Analysis of C9orf72 repeat expansions in Georgian patients with ALS

Mariam Kekenadze (mariam.kekenadze@gmail.com)
Tbilisi State Medical University  https://orcid.org/0000-0002-5578-7213

Clarissa Rocca
University College London

Rauan Kaiyrzhanov
University College London

Sara Nagy
University of Basel: Universitat Basel

Nana Kvirvelia
Ivane Javakhishvili Tbilisi State University

Shorena Vashadze
Batumi Shota Rustaveli State University Faculty of Natural Sciences and Health Care

Eka Kvaratskhelia
Tbilisi State Medical University

Maia Beridze
Tbilisi State Medical University

Henry Houlden
University College London

Research Article

Keywords: ALS, MND, Gene, C9orf72, Georgia

Posted Date: July 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1791361/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

BACKGROUND

Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disorder that affects the upper and lower motor neurons. Several genetic risk factors have been identified in the past decade with a hexanucleotide repeat expansion in the C9orf72 gene being the most significant. However, the presence of c9orf72 repeat expansion has not been examined in the Transcaucasian region, therefore we aimed to analyze its frequency in Georgian patients with ALS.

Methods

We included 47 self-reported Georgian patients with ALS from different parts of the country, fulfilling the Gold Coast criteria. To investigate the presence of an expanded GGGGCC hexanucleotide repeat in the non-coding region of the C9orf72 gene, we performed Repeat-Primed PCR.

Results

45 sporadic and 2 familial ALS cases were identified. Patients were aged 26 to 84 years with a mean age of 58.3 years at disease onset. Bulbar onset was observed in 21.3%, upper limb onset in 38.3%, and lower limb onset in 40.4% of the patients. Frontotemporal dementia (FTD) fulfilling the Strong criteria was diagnosed in 6 patients (12.7%). C9orf72 repeat expansion could not be detected in any of the cases using RP-PCR.

Conclusions

Our results indicate that c9orf72 hexanucleotide expansion does not belong to the genetic risk factors of ALS in Georgian patients. Further genetic studies in a bigger study population are needed to reveal the genetic causes of ALS in the Transcaucasian population.

As part of this research, Whole Exome Sequencing testing will be performed for identifying other gene mutations that might contribute to developing ALS in those patients.

Background

“Does it take place through simple propagation, extending gradually across the neuroglia?”¹. This is what French Neurologist -J.M Charcot has been questioning regarding the disease development of amyotrophic lateral sclerosis (ALS) almost 150 years ago. Although the pathogenesis of ALS is still unknown, extensive studies have revealed important genetic risk factors in the past decade. C9orf72 (#MIM 105550), SOD1 (#MIM105400), TARDBP (#MIM612069, #MIM612069), and FUS (#MIM608030) are the most frequently mutated genes that have been shown in ALS ²,³. with the hexanucleotide repeat expansion in C9orf72 being the most significant and accounting for 30–50% of familial and 7% of sporadic ALS cases in the European population.²,³ However, the genetic basis of ALS has not been
investigated in the Transcaucasian region. Therefore, we aimed to determine the frequency of \textit{C9orf72} repeat expansion in Georgian patients with ALS.

**Methods**

47 self-reported ethnically Georgian patients with ALS have been included in the study. Ethical approval was obtained from Tbilisi State Medical University (TSMU) ethics committee and University College London (UCL) institutional board. Written informed consent was obtained from all subjects or their legal representatives before the study. A database of the First University Clinic of TSMU as well as of collaborative clinics were utilized for patient recruitment.

Diagnosis of ALS was based on the new Gold coast criteria - incorporating progressive motor impairment documented by history or repeated clinical assessment, preceded by normal motor function, and presence of upper and lower motor neuron dysfunction in at least 1 body region, or lower motor neuron dysfunction in at least 2 body regions, most importantly excluding other diseases.\(^4\) Patients diagnosed with conditions such as spinal muscular atrophy, Kennedy syndrome, monomelic amyotrophy, Hirayama syndrome, or multifocal motor neuropathy were excluded from the study.

Venous blood samples were collected in Georgia and transferred to UCL Queen Square Institute of Neurology, Neurogenetics Laboratory for further research. Genomic DNA of the included subjects was extracted from whole blood using the Promega ReliaPrep™ Blood gDNA Miniprep System.

