Prevalence of *C9orf72* hexanucleotide repeat expansion in Greek patients with sporadic ALS

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Abstract

A total of 178 consecutive patients with definite sALS without frontotemporal dementia (FTD) were enrolled in this study, after complete clinical evaluation. A Repeat-Primed Polymerase Chain Reaction (RP-PCR) protocol was applied to detect the G_4C_2 repeats expansions. In the studied sALS patients, 5.06% (n=9) carried the *C9orf72* mutation. Among carriers, 2/3 of them were females and spinal onset accounted for 78% and bulbar for 22%, while the mean age of onset was about 60 years. Our study showed that the prevalence of *C9orf72* repeat expansion in Greek sALS patients is similar to the overall frequency of the mutation in European populations. The pathogenic mutation remains a promising biomarker for genetic testing and targeted treatment.

Key words: *C9orf72* expansion, hexanucleotide repeats, Greek cohort, sporadic Amyotrophic Lateral Sclerosis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by death of the motor neurons in the brainstem and the spinal cord (1). In 90-95% of patients, the disease is sporadic (sALS), involving interplay between genes and environmental factors, while the rest 5-10% stands for hereditary or familial ALS (fALS) (2).

Recently, an updated meta-analysis was published by our research team, concerning the role of *C9orf72* in ALS and the development of an online database (3). Up to now, one study has been published containing results from Greek patients with fALS and sALS (4). To provide a more robust aspect on the frequency of *C9orf72* mutation in Greek population, we carried out a research in a larger, well-characterized Greek cohort of sALS patients and compared our results with the previous study by Mok *et al.*

2. Methods

2.1 Study Participants

A total of 178 consecutive patients with sALS were enrolled in this study. All patients were admitted to the Department of Neurology, University Hospital of Larisa, Greece and the University Hospital of A.H.E.P.A in Thessaloniki. Only patients with definite ALS, with no known family history of ALS and without FTD were recruited. The study was ethically approved by the University of Thessaly and informed consent was signed by all participants.

2.2 Experimental procedure

Genomic DNA was isolated and the G_4C_2 repeat expansion in *C9orf72* was screened using a Repeat-Primed Polymerase Chain Reaction (RP-PCR) protocol (4, 5). Fragment analysis of the PCR amplicons was conducted through capillary electrophosesis with ABI3730 Genetic Analyser and the products were visualized by using the GeneMapper software v5.0. G_4C_2 expansion repeats of more than 30 were considered pathogenic (5).

2.3 Statistical analysis

Patients were stratified according to mutation carriage. Sex and phenotype between carriers and non-carriers, were compared using Fisher's exact test, while for age at sample collection, age of onset and disease duration, 2-tailed t-test was applied through the IBM SPSS v21 software. The categorical variables of our study were compared with those provided by Mok *et al.*, using chi-square 2x2 contingency tables.

3. Results

In total, 5.06 % (n=9) of our ALS cases carried the *C9orf72* hexanucleotide mutation, in more than 30 repeats. All of our cases were sporadic and of Greek origin. The main demographic and clinical characteristics of our patients, carriers and non-carriers of the repeat expansion, are presented in **Table 1**. No significant differences were noticed in gender, current age, age of onset, disease duration and phenotype between carriers and non-carriers of the pathogenic mutation ($p>0.05$); (**Supplemental Table 1**).

In comparison to Mok *et al.*, statistical significance concerned only the sex ratio of the total cohorts, as we included significantly more females than Mok *et al.* (51.1% versus 23.5%, respectively), $p=0.00083$ (**Supplemental table 2**).

4. Discussion

Our results demonstrate that the prevalence of the mutation in Greece is congruent with the overall mutation frequency in Europeans and Caucasians with sALS. This has been supported both by Zou *et al.* and by our recent meta-analysis (3, 6) (**Supplemental Figure 1**). However, it is in contrast to Mok *et al.* that revealed a high prevalence of the mutation in Greek sALS patients (8.2%), the second highest in European populations after Finland (4).

The discrepancy between our results and those of Mok *et al.* could be attributed to the composition of the two cohorts or even regional genetic differences. Notably, our cohort included significantly more females. Higher prevalence of female ALS patients carrying G_4C_2 repeats expansions has been reported in Curtis *et al.*'s meta-analysis, suggesting that sex-related risk factors might moderate *C9orf72* mediated phenotypic expression (7). In our study, female carriers also outnumbered male carriers.

The spread of *C9orf72* mutation across Europe has been attributed to Vikings' migration (8). Geographic isolation of some regions together with low racial admixture could be a possible factor for mutagenesis in the Greek population (4).

The majority of our carriers had spinal onset of the disease. This is in agreement with Mok *et al* and in contrast with previous studies reporting a preponderance of the bulbar phenotype among G_4C_2 mutation carriers (9).

A cut-off limit of 30 repeats of G_4C_2 expansion was used, since RP-PCR is widely accepted in distinguishing carriers at this level (5). However, interesting findings concerning intermediate expansions (20-30 repeats) suggest that the pathogenicity cut-off point could be lowered to over 24 repeats (10). Future studies should emphasize on the effect of the expansion repeats on survival and improve the detection method to provide us with data concerning carriers of long repeats.

Disclosure statement

All authors declare that there is no conflict of interests.

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