Neurobiology of Aging 67 (2018) 201.e5–201.e7

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Analysis of *ATXN2* trinucleotide repeats in Korean patients with amyotrophic lateral sclerosis

Young-Eun Kim [a], Ki-Wook Oh [b,c], Min-Young Noh [b,c], Jinseok Park [b,c], Hee-Jung Kim [d], Jong Eun Park [e], Chang-Seok Ki [e,*], Seung Hyun Kim [b,c,**]

[a] Department of Laboratory Medicine, Hanyang University College of Medicine, Seoul, Republic of Korea
[b] Department of Neurology, Hanyang University College of Medicine, Seoul, Republic of Korea
[c] Cell Therapy Center, Hanyang University Hospital, Seoul, Republic of Korea
[d] Department of Laboratory Medicine, CHA Gangnam Medical Center, CHA University School of Medicine, Seoul, Republic of Korea
[e] Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 23 August 2017
Received in revised form 19 February 2018
Accepted 16 March 2018
Available online 23 March 2018

Keywords:
Amyotrophic lateral sclerosis
ATXN2
CAG repeat expansion
Spinocerebellar ataxia type 2

ABSTRACT

ATXN2 intermediate-length trinucleotide repeat expansions have been reported as a risk factor for amyotrophic lateral sclerosis (ALS) in various ethnicities. We tried to confirm this finding in Korean patients with ALS by screening *ATXN2* cytosine-adenine-guanine nucleotide sequences (CAG) repeat lengths in 464 unrelated ALS patients and 703 controls. The most common and the highest CAG repeat lengths in the controls were 22 and 28, respectively, whereas those in ALS patients were 22 and 33, respectively. The frequency of CAG repeat lengths of 30 or more was significantly different between the 2 groups after Bonferroni correction (1.5% in ALS vs. 0% in controls, corrected $p = 0.0075$). There were no significant differences in gender, age at onset, site of onset, functional rating scale–revised score at initial visit, calculated progression rate, or survival between patients with CAG repeat lengths of 30–33 and patients with CAG repeat lengths <30. These findings support the notion that intermediate-length *ATXN2* repeat expansions might be a risk factor in Korean patients with ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disorder characterized by progressive loss of cortical, bulbar, and spinal motor neurons (Rowland and Shneider, 2001). Approximately, 5%–10% of patients are familial (fALS) cases, whereas the remaining 90% are apparently sporadic (sALS) cases (Rowland and Shneider, 2001). To date, a number of genetic loci and disease-causing genes have been reported to be associated with ALS, including *SOD1*, *FUS*, *TARDBP*, *OPTN*, *SQSTM1*, *VCP*, and hexanucleotide expansion of *C9orf72*. However, pathogenic variants in these genes have been identified in only two-thirds of all fALS cases and about 11% of all sALS cases (Renton et al., 2014).

Spinocerebellar ataxia type 2 is an autosomal dominant hereditary ataxia caused by cytosine-adenine-guanine nucleotide sequences (CAG) trinucleotide repeat expansion in the *ATXN2* gene (OMIM #183090). Normal alleles typically contain between 21 and 23 CAG repeats, and affected individuals have alleles with 35 or more repeats (Geschwind et al., 1997). Recently, intermediate-length CAG expansions (27–33 repeats) were revealed to have a significant association with ALS, which suggested *ATXN2* as an ALS susceptibility gene leading to the mislocalization of TAR DNA-binding protein 43 (TDP-43) from the nucleus to the cytoplasm under stress conditions (Elden et al., 2010).

In Korean patients with ALS, the *C9orf72* repeat expansion has not been identified, which is the most common cause of ALS in people of European ancestry (Jang et al., 2013). Instead, *SOD1* and *FUS* appear to be the 2 major genes involved in ALS (Kim et al., 2014, 2015, 2016, 2017; Kwon et al., 2012). In this study, we aimed to determine the frequency and spectrum of *ATXN2* repeat length in Korean patients with ALS.

2. Methods

2.1. Subjects

A total of 464 unrelated Korean ALS patients, including 447 sALS patients and 17 fALS (with an affected third degree relative)

YEK and KWO contributed equally to this work.

* Corresponding author at: Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Republic of Korea. Tel.: +82 2 3410 2709; fax: +82 2 3410 2719.
** Department of Neurology, College of Medicine, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul 04763, Republic of Korea. Tel: +82 2 2290 8371; fax: +82 2 2296 8370.
E-mail addresses: changski@skku.edu (C.-S. Ki), kimsh1@hanyang.ac.kr (S.H. Kim).

0197-4580/$ – see front matter © 2018 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2018.03.019

patients, were collected from a motor neuron disease registry obtained from the ALS Clinic at Hanyang University Hospital in Seoul, Korea. Diagnosis of ALS was based on the El Escorial revised criteria (Brooks et al., 2000). Patients with clinically definite, probable, probable-laboratory supported, or possible ALS were enrolled in this study. In addition, 703 control DNAs from healthy Korean adults who undertook routine health checks at a Health Promotion Center were included. The following data were reviewed: patient demographics (age, sex, and family history of ALS), degree of diagnostic certainty, site of symptom onset, revised ALS Functional Rating Scale (ALSFRS-R) score (Cedarbaum et al., 1999), calculated progression rate (delta-FS) (Gordon and Cheung, 2006), and comprehensive neuropsychological battery of the Seoul Neuropsychological Screening Battery (Jahng et al., 2015). However, age, sex, and other phenotype data of each control were not available due to Korean Law of Personal Information Protection Act. This study was approved by the Institutional Review Boards of Hanyang University Hospital (#HYI-10-01e3) and Samsung Medical Center (#2013-04-131-002). All participants provided informed consent for genetic testing.

