RESEARCH NOTE

Lack of variation of ATTCT pentanucleotide repeats at *ATXN10* gene between clinically diagnosed ataxia patients and normal individuals originated from Chinese Han

JUN-LING WANG[1,2], HONG JIANG[1,2], SHEN ZHANG[1,2], QIAN XU[1,2], YA-FANG ZHOU[1,2], SHU-SHENG LIAO[1,2], LU SHEN[1,2], XIN-XIANG YAN[1,2], HUAI-XU ZHU[3], QIAN PAN[3], KUN XIA[3] and BEI-SHA TANG[1,2,3*]

[1]*Department of Neurology, Xiangya Hospital,* [2]*Neurodegenerative Disorders Research Centre, Central South University, Changsha 410008, People's Republic of China,*
[3]*National Lab of Medical Genetics of China, Changsha, 410008, People's Republic of China*

Introduction

The autosomal dominant spinocerebellar ataxias (SCA) are clinically and genetically heterogeneous group of debilitating neurodegenerative diseases characterized by a generalized incoordination of gait, speech and limb movements (Tang *et al*. 2000; Soong and Paulson 2007). To date, sixteen different genes related to SCA have been identified: *ATXN1, ATXN2, MJD1, PLEKHG4, SPTBN2, CACNA1A, ATXN7, ATXN8OS, ATXN10, TTBK2, PPP2R2B, KCNC3, PRKCG, TBP, FGF14* and *DRPLA* (dentatorubral-pallidoluysian atrophy) (Soong and Paulson 2007). Most subtypes of SCA are caused by unstable CAG repeat expansions encoding polyglutamine tracts, while SCA10 is the only subtype caused by an intronic ATTCT pentanucleotide repeat expansion (Matsuura *et al*. 2006). Zu *et al*. (1999) first mapped the SCA10 locus to chromosome 22q13-qter and thereafter cloned the causative gene *ATXN10*. The expansion of pentanucleotide (ATTCT) repeat is located in intron 9 of the *ATXN10* gene (Zu *et al*. 1999; Matsuura *et al*. 2000). In SCA10, normal alleles carry 10–22 ATTCT repeats, intermediate alleles with reduced or no penetrance range from 280 to 370 ATTCT repeats, and pathological alleles contain 800–4500 ATTCT repeats (Alonso *et al*. 2006).

An epidemiological study found that SCA10 subtype, first reported in individuals of Mexican ancestry with ataxia, was rare in the world. Subsequently, in a cohort of families from Mexico who had inherited ataxia, SCA10 was determined to be the second most common subtype of inherited ataxia, after SCA2 (Xi and Ashizawa 2005; Rasmussen *et al*. 2001). So far, all the SCA10 patients are been from Latin America. The genealogical histories and physical characteristics of these patients suggested an admixture of Native American with Spanish/Portuguese ancestry in all SCA10 families (Teive *et al*. 2004; Seixas *et al*. 2005). In contrast, *ATXN10* mutations have not been found in certain Caucasian (including American white, French, Italian, Spanish, and Portuguese) or Asian (Japanese, Chinese, and Indians) populations with ataxia, in which other known SCA have been excluded (Rasmussen *et al*. 2001; Chakravarty and Mukherjee 2002; Fujigasaki *et al*. 2002; Matsuura *et al*. 2002; Sasaki *et al*. 2003; Brusco *et al*. 2004; Sulek *et al*. 2004; Jiang *et al*. 2005).

Most subjects studied thus far have been of European and American ancestry. Therefore, it is important to screen additional series of patients for expansions to determine the relative prevalence of SCA10 among ataxias. Here, we explore the relative frequency of SCA10 among SCA patients in Chinese Han people, in whom other known SCA have been excluded, and investigate the distribution and frequency of ATTCT repeats in SCA10 patients and healthy controls. We used fluorescence-PCR, capillary electrophoresis and Southern blot to analyses ATTCT repeat in a series of unassigned SCA patients and healthy controls in Chinese Han ethnic groups.

Materials and methods

Patients

Among 393 SCA patients (269 familial and 124 sporadic), we identified 21 with SCA1 (5.34%), 32 with SCA2 (8.14%), 185 with SCA3/MJD (47.07%), 11 with SCA6 (2.80%), 7 with SCA7 (1.78%) and 1 with SCA17 patients (0.25%).

*For correspondence. E-mail: bstang7398@yahoo.com.cn.

