Questioning the Association of the STMN2 Dinucleotide Repeat With Amyotrophic Lateral Sclerosis

Jay P. Ross, BSc, Fulya Akçimen, MSc, PhD, Calwing Liao, PhD, Dan Spiegelman, MSc, Ben Weisburd, BSc, Nicolas Dupré, MD, Patrick A. Dion, PhD, Guy A. Rouleau, MD, PhD, and Sali M.K. Farhan, PhD

Neurol Genet 2022;8:e678. doi:10.1212/NXG.0000000000000678

Abstract

Objectives
Recently, the number of dinucleotide CA repeats in an intron of the STMN2 gene was reported to be associated with an increased risk for amyotrophic lateral sclerosis (ALS). Therefore, we sought to replicate this observation in an independent group of ALS patients and a much larger control group.

Methods
Here, we used whole-genome sequencing and tested the STMN2 CA repeat in a case-control cohort of the European genetic background and in genomes from various populations in the gnomAD cohort to attempt to replicate this proposed association.

Results
We find that repeats well above the previously reported pathogenic threshold of 19 are commonly observed in unaffected individuals across different populations. Furthermore, we did not observe an association between longer STMN2 CA repeats and ALS phenotype.

Discussion
In summary, our results do not support a role of STMN2 CA repeats toward ALS risk. As TDP-43 aggregation is central to ALS pathogenesis, lowered expression of STMN2 could be used as a biomarker for ALS. Therefore, a variant associated both with the risk for ALS and the level of STMN2 expression would be clinically useful. However, for a variant to be actionable, it must be strongly replicated in independent cohorts and exceed the rigorous statistical thresholds applied.
Altered stathmin-2 (STMN2) expression has been implicated in amyotrophic lateral sclerosis (ALS). On decrease of TDP-43, the STMN2 transcript becomes truncated and produces a nonfunctional stathmin-2 protein. This dysfunction results in altered neural response to cell damage and reduced axonal regrowth.

Recently, an intronic dinucleotide CA repeat between exons 3 and 4 of STMN2 was reported to be associated with ALS. Specifically, alleles longer than 19 CA repeats were reported to increase the risk for ALS, and those carrying a 24-repeat allele alongside another long allele had the highest risk. In our study, we observed carriers of STMN2 CA repeats well beyond the reported pathogenic repeat threshold in both case-control and gnomAD cohorts, and we did not reproduce the association between expanded STMN2 repeats and ALS. Although STMN2 dysfunction may contribute to ALS, its dinucleotide repeat does not impart a significant risk to ALS.

**Methods**

We used the STREGA checklist.

**Samples and Sequencing**

Patients and controls were recruited in clinics across Québec, Canada. One hundred fifty-four patients (average age: 59.7 ± 11.7 years, male:female ratio 1.68) were included. Two hundred sixteen controls (average age: 67.8 ± 13.3 years, male:female ratio 0.56) were included. gnomAD was used as an external data set. All individuals included gave written informed consent.

Whole-genome sequencing (WGS) was performed on Illumina HiSeq X-Ten and NovaSeq 6000 sequencers at the Génome Québec Centre d’Expertise et de Services. Bioinformatic analyses were performed on the Béluga cluster of Compute Canada and Calcul Québec using DRAGEN Bio-IT v3.8 (Illumina, Inc., San Diego, CA). After alignment to the hg38 human reference genome, an average depth of 34.1X was observed.

**Results**

STMN2 CA repeats were successfully genotyped by ExpansionHunter in 153 ALS and 207 controls. No allele combination with the current case-control cohort suggested an association of long or long with 24 repeats (L/L with 24CA) with the ALS phenotype (Table 1). Although there was a nominally significant p value of L/L with 24CA using the CMH test combining allele counts from the current cohort and the Australian cohort from the previous study (p = 0.041), this result does not pass the multiple testing correction threshold (α = 0.05/10; p = 0.005). The longest repeats were more often observed in female samples, and the largest repeats were observed in female control samples (eTable 2, links.lww.com/NXI/A735).

Notably, STMN2 CA repeats much longer than the previously reported ALS-associated threshold were frequently observed in the gnomAD (eFigure 1, links.lww.com/NXI/A735).

**Table 1 Replication Results of Theunissen et al.’s Associations of STMN2 CA Repeat Lengths and ALS Phenotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Status</th>
<th>Fisher exact test</th>
<th>CMH test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALS</td>
<td>p Value OR 95% CI</td>
<td>p Value OR 95% CI</td>
</tr>
<tr>
<td>Long/long (L/L)</td>
<td>84 (54.9%) 123 (59.4%)</td>
<td>0.4504 1.20 0.77–1.87</td>
<td>0.1775 1.19 0.93–1.53</td>
</tr>
<tr>
<td>L/L (with 24 CA)</td>
<td>59 (38.6%) 77 (37.2%)</td>
<td>0.8263 0.94 0.60–1.48</td>
<td>0.0407 1.44 1.02–2.03</td>
</tr>
<tr>
<td>L/L (without 24 CA)</td>
<td>25 (6.94%) 46 (12.8%)</td>
<td>0.1819 1.46 0.82–2.62</td>
<td>0.9179 0.97 0.75–1.27</td>
</tr>
<tr>
<td>Long/short (L/S)</td>
<td>44 (12.2%) 62 (17.2%)</td>
<td>0.8163 1.06 0.65–1.72</td>
<td>0.0935 0.79 0.61–1.03</td>
</tr>
<tr>
<td>Short/short (S/S)</td>
<td>25 (6.94%) 22 (6.11%)</td>
<td>0.1167 0.61 0.31–1.18</td>
<td>0.2504 1.36 0.84–2.20</td>
</tr>
</tbody>
</table>

Abbreviations: ALS = amyotrophic lateral sclerosis; CI = confidence interval; CMH = Cochran-Mantel-Haenszel; OR = odds ratio.

