysis and scientific research. A request was made to C-BIG to access samples from ALS patients actively being followed in the clinic (n = 116). This request was approved by their Tissue and Data Committee under the ethical framework approved by the McGill University Health Centre’s Research Ethics Board—Neuroscience and Psychiatry Panel (MUHC-15-944 (CRU)/2017-330, 15-944-MUHC).

DNA was extracted from blood samples using standard procedures. The ATXN2 CAG repeat region was amplified following published methodology [6], with minor changes (M13 universal sequence not added to primers). For 11 samples, the repeat was estimated from whole genome sequencing data using Expansion-Hunter v3.2.0 [7]. Repeat alleles were considered “intermediate” length with at least 27 repeats.

CHARACTERISTICS OF PATIENTS WITH INTERMEDIATE ATXN2 REPEATS

Since adding ATXN2 intermediate repeat expansion testing to the clinic’s standard genetic testing practices, five patients have been identified (Table 1): four were identified through the retrospective screening of banked DNA samples, and one newly diagnosed patient was identified through prospective clinical testing. Consistent with previous studies [5], none of the patients had a family history of ALS, or related disorders, further emphasising the need to offer genetic testing to all people living with ALS.

GENETIC COUNSELLING IMPLICATIONS FOR ATXN2

Intermediate repeats in ATXN2 are associated with increased risk of developing ALS, as opposed to a monogenetic, inherited cause.
Should a test result indicate that intermediate repeats are present, this introduces challenges in conveying the risk to family members in post-test counselling, as the risk and implications are unclear. Genetic counselling, provided by an appropriate specialist, should always accompany the offer of genetic testing for people living with ALS.

**VALUE OF ATXN2 TESTING IN CLINICAL PRACTICE**

Genetics has long contributed to our understanding of ALS, and is translating into the development of targeted therapies at an increasing rate. As such, genetic testing practices in ALS need to evolve with emerging clinical trials. Implementation of routine ATXN2 testing in our clinical setting identified people living with ALS who were potentially eligible for a targeted genetic therapy trial. Use of banked DNA samples to conduct a retrospective screen of those patients who had previously had genetic testing reduced the burden on both financial and human resources, in our public healthcare setting. This model could be applied in other clinical settings, and allows for adaptability in clinical practice in an evolving therapeutic landscape.

**DATA AVAILABILITY**

All data analysed during this study are included in this published article. The data generated during the study are available in the C-BIG repository, [https://www.mcgill.ca/neuro/open-science/c-big-repository](https://www.mcgill.ca/neuro/open-science/c-big-repository).

**REFERENCES**

1. Goutman SA, Hardiman O, Al-Chalabi A, Chio A, Saveleff MG, Kiernan MC, et al. Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis. Lancet Neurol. 2022;21:465–79.

2. Salmon K, Kiernan MC, Kim SH, Andersen PM, Chio A, van den Berg LH, et al. The importance of offering early genetic testing in everyone with amyotrophic lateral sclerosis. Brain. 2022;145:1207–10.

3. Elden AC, Kim HJ, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature. 2010;466:1069–75.

4. Bonini NM, Gilder AD. Model organisms reveal insight into human neurodegenerative disease: ataxin-2 intermediate-length polyglutamine expansions are a risk factor for ALS. J Mol Neurosci. 2011;45:676–83.

5. Wang MD, Gomes J, Cashman NR, Little J, Krewski D. Intermediate CAG repeat expansion in the ATXN2 gene is a unique genetic risk factor for ALS-a systematic review and meta-analysis of observational studies. PLoS ONE. 2014;9:e105534.

6. Daoud H, Belzil V, Martini S, Sabbagh M, Provencier P, Lacomblez L, et al. Association of long ATXN2 CAG repeat sizes with increased risk of amyotrophic lateral sclerosis. Arch Neurol. 2011;68:739–42.

7. Dolzhenko E, Deshpande V, Schlesinger F, Krusche P, Petrovski R, Chen S, et al. ExpansionHunter: a sequence-graph-based tool to analyze variation in short tandem repeat regions. Bioinformatics. 2019;35:4754–6.

**AUTHOR CONTRIBUTIONS**

KS conceived the concept, synthesised data, and wrote the manuscript. JPR and PAD designed and conducted experiments, extracted data, and reviewed the manuscript. VB, MG, and NA contributed to data extraction, and reviewed the manuscript. GAR and JK supervised experiments, and reviewed the manuscript. AG supervised the project, reviewed, and approved the manuscript.

**FUNDING**

Funding for ATXN2 repeat expansion analysis in banked DNA samples was provided by Biogen. JPR has received a Canadian Institutes of Health Research Graduate Scholarship (FRN 159279).

**COMPETING INTERESTS**

KS, JPR, VB, MG, NA, JK, PAD, GAR declare no competing interests. AG reports consultancies or advisory boards for Alexion, AL-S Pharma, Amylyx, Anelixis, Anexon, Apellis, Atlantic Research Group, Biogen, Calico, Cytokinetics, Eli Lilly, Ionis, Mitsubishi Tanabe Pharma, Orion Pharma, QurAlis, Roche, Sanofi, UCB, and Wave Life Sciences.
ETHICAL APPROVAL
The request for banked samples was approved by the C-BIG Repository’s Tissue and Data Committee under the ethical framework approved by the McGill University Health Centre’s Research Ethics Board—Neuroscience and Psychiatry Panel (MUHC-15-944 (CRU)/2017-330, 15-944-MUHC).

ADDITIONAL INFORMATION
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