2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using a Wizard Genomic DNA purification kit according to the manufacturer's instructions (Promega, Madison, WI, USA). The CAG repeats of *ATXN2* were amplified using polymerase chain reaction with fluorescently labeled primers following a previously published method (Elden et al., 2010). Polymerase chain reaction products were mixed with a GeneScan 500 ROX dye size standard (Applied Biosystems, Foster City, CA, USA) and analyzed by capillary electrophoresis on an ABI 3130XL genetic analyzer (Applied Biosystems).

2.3. Statistical analysis

The chi-square test was used to test the association between *ATXN2* CAG repeat length and ALS, whereas Fisher's exact test was applied when the 2×2 table consisted of a cell where the expected number of frequencies is fewer than 5. Odds ratio (OR) with 95% confidence interval was calculated for the expanded alleles compared with the low repeat alleles. A p-value < 0.05 was considered statistically significant, and corrected p-value by multiplication of 5 was used after Bonferroni correction for the adjustment of multiple comparisons. We could not perform further analysis including age and gender as covariates because these data were not available from controls. To compare clinical characteristics between patients with CAG repeat lengths of 30–33 and patients with CAG repeat lengths <30, the chi-square test or Fisher's exact test for categorical variables and Student t test for continuous variables were used. In addition, Kaplan-Meier survival analysis was conducted using the log-rank test to compare the survival time between the 2 groups. All analysis was performed using SPSS statistics (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. ATXN2 *CAG repeats expansion*

The most common CAG repeat length in the 703 controls was 22 (93.9%), followed by 19 (1.8%) and 23 (1.2%) repeats (Supplementary Table 1). The maximum length was 29 heterozygous repeats, which was seen in only 3 (0.4%) normal controls. In 464 ALS patients, 22 CAG repeats was the most common allele (92.2%) with a range from 15 to 33 (Fig. 1). As shown in Table 1, there were no statistical differences between ALS and the controls using a cutoff of ≥26 or ≥27 for the chi-square test, whereas CAG repeats with ≥28, ≥29, and ≥30 showed significant differences between the 2 groups. However, a CAG repeat cutoff of ≥30 remained significant after Bonferroni correction ($p = 0.0075$). CAG repeats of ≥30 were observed only in the ALS patients so that the OR could not be calculated.

3.2. Clinical features of ALS patients

Among 464 patients, the mean onset age was 55.5 ± 11.0 years (median 55, range 19–83 years). Two hundred fifty-eight patients (55.6%) were male and 206 (44.4%) were female. In the control group, 343 (48.7%) were male and 360 (51.2%) were female. Three hundred nineteen patients (68.8%) had limb-onset, and 132 (28.4%) had bulbar-onset ALS; of the remaining patients (n = 13, 2.8%), 6

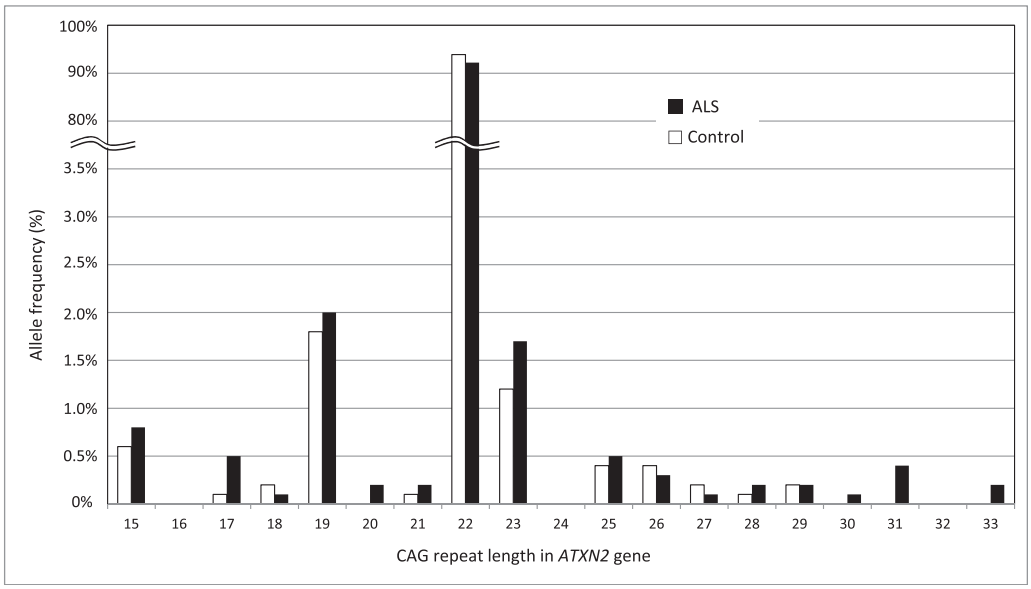

Fig. 1. The distribution of *ATXN2* CAG repeats in ALS patients and controls. Abbreviation: ALS, amyotrophic lateral sclerosis.