Tests of association were performed using either the Fisher exact test or the CMH test. p Values are reported uncorrected. Counts and percentages of individual carriers are listed for ALS and control cohorts for each combination of allele length. OR and 95% CIs are given separately for each test and combination.
Repeat lengths as long as 89 were observed in the non-Finnish European cohort, which is likely the closest match to ours and the previously reported cohort. 3 The frequency of the different allele combinations in Figure 1 varied slightly between gnomAD populations (eTable 1).

**Discussion**

We used WGS data to estimate the STMN2 CA repeat length and observed large repeats above the purportedly pathogenic threshold in phenotypically normal individuals. We did not observe an association between longer alleles and ALS risk, nor did we replicate the necessity of the 24-repeat allele for this association.

The previous study reported a trend of large STMN2 CA repeat length with decreased expression of STMN2.3 However, this trend was not statistically significant. Furthermore, it is unclear whether larger repeats are linearly associated with decreased STMN2 levels, or whether the decrease is comparable with that resulting from TARDBP variation or lowered TARDBP expression.1,3 Although the expression level and pathologic truncation of STMN2 are important in ALS and TDP-43 pathology, our current results refute the association of the STMN2 CA dinucleotide repeat with ALS.

The gnomAD browser is useful to assess the maximum credible allele frequency of a variant.7 However, as structural variants are not as well documented, it is still possible to find associations between CNVs and a given phenotype that do not replicate. Samples in gnomAD or The 1000 Genomes Project8 may carry large repeat alleles of risk variants,9 but without prior evidence to support variant pathogenicity, an individual might also coincidentally carry a large repeat allele. It is important that these known limitations did not hinder our evaluation of the proposed association of the STMN2 CA repeat size and ALS.

**Study Funding**

J.P. Ross has received a Canadian Institutes of Health Research (CIHR) Frederick Doctoral Scholarship (FRN 159279). F. Akçimen has received funding from the Fonds de Recherche du Québec–Santé (FRQS). C. Liao has received a CIHR Vanier Graduate Scholarship (FRN 169885). P.A. Dion has received project funding from the Radala Foundation for ALS Research and jointly from the ALS Society of Canada and the Brain Canada Foundation. G.A. Rouleau has...
received funding from the ALS Society of Canada and holds a Canada Research Chair in Genetics of the Nervous System and the Wilder Penfield Chair in Neurosciences.

**Disclosure**
The authors report no disclosures relevant to the manuscript. Go to Neurology.org/NG for full disclosures.

**Publication History**
Received by *Neurology: Genetics* January 26, 2022. Accepted in final form April 5, 2022. Submitted and externally peer reviewed. The handling editor was Raymond P. Roos, MD, FAAN.

### Appendix Authors

<table>
<thead>
<tr>
<th>Name</th>
<th>Location</th>
<th>Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jay P. Ross, BSc</td>
<td>Department of Human Genetics, Montreal Neurological Institute and Hospital, McGill University, Quebec, Canada</td>
<td>Drafting/revision of the manuscript for content; including medical writing for content; major role in the acquisition of data; study concept or design; and analysis or interpretation of data</td>
</tr>
<tr>
<td>Fulya Akçimen, MSc, PhD</td>
<td>Department of Human Genetics, Montreal Neurological Institute and Hospital, McGill University, Quebec, Canada</td>
<td>Analysis or interpretation of data</td>
</tr>
<tr>
<td>Calving Liao, PhD</td>
<td>Department of Human Genetics, Montreal Neurological Institute and Hospital, McGill University, Quebec, Canada</td>
<td>Analysis or interpretation of data</td>
</tr>
<tr>
<td>Dan Spiegelman, MSc</td>
<td>Montreal Neurological Institute and Hospital, and Department of Neurology and Neurosurgery, McGill University, Montréal, Quebec, Canada</td>
<td>Analysis or interpretation of data</td>
</tr>
<tr>
<td>Ben Weisburd, BSc</td>
<td>Program in Medical and Population Genetics, The Broad Institute of MIT and Harvard, Cambridge, MA</td>
<td>Analysis or interpretation of data</td>
</tr>
<tr>
<td>Nicolas Dupré, MD</td>
<td>Division of Neurosciences, CHU de Québec, Université Laval; Department of Medicine, Faculty of Medicine, Université Laval, Québec City, Canada</td>
<td>Major role in the acquisition of data; additional contributions: providing clinical data</td>
</tr>
</tbody>
</table>

### References

Questioning the Association of the STMN2 Dinucleotide Repeat With Amyotrophic Lateral Sclerosis

Jay P. Ross, Fulya Akçimen, Calwing Liao, et al.

Neurol Genet 2022;8;
DOI 10.1212/NXG.0000000000000678

This information is current as of July 13, 2022

Updated Information & Services
including high resolution figures, can be found at:
http://ng.neurology.org/content/8/4/e678.full.html

References
This article cites 10 articles, 0 of which you can access for free at:
http://ng.neurology.org/content/8/4/e678.full.html#ref-list-1

Citations
This article has been cited by 1 HighWire-hosted articles:
http://ng.neurology.org/content/8/4/e678.full.html#otherarticles

Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
Amyotrophic lateral sclerosis
http://ng.neurology.org//cgi/collection/amyotrophic_lateral_sclerosis_
Association studies in genetics
http://ng.neurology.org//cgi/collection/association_studies_in_genetics

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://ng.neurology.org/misc/about.xhtml#permissions

Reprints
Information about ordering reprints can be found online:
http://ng.neurology.org/misc/addir.xhtml#reprintsus

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.