To investigate the presence of an expanded GGGGCC hexanucleotide repeat in the non-coding region of the C9orf72 gene, we performed Repeat-Primed PCR (RP-PCR) in all patients and in 3 positive controls. The primers and thermocycling conditions used for the assay have been previously described \(^5\). RP-PCR is able to determine whether an expanded allele is present in an individual, in which case a characteristic stutter pattern is seen (Fig. 1B).\(^5\)

All primers were used with the same molar concentrations. Fragment length analysis was performed on an ABI 3730xl Genetic Analyzer (Applied Biosystems), and data were analyzed with the GeneMapper software (v. 4.0, Applied Biosystems).

**Results**

Patients with ALS were aged 26 to 84 years with a mean age of 58.3 years at disease onset. 63.8% of the patients were 50–69 years old. 51% of the patients were male, 49% female with a male to female ratio of 1:1. Bulbar onset ALS was observed in 21.3%, upper limb onset (UL-ALS) in 38.3%, and lower limb onset (LL-ALS) in 40.4% of the patients (Table 1). Frontotemporal dementia (FTD) fulfilling the Strong criteria was diagnosed in 6 patients (12.7%). Two patients have been identified to have familial ALS based on family history.

**Table 1.** ALS Phenotypes in Georgian patients
<table>
<thead>
<tr>
<th>Phenotypic Variant</th>
<th>Neuronal level</th>
<th>UMN</th>
<th>LMN</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical ALS</td>
<td>+</td>
<td>+</td>
<td>44</td>
<td>93.6%</td>
<td></td>
</tr>
<tr>
<td>PLS</td>
<td>++</td>
<td>-</td>
<td>1</td>
<td>2.1%</td>
<td></td>
</tr>
<tr>
<td>PMA</td>
<td>-</td>
<td>++</td>
<td>2</td>
<td>4.3%</td>
<td></td>
</tr>
<tr>
<td>PBP</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Site of onset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulbar ALS</td>
<td></td>
<td>10</td>
<td>21.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UL-ALS</td>
<td></td>
<td>18</td>
<td>38.3%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LL-ALS</td>
<td></td>
<td>19</td>
<td>40.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mill’s (hemiplegic) variant</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flail Arm</td>
<td></td>
<td>0</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flail Leg</td>
<td></td>
<td>0</td>
<td>0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of FTD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALS/FTD</td>
<td></td>
<td>6</td>
<td>12.7%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; UMN, upper motor neuron; LMN, lower motor neuron; PLS- primary lateral sclerosis; PMA - progressive muscular atrophy, PBP- progressive bulbar palsy, UL-ALS- upper limb onset ALS, LL-ALS - lower limb onset ALS, FTD-frontotemporal dementia. + typical to a variable degree; ++ primary feature, - not a feature,

Table 1: shows numbers and percentage of different ALS phenotypes in Georgian patients according to Neuronal level, site of onset, and presence of ALS/FTD

We screened all patients for GGGGCC hexanucleotide expansions between two-five prime non-coding exons of the C9ORF72 locus using RP-PCR. We used a reliable assay that confidently differentiates between positive and negative cases by detecting up to 40 repeats, thus categorizing them as pathogenic expansions. An accurate number of repeats in each allele can be detected in the negative cases. The repeat size in the general population has been observed to vary between 2 to 30 for healthy individuals, while affected people present at least one expanded allele with repeats ranging between 30 to several hundred hexanucleotides. After performing RP-PCR, GGGGCC expansions were not observed in any of the 47 patients. Most of our cases presented a homozygous two-repeats expansion. The largest expansion with twelve hexanucleotides in a heterozygous state was found in only one patient. The mean expansion in our cohort was 2+3.11 repeats (Allele1 2-2, Allele2 2-12).