Keywords. spinocerebellar ataxia (SCA); *ATXN10* gene; nucleotide repeat; mutation detection; capillary electrophoresis; human genetics.

This study also included 136 unassigned patients (55 familial and 81 sporadic cases), in whom the subtypes mentioned above were excluded. These 136 subjects consisted of 82 men and 54 women, from whom the mean (± SD) age-at-onset of the first neurological symptoms related to ataxia could be obtained (familial patients, 35.52 ± 9.16 years; sporadic patients, 38.64 ± 7.25 years; range 2–64 years). All the patients were obtained from the outpatient neurology clinics of Xiangya Hospital and the National Lab of Medical Genetics of China from April 1995 to August 2006, and 264 healthy mainland Chinese individuals were selected as controls. The diagnosis of SCA was determined clinically by two or more experienced neurologists according to the Harding criteria (Harding 1993). Patients with ataxias caused by alcohol, other substances abuse, paraneoplasia, malformation, vascular defects, inflammation or autoimmune diseases were excluded from this study. All the patients and healthy controls were of Han nationality. Clinical data were provided by the patients or by their close relatives. The 264 controls were healthy volunteers unaffected by any neurological conditions. Written informed consent was obtained from every individual before participation.

Molecular analysis

High molecular weight deoxyribonucleic acid (DNA) was extracted from peripheral blood leukocytes. The ATTCT repeat size was determined by fluorescent PCR using previously published primers (Matsuura *et al*. 2000). The primer was labelled with a HEX fluorophore. The capillary electrophoresis (CE) analysis of fluorescent PCR products was performed by an ABI-Prism 377 automatic sequencer (Perkin Elmer, Foster City, USA). Data were examined by Genescan 3.1 software. Using a sequenced allele (12 ATTCT repeat, 186.35 amplicon length) as the control, the sample ATTCT number was calculated as = (sample length – 186.35)/5 + 12 (figure 1,a&b).

When a single ATTCT repeat allele was amplified, Southern blot analysis using *Eco*RI was performed to distinguish homozygosity from heterozygosity containing a large ATTCT repeat expansion, as described elsewhere (Matsuura *et al*. 2000). A radio-labelled genomic probe upstream of the ATTCT repeat was used for hybridization.

Statistical analysis

The data were analysed by SPSS 11.0. The mean allele frequencies between patients and normal control groups were analysed by the two sample *t*-tests. The *P* value was two-tailed, and the level of significance was 0.05.

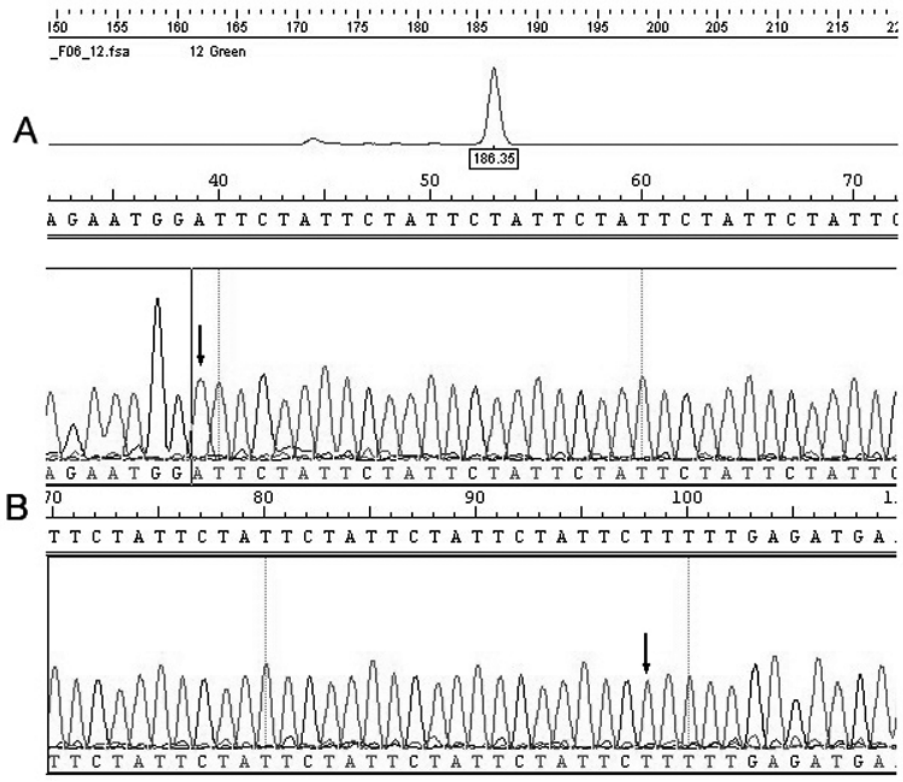

Figure 1. (A) Capillary electrophoresis, and (B) sequencing result for a healthy control.

