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

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Neurology Genet 2022;8:e678. doi:10.1212/NXG.000000000000678

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).\(^1,2\) 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.\(^3\) 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.\(^10\)

### 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.\(^4\) 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.

### Estimation of STMN2 CA Repeat Length

ExpansionHunter v4.0.2\(^5\) was used to calculate the number of CA repeats.\(^3\) Options applied were ReferenceRegion: chr8:79641628-79641672, VariantType: “Repeat,” and LocusStructure: “(CA)\(^n\).” The reported or imputed sex of individuals was used as an input option.

### Statistical Analyses

Statistical tests were performed using R v4.0.3. A Fisher exact test (fisher.test) was used to test for differences between cases and controls. A Cochran-Mantel-Haenszel (CMH) test (mantelhaen.test) was used to incorporate the current results with those of the previous Theunissen study.\(^5\) All reported \(p\) values are uncorrected. Per-sample allele lengths are reported in eTable 2 (links.lww.com/NXI/A735).

### Data Availability

Raw data for genome sequencing used in the study are available through Project MinE (projectmine.com/) or available on request. Raw data for ExpansionHunter variant calling are also available on request.

### 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 (\(\alpha = 0.05/10; \ p = 0.005\)). 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

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

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.1,5 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

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**Figure 1** Distributions of STMN2 CA Repeat Length Calculated From ALS (A), Control (B), and Non-Finnish European gnomAD Genomes (C)
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.

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### Appendix Authors

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DOI 10.1212/NXG.0000000000000678

This information is current as of July 13, 2022