ATXN2 CAG repeat expansions increase the risk for Chinese patients with amyotrophic lateral sclerosis

Xiaolu Liu a, Ming Lu a, Lu Tang a, Nan Zhang a, Dehua Chui b,**, Dongsheng Fan a,***

a Department of Neurology, Peking University Third Hospital, Beijing, China
b Neuroscience Research Institute, Peking University, Beijing, China

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with unclear etiology. Recently, intermediate CAG repeat expansions in ATXN2, the gene responsible for spinocerebellar ataxia type 2 (SCA2), have been identified as a possible genetic risk factor for ALS. In this study, we analyzed the ATXN2 CAG repeat length in Chinese patients with ALS to evaluate the relationship between the genotype and phenotype. We studied 1,067 patients with ALS and 506 controls from mainland China (excluding Tibet). We collected clinical data and analyzed fluorescent PCR products to assess ATXN2 CAG repeat length in all of the samples. We observed that intermediate CAG repeat expansions in ATXN2 (CAG repeat length >30) were associated with ALS (p = 0.004). There was no significant difference in clinical characteristics between the groups with and without intermediate CAG repeat expansions in ATXN2. Our data indicate that, for ALS patients from mainland China, intermediate CAG repeat expansions in ATXN2 increase the risk of ALS but have no effect on disease phenotype.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, always fatal neurodegenerative disease caused mainly by the degeneration of upper and lower motor neurons in the motor cortex, brainstem, and spinal cord (Brooks et al., 2000). The exact incidence of ALS worldwide is unknown, but the incidence is 2.16 per 100,000 person-years in Europe (Logroscino et al., 2010). Fifty percent of patients die from respiratory failure within 30 months of symptom onset (Kierman et al., 2011). Approximately 10% of patients have a self-reported family history of ALS (FALS), and 90% of patients have sporadic ALS (SALS). Superoxide dismutase 1 (SOD1) was the first gene that was found to be associated with FALS, and mutations of SOD1 are responsible for 12% to 23% of FALS and 1% to 4% of SALS (Burgunder et al., 2011). Mutations of ALSIN, SETX, FUS/TLS, DCTN1, ANG, and TARDBP have also been identified in FALS patients (Gros-Louis et al., 2006). An expanded hexanucleotide repeat within the C9ORF72 gene accounts for 23% to 49% of FALS and 12% to 29% of familial frontotemporal dementia (Dejesus-Hernandez et al., 2011; Renton et al., 2011).

Spinocerebellar ataxia type 2 (SCA2) is one of the most common autosomal-dominant hereditary ataxias and is caused by CAG trinucleotide repeat expansions in ATXN2. The triplet length in this gene is typically 22 to 23 (Imbert et al., 1996) but increases to 35 to 59 in SCA2 (Sanpei et al., 1996). Recently, intermediate CAG repeat expansions (>27) in ATXN2 were identified as a genetic risk factor for ALS in a large cohort of Americans (Elden et al., 2010). Thereafter, studies in patients of different ethnicities, including people from southwestern China, supported that finding, and the statistical cutoff was determined to be a triplet length of 27 to 30 (Chen et al., 2011; Lee et al., 2011a,b; Soraru et al., 2011; Van Damme et al., 2011). The triplet length of ataxin-1, ataxin-3, ataxin-6, ataxin-7, TBP, atrophin-1, huntingtin and the androgen receptor was found not to be relevant to ALS (Garofalo et al., 1993; Lee et al., 2011a, 2011b; Ramos et al., 2012).

In this study, we tested the CAG repeat length in a large cohort of Chinese mainland ALS patients and controls, with the purpose of confirming previous findings.

2. Methods

2.1. Patients

All of the patients were from the Neurology Department of Peking University Third Hospital and diagnosed with ALS according to the El Escorial revised criteria (Brooks et al., 2000). They came...
from 30 provinces (excluding Tibet) of mainland China. We collected clinical features, including sex, age at examination, age at onset, site of onset, brainstem involvement, level of diagnostic certainty, fasciculation, and the ALS Functional Rating Scale-Revised (ALSFRS-R). We calculated the progression rate of ALSFRS-R (ΔFS) at the time of diagnosis. Individuals without neurological disease history were anonymous blood donors of Chinese origin and were used as controls. All subjects provided written consent for DNA genetic testing.

2.2. Genetic analysis

Genomic DNA was collected from peripheral blood lymphocytes using a standard method. The CAG repeats of ATXN2 were amplified using the polymerase chain reaction (PCR) with fluorescently labeled primers. The forward primer was 5'- FAM-CCC CGC CCG GCG TGC GAG CCG GTG TATG - 3', and the reverse primer was 5' - CGG GCT TGC GGA CAT TGG-3'. PCR cycling was as follows: 2 min at 94°C, 35 cycles (1 minute at 94°C, 1 minute at 66°C, and 1 minute at 72°C), and 5 minutes at 72°C (Eden et al., 2010). PCR products were mixed with a Liz-500 size standard and sized by capillary electrophoresis on an ABI 3730XL genetic analyzer (Foster City, CA, USA). A subject with a 22/22 homozygous genotype, as confirmed by clonal sequencing, was used as a standard calibrator.

