Identification of C9orf72 repeat expansions in patients with amyotrophic lateral sclerosis and frontotemporal dementia in mainland China

Bin Jiao a, Beisha Tang a,b,c, Xiaoyan Liu a, Xinxian Yan a,c, Lin Zhou a,c, Yi Yang a,c, Junling Wang a,c, Kun Xia a,b,c, Lu Shen a,b,c,*

a Department of Neurology, Xiangya Hospital, Central South University, Changsha 410008, China
b State Key Laboratory of Medical Genetics, Changsha 410008, China
c Key Laboratory of Hunan Province in Neurodegenerative Disorders, Central South University, Changsha 410008, China

Abstract

The GGGGCC repeat expansion in the C9orf72 gene was recently identified as a major cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) in white populations. To estimate the frequency of hexanucleotide repeats in patients with ALS and FTD from mainland China, we screened for C9orf72 in a cohort of 128 patients and 150 control subjects using the repeat-primed polymerase chain reaction method. We observed pathogenic repeat expansions in a family with ALS-FTD and in a patient with sporadic FTD. In the family with ALS-FTD, the proband and the 2 asymptomatic siblings exhibited C9orf72 repeat expansions, and the clinical feature of the proband was characterized by pure motor syndrome with no cognitive impairment. The patient with sporadic FTD presented primarily with deteriorating behavior and mental status. Genotype analysis revealed that the proband shared the previously reported 20-single nucleotide polymorphism risk haplotype, whereas the patient with sporadic FTD carried all single nucleotide polymorphisms except rs2814707-A. To our knowledge, this study is the first to report 2 C9orf72 mutation patients in mainland China, and they shared the similar risk haplotype identified in white populations, suggesting that ALS and FTD associated with C9orf72 mutation was probably derived from a single founder.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive adult-onset disorder that affects upper and lower motor neurons and leads to progressive muscular weakness and atrophy, with eventual death within 3–5 years after the onset primarily as a result of respiratory failure (Koppers et al., 2012). Frontotemporal dementia (FTD) is the second most common cause of young-onset dementia, which primarily affects individuals younger than 65 years old. FTD involves a range of progressive cognitive impairment syndromes associated with frontal and temporal lobe atrophy, characterized by behavioral changes, executive dysfunction, and language difficulties (Cerami et al., 2012). Recent compelling evidence has suggested that these 2 neurodegenerative disorders are associated through overlapping clinical features and pathologic spectrums. Approximately 10% of patients with FTD presented clinical and pathologic evidence of ALS. Similarly, 5%-15% of patients with ALS show severe behavioral changes and/or language dysfunctions that meet the diagnosis of FTD (Lillo et al., 2011; Piguet et al., 2011; Trojsi et al., 2012).

ALS and FTD have a strong genetic basis, with familial forms occurring in approximately 10% of ALS patients and 30%-50% of FTD patients, with an autosomal dominant pattern of inheritance (Borroni et al., 2013; Hardiman et al., 2011). The causative genes have been identified in patients with ALS or FTD and, among them, MAPT and GRN account for a significant number of patients with familial FTD (Pickering-Brown et al., 2008). SOD1, TARDBP, and FUS mutations are more common in patients with familial ALS (Andersen and Al-Chalabi, 2011). A recent breakthrough finding revealed GGGGCC repeat expansions in the noncoding region of C9orf72 in patients with familial or sporadic ALS and FTD in white populations. The distribution of GGGGCC repeats in healthy individuals commonly ranges from 2–23 repeats; the expansion sequence contains up to 500–1600 repeats in patients with ALS and FTD. The prevalence of expanded repeats was reported to be as high...
as 23.5%–47% for familial ALS or FTD and 4.1%–21.0% for sporadic ALS in Italy, Germany, Belgium, the United Kingdom, and the United States (DeJesus-Hernandez et al., 2011; Gijselinck et al., 2012; Renton et al., 2011). However, studies in East Asia suggest that the prevalence of pathogenic repeats was comparatively lower than that in Europe. Across these studies, the C9orf72 mutation accounted only for 18% of familial ALS and 2% of sporadic ALS in Taiwan (Tsai et al., 2012), and an even lower prevalence was observed in a cohort of patients with sporadic ALS in Japan (Ogaki et al., 2012), whereas no pathogenic mutation was observed in patients with ALS in mainland China and Korea (Jang et al., 2013; Zou et al., 2013), indicating that the expanded repeat sequences in the C9orf72 gene probably vary according to geographic region.

In addition, Mok et al. (2012) demonstrated that most of the patients with ALS and FTD carrying C9orf72 expanded repeats shared the same 20-single nucleotide polymorphism (SNP) risk haplotype associated with the C9orf72 gene in populations from Finland, Ireland, Italy, the United Kingdom, and the United States, suggesting that ALS/FTD associated with the C9orf72 mutation is probably derived from a single founder. However, it remains unclear whether patients with ALS/FTD in mainland China carry the same risk haplotype identified in white populations. To resolve this issue, in the current study, we examined the repeat expansions and risk haplotype of C9orf72 in patients with ALS/FTD from mainland China.

