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

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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.

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 (Kiernan 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

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Table 1
Characteristics of amyotrophic lateral sclerosis (ALS) patients and controls

	No.	% Male	Age at examination, y, mean ± SD	Age at onset, y, mean ± SD	Diagnostic delay, mo, mean ± SD
ALS	1,067	65.7	51.03 ± 11.93	49.85 ± 11.79	19.49 ± 19.36
Controls	506	57.1	53.25 ± 12.95	-	-

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 (Elden 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, χ^2 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 χ^2 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 53.25 ± 12.95 years. In all, 122 patients (16.8%) had the bulbar onset form, and 602 (83.1%) had the spinal onset form. One patient (0.1%), who initially presented with memory impairment, was diagnosed with FTD-ALS. The median ΔFS was 0.500 and the maximal value was 5.667. Of the patients, 167 (23.3%) had difficulty breathing at the time of diagnosis.

Table 2
Regional distribution of the amyotrophic lateral sclerosis (ALS) patients and controls

Province	ALS n = 1,067	Controls n = 506
Anhui	27	7
Beijing	121	192
Chongqing	9	2
Fujian	14	3
Gansu	8	3
Guangdong	11	3
Guangxi	5	0
Guizhou	11	3
Hainan	1	0
Hebei	105	78
Heilongjiang	38	21
Henan	62	21
Hubei	28	2
Hunan	20	3
Inner Mongolia	26	12
Jiangsu	26	8
Jiangxi	17	4
Jilin	22	7
Liaoning	43	8
Ningxia	8	3
Qinghai	2	0
Shaanxi	22	1
Shandong	63	22
Shanghai	1	0
Shanxi	38	24
Sichuan	18	2
Tianjin	25	5
Xinjiang	8	0
Yunnan	4	1
Zhejiang	26	3
NA	258	68

Key: NA, not available.

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 ($\chi^2 = 8.150$, $p = 0.004$). The distribution of repeat length >22 in ALS and control cases is shown in Fig. 1. All

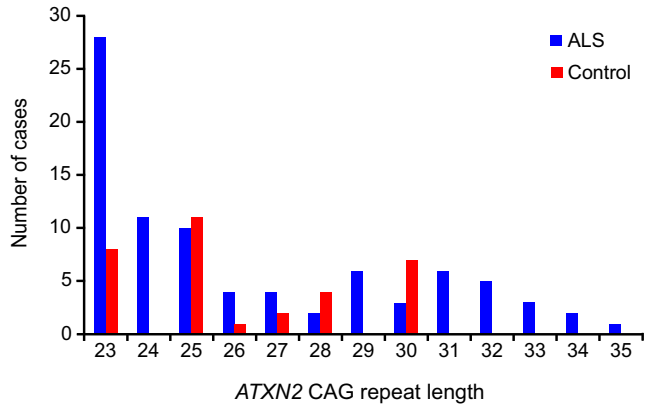

Fig. 1. Distribution of ATXN2 repeat length >22 in amyotrophic lateral sclerosis (ALS) patients and controls.