2.3. Statistical analysis

To assess the association between intermediate ATXN2 CAG repeat length and ALS, χ² statistics or two-tailed Fisher’s exact tests were used. Rank-sum tests were used to analyze the difference in age at onset between the 2 groups with and without intermediate-length ATXN2 CAG repeats. The χ² statistics were used to compare sex, site of onset, and levels of diagnostic certainty between the 2 groups. Only patients with available clinical data were included in the statistical analysis.

3. Results

3.1. Clinical data

We analyzed the ATXN2 CAG repeat lengths in 1067 ALS patients and 506 neurologically healthy controls. Demographic descriptions of the patients and controls are summarized in Table 1, and regional distribution is given in Table 2. Among the ALS subjects, 1061 patients had SALS, and 6 patients had FALS. Two FALS patients carried SOD1 mutations, and the other 4 FALS patients were genetically undefined. ALS patients had a mean age at onset of 49.85 ± 11.79 years (range, 16–79 years) and a mean age at examination of 51.03 ± 11.93 years. Controls had a mean age at examination of 49.85 ± 11.93 years (range, 16–79 years). The maximal value was 5.667. Of the patients, 187 (23.3%) had difficulty breathing at the time of diagnosis.

3.2. ATXN2 CAG repeat expansions

In controls, the most common (92.7%) repeat length was 22, and the remaining alleles carried repeat lengths ranging from 13 to 30. As previous studies reported, 87.7% of controls were homozygotes (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996). Although the maximal repeat length in the ALS cohort was 35, the most abundant repeat length was 22, and 84.3% of the ALS cohort were homozygotes, which was similar to the characteristics of the controls. Using a repeat length cut-off >30 units, 17 of 1067 patients (1.6%) and none of 506 controls were identified, and this difference was statistically significant (χ² = 8.150, p = 0.004). The distribution of repeat length >22 in ALS and control cases is shown in Fig. 1.
subjects with CAG repeat length >30 were heterozygotes; the other alleles had a repeat length <26. Six FALS patients did not have a repeat length >22.

Clinically, patients with expanded CAG repeat length did not have a uniform manifestation. There was no significant difference in age at onset (p = 0.582) and ΔFS (p = 0.723) between ALS patients with CAG repeat length >30 and ≤30. Analogously, we did not find an association between CAG repeat length >30 and clinical features, including sex (p = 0.933), site of onset (p = 0.841), brainstem involvement (p = 0.081), levels of diagnostic certainty (p = 0.449), fasciculation (p = 1.000) and difficulty breathing (p = 0.078). The FTD-ALS patient was a homozygote with a repeat length of 22 (Table 3).

4. Discussion

We analyzed ATXN2 CAG repeat length in 1067 ALS patients and 506 neurologically healthy controls, which makes this the largest-scale study of ATXN2 CAG repeat expansion in Chinese ALS patients. The patients and controls represent the population of the Chinese mainland. We found that alleles with >30 repeats were significantly more frequent in ALS patients than in controls. Our data indicate that, among ALS patients from mainland China, intermediate CAG repeat expansions in ATXN2 are associated with the disease. It has been reported that for the southwestern Chinese population, ATXN2 CAG repeat length ≥27 was weakly associated with SALS (Chen et al., 2011). Combining the evidence of these two studies, we can conclude that intermediate CAG repeat expansions in ATXN2 increase the risk of ALS in the Chinese mainland population. Thus far, intermediate CAG repeat length in ATXN2 has been found in 0.6% to 9.7% of ALS patients in different ethnic populations (Chen et al., 2011; Corrado et al., 2011; Daoud et al., 2011; Elden et al., 2010; Gellera et al., 2012; Gispert et al., 2012; Lee et al., 2011a, 2011b; Ross et al., 2011; Soraru et al., 2011; Van Damme et al., 2011; Van Langenhove et al., 2011).

In SCA2, a strong inverse correlation between age at onset and repeat length was observed (Imbert et al., 1996). Other studies have indicated that patients with CAG repeat length ≥24 have an earlier onset age, but this was nonsignificant in Chinese individuals (Chen et al., 2011; Elden et al., 2010). In our study, however, the patients with expanded CAG repeat length did not have a uniform manifestation. We found no association between clinical features and CAG repeat length, which was in agreement with most previous studies (Lee et al., 2011a, 2011b; Soraru et al., 2011; Van Damme et al., 2011). Sequencing of the repeat region has shown that ALS patients have CAG repeat regions (Imbert et al., 1996; Pulst et al., 1996; Sanpei et al., 1996). The CAA interruptions determine the folding of the ATXN2 transcript into branched hairpins, whereas pure CAG repeats in large ATXN2 expansions form slippery hairpins (Imbert et al., 1996). The structure of the DNA may also determine the protein function.

In summary, our data indicate that, in mainland China, intermediate CAG repeat expansions in ATXN2 increase the risk of ALS and have no effect on disease phenotype.

Disclosure statement

The authors declare no conflicts of interest.

Acknowledgements

We thank the patients and their families for their participation in this project.

This study was supported by grants from the National Natural Sciences Foundation of China (81030019), the Beijing Natural Science Foundation (7102161), Doctoral Fund of Chinese Ministry of Education (20100000110084), and the Major Projects of the National Science and Technology of China (2011ZX09307-001-07).

References