2. Materials and methods

2.1. Study participants

We recruited 128 patients with ALS and/or FTD (60.9% men; mean age at onset, 41.1 ± 11.5 years) and 150 healthy control subjects from mainland China for this study. All patients, including 110 with ALS (10 familial ALS) and 18 with FTD (5 familial FTD), were recruited consecutively from the outpatient neurology clinic of Xiangya Hospital, and standard clinical neurologic examinations were performed on each patient. All patients met the El Escorial criteria for ALS and the Lund-Manchester criteria for FTD (Brooks, 1994; Lund and Manchester Groups, 1994). All healthy control subjects were recruited from the Xiangya Wellness Center and were matched for sex and age (56.8% men; mean age, 39.7 ± 8.9 years) with the patient group. The protocol of the study was approved by the ethics committee of Xiangya Hospital, Central South University, and informed consent was obtained from all participants.

2.2. Repeat-primed polymerase chain reaction assay

Genomic DNA was isolated from peripheral blood leukocytes using a QIAGEN kit. First, we performed a FAM fluorescent-labeled polymerase chain reaction (PCR) assay to determine the genotypes in all patients and control subjects using genotyping primers, as reported previously (Dejesus-Hernandez et al., 2011). Second, to obtain a qualitative estimation of the presence of C9orf72 expanded repeats, we performed repeat-primed PCR, as described previously (Dejesus-Hernandez et al., 2011).

2.3. SNP genotyping

The available information concerning the 20 SNPs associated with the C9orf72 gene was extracted from the National Center for Biotechnology Information (NCBI) database of Single Nucleotide Polymorphism (dbSNP). The 20 SNPs were genotyped using multiplex SNaPshot technology. The primers for PCR amplification and SNaPshot extension reactions were designed with the NCBI sequence database using Primer 5 software.

3. Results

3.1. Detection of expanded repeats in the C9orf72 gene

The C9orf72 expanded repeats were observed in the proband and 2 asymptomatic siblings from an family with ALS-FTD and a patient with sporadic FTD (Fig. 1A and Fig. E1); no abnormal repeat expansion was identified in other patients and control individuals. A wide range of 2–20 repeats was observed in these patients, except for the 2 mutation carriers, and the frequency of repeats was 2 U (30.5%), followed by 7 U (29.7%) and 6 U (13.3%), with an average repeat number of 6.2 ± 4.8. A wide range (2–11) of repeats was observed in control individuals, with a similar frequency of 2 U (32.0%) followed by 7 U (22.7%) and 8 U (16.0%), and an average repeat number of 6.0 ± 3.2 (Fig. 2). There was no significant difference in the distributions of the repeat numbers between patients and control subjects using the Mann-Whitney U test (p = 0.23). The genotypes of the patients with the C9orf72 mutation were determined for the 20-SNP risk haplotype. The proband from the family with ALS-FTD shared the same risk haplotype identified in whites, and the patient with sporadic FTD carried all SNPs, except rs2814707-A (Table E1).

3.2. Clinical features of mutation cases

3.2.1. The ALS proband from a family with ALS-FTD

The proband was a 47-year-old man that first presented progressive weakness in the left extremity. Seven months later, he experienced worsening of weakness on the left side and atrophy on the right side. Ten months later, dysphagia and dysarthria occurred. On admission, neurologic examination revealed fasciculations and hyperreflexia in the affected limb, muscle weakness, and more severe atrophy in the lower limbs than in the upper limbs. The muscle

Fig. 1. (A) The pedigree of the family with amyotrophic lateral sclerosis (ALS)-frontotemporal dementia (FTD) carrying the C9orf72 hexanucleotide repeat expansion. The proband is indicated with an arrow. (B, C) T1-weighted magnetic resonance imaging of the brain of the proband in family with ALS-FTD (B) and the patient with sporadic FTD (C).
test revealed a score of 4 of 5 points in the proximal muscles of the 4 extremities, and the bilateral Babinski signs were positive. No sensory deficits or other associated neurologic signs were observed. The electromyogram showed spontaneous denervation activity, and a loss of motor units in the muscles of the rectus femoris and tibialis anterior. Magnetic resonance imaging of the brain was conducted and no structural damage was observed (Fig. 1B). His Mini-Mental State Examination score was 28 of 30 points; his Frontal Assessment Battery score was 15 of 18 points. This patient was diagnosed as having clinically possible ALS according to the El Escorial criteria. Further genetic analyses excluded the TARDBP or FUS gene mutations. His 2 asymptomatic younger siblings also exhibited C9orf72 expanded repeats. His father was diagnosed clinically with ALS and died of respiratory insufficiency at age 53; his 2 aunts and 1 uncle were diagnosed with clinical FTD and died at age 60.