**Discussion**

A pathogenic repeat expansion in the non-coding region of the C9orf72 gene has been described to be the most common risk to develop familial ALS. However, most studies have been performed in European study populations and little is known from Non-European countries. Based on these, a significant variation in c9orf-ALS frequency and distribution throughout Eurasia can be observed, with East Asian populations experiencing lesser cases, and India and Taiwan being exemptions (Fig. 1).
In our study, we aimed to investigate the frequency of c9orf-ALS in Georgian patients. However, none of our 47 patients were positively tested for pathogenic GGGGCC repeat expansion, possibly indicating a different genetic background and the presence of distinct risk factors for ALS in this ethnic group. The Georgian geography with its isolation and small population size, particularly in the highlands, might have led to the bottleneck effect and enhanced genetic differentiation seen in our data.

However, our results are limited due to the small cohort size. Further, the nonexistence of the pathogenic repeat expansion could also be due to a previous single founder mutation. Smith et al (2013) identified a haplotype that proves this point, that all massive GGGGCC hexanucleotide repeat expansion mutations - identified within intron 1 of C9ORF72- carriers arose from a single common founder. 10 The most sensible Explanation would therefore be that the expansion mutation arose on just one occasion in the European population. The results could further be explained by a close link between Georgian and Asian genetic pools, however, researchers reported that Caucasian groups were much closer to European than to West Asian groups with respect to mtDNA, opposite to be true for Y chromosome, indicating a predominantly West Asian influence 11.

**Conclusion**

Further genetic studies in a larger cohort are needed to confirm our results and to reveal genetic risks for ALS in the Transcaucasian population.

**List Of Abbreviations**

**ALS**- Amyotrophic Lateral Sclerosis  
**TSMU**- Tbilisi State Medical University  
**UCL**- University College London  
**RP-PCR** - Repeat-Primed Polymerase Chain Reaction  
**UL-ALS**- Upper Limb onset Amyotrophic Lateral Sclerosis  
**LL-ALS**- Lower Limb onset Amyotrophic Lateral Sclerosis  
**FTD**- Frontotemporal dementia  
**UMN**- Upper Motor Neuron  
**LMN**- Lower Motor Neuron  
**PLS**- Primary Lateral Sclerosis  
**PMA**- Progressive Muscular Atrophy
Declarations

1. Ethics approval and consent to participate

Ethical approval was obtained from Tbilisi State Medical University (TSMU) ethics committee and University College London (UCL) institutional board. All participants consented in written form to participate in the study.

2. Consent for publication

I confirm that the manuscript has been read and approved for submission by all the named authors.

3. Data and material availability

Supporting data is available from the corresponding author upon a reasonable request.

4. Competing interests

The authors declare that there are no conflicts of interest relevant to this work.

5. Funding

Funding Sources and Conflict of Interest: This research was funded in part, by the Wellcome Trust [WT093205MA, WT104033AIA, and the Synaptopathies Strategic Award, 165908]. This study was funded by the Medical research council (MRC) (MR/S01165X/1, MR/S005021/1, G0601943). Funding was used to cover the expenses of genetic testing.

6. Authors' contributions

MK contributed to the conception and design of the study, acquisition of the samples, and drafted article, CR carried out the molecular genetic analysis and participated in drafting the manuscript. RK, SN, NK contributed to the analysis and interpretation of data, drafting the article. MB, HH, Sh.V, and EK have been involved in drafting the manuscript or revising it and have given final approval for the version to be published.

7. Acknowledgments

Families were collected as part of the SYNaPS Study Group collaboration funded by The Wellcome Trust and strategic award (Synaptopathies) funding (WT093205 MA and WT104033AIA) and research was conducted as part of the Queen Square Genomics group at the University College London, supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. MK was supported by the EAN clinical fellowship award and Guarantors of Brain award to
conduct the visit to the UK. We are also grateful to Queen Square genomics at the Institute of Neurology University College London, supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre, for the bioinformatics support. For the purpose of Open Access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission.

References


Figures
**Figure 1**

Eurasia map depicting c9orf-ALS plus cases (in blue) and c9orf-ALS Negative cases (in yellow), blue circle sizes are proportional of positive cases, the bottom part shows the list of c9orf72 sALS and fALS cases in Eurasian countries.

Figure 1B RP-PCR targeting the GGGGCC repeated hexanucleotide the plot in the top panel shows results from a positive control with the expanded repeats, the bottom panel shows results from one of the non-expanded Georgian cases.