3.2.2. Patient with FTD

The patient with FTD was a 67-year-old man with a 2-year history of progressive deteriorating behavior and mental status. His wife described the initial symptoms, including the use of obscene words, lack of initiation during social gatherings, and no communication with family members and friends. One year later, this patient presented random public defecation and mild executive function impairment. However, he did not exhibit a decline in memory, judgment, or language function. His familial history was negative. Neurologic examination revealed no neurologic signs. His Mini-Mental State Examination score was 28 of 30 points and his Frontal Assessment Battery score was 10 of 18 points. Brain magnetic resonance imaging revealed bilateral frontotemporal atrophy (Fig. 1C). This patient was diagnosed as having clinical FTD according to the Lund-Manchester criteria. Further genetic analyses excluded MAPT or GRN gene mutations.

4. Discussion

A hexanucleotide repeat expansion in the first intron of the C9orf72 gene was recently identified as a major cause of the chromosome 9p21-associated diseases ALS and FTD. Although the detailed pathologic mechanism is unknown, the general causes include primarily an inhibition of normal expression of the encoded protein or the loss of protein function through the generation of abnormal, toxic RNA foci that disrupt normal cellular pathways (Renton et al., 2011). A recent pathologic study has revealed that patients with repeat expansions have several unique pathologic features in cytoplasmic inclusions, characterized by dipeptide-repeat proteins, which were probably generated through non-ATG-initiated translation from the expanded GGGGCC repeats in 3 reading frames (Mori et al., 2013). An additional functional study of the C9orf72 gene has demonstrated that (GGGGCC)n RNA forms extremely stable G-quadruplex structures, which are strongly associated with the number of repeats and RNA concentration. The RNA-RNA interactions facilitated through G-quadruplex formation might influence transcript aggregation and foci formation in ALS and FTD cells (Reddy et al., 2013). Thus, length-dependent G-quadruplex formation involving C9orf72 RNA might play a role in patients with ALS and FTD.

GGGCC repeat expansions in the C9orf72 gene in patients from mainland China with FTD or ALS revealed that only 1 sporadic case of FTD and 1 familial case of ALS harbored the pathogenic mutation, and the prevalence of this mutation was similar to that reported previously in other Asian countries. Although the frequency was much lower than that in white populations, to our knowledge, this study is the first to report patients with the C9orf72 gene mutation in mainland China, thereby providing an opportunity to explore the pathologic mechanism further.

Mok et al. (2012) identified a 20-SNP risk haplotype strongly associated with patients with ALS and FTD carrying the C9orf72 mutation in several populations of northern European ancestry. Intriguingly, our findings that the patient with ALS shared the same haplotype and the patient with FTD carried all SNPs except rs2814707-A are nearly consistent with observations in European populations. Two studies from Japan have reported that most patients with ALS with C9orf72 mutations also share the same risk haplotype. Altogether, these observations suggest that ALS and FTD associated with C9orf72 mutations are probably derived from a single founder.

With regard to the clinical phenotypes associated with C9orf72 mutations, emerging evidence has provided various descriptions from different cohorts. In this study, the symptoms of the patient with FTD primarily included behavioral abnormalities and mild executive impairments, but not in combination with other cognitive impairments or motor syndromes, consistent with a previous study on a cohort from a white population (DeJesus-Hernandez et al., 2011). Interestingly, although the patient with ALS in the current study had a positive family history of ALS-FTD, the clinical signs were focused primarily on upper and lower motor neurons, without cognitive function impairment. Nevertheless, our findings suggested that potential C9orf72 mutations should be detected in patients with FTD with prominent behavioral changes or in patients with ALS with pure motor neuron signs.

Although we did not detect the expanded repeats in healthy control subjects, the fact that the GGGGCC repeat was observed in 2 asymptomatic individuals from a family with ALS-FTD suggests that this phenomenon was probably a result of incomplete penetrance or a later age of onset. Therefore, further longitudinal follow-up studies should focus on family members of patients with the C9orf72 expanded repeats to determine the true penetrance.

In summary, although the prevalence of mutations in the C9orf72 gene varies greatly among ethnic populations, the finding of 2 mutation cases in our cohort suggests that the C9orf72 gene might also play a key role in patients with ALS and FTD from mainland China. In addition, the 20-SNP risk haplotype in the 2 reported cases provide further evidence that cases with ALS and FTD with GGGGCC expanded repeats in mainland China were probably derived from the same founder, as observed in European populations. However, the limited mutation samples hindered further study. Larger samples, particularly patients carrying the
C9orf72 mutation, are required to investigate the potential associations between the number of repeats and disease susceptibility.

Disclosure statement

The authors have no actual or potential conflicts of interest. The study was approved by the expert committee (equivalent to an institutional review board) of Xiangya Hospital, Central South University, China, and written informed consent was obtained from all patients or their guardians.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.10.001.

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