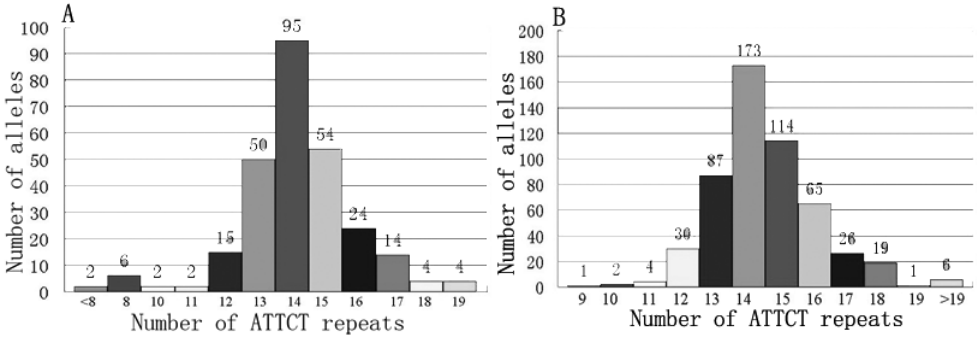

Figure 2. Distribution of ATTCT pentanucleotide repeat alleles in *ATXN10* genes in (A) 136 patients (272 chromosomes), and (B) 264 healthy controls (528 chromosomes).

Results

ATXN10 analysis in SCA patients

There were no obvious abnormal changes found in 136 SCA cases by 8% denaturing polyacrylamide gel and capillary electrophoresis. We have discovered 31 cases of homozygosis (22.8%) in 136 SCA cases by capillary electrophoresis analysis, and the extent of ATTCT repeat numbers were from 5 to 19 (mean ± SD: 13.96 ± 2.14), in which 14 repeats of CAG appears most frequently (figure 2,a). Southern blot analysis of 31 homozygosis patients showed no expanded alleles of the ATTCT repeat (figure 3). Thus, no ATTCT expansion was detected.

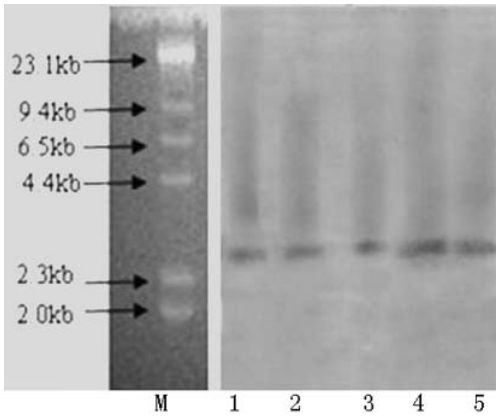

Figure 3. Southern blot result of part of SCA patients (1–5) with homozygous alleles. All detected subjects showed the same 2.5-kb *Eco*RI fragment, thus, no ATTCT expansion was detected.

ATXN10 analysis in healthy controls

There was no obvious abnormal change found in 264 healthy controls. We have identified 70 cases of homozygosis (26.5%) in 264 healthy controls by CE analysis, and the extent of ATTCT repeat numbers were from 9 to 32 (mean ± SD: 14.51 ± 1.60), in which 14 repeats of ATTCT appears most frequently (figure 2,b). There was a decreasing tendency in the mean ATTCT repeat number of 13.96 in SCA patients compared with 14.51 in healthy controls ($t = -4.089$; $P < 0.05$).

Discussion

To date, SCA10 is the only SCA subtype caused by expansion of ATTCT pentanucleotide repeat in intron 9 of *ATXN10* gene. The normal repeat number is from 10 to 29, 82% of unaffected individuals are compound heterozygotes for ATTCT repeat sizes in this range, whereas 18% are homozygotes (Matsuura *et al*. 2000). Alleles with 280–370 ATTCT repeats may be an intermediate alleles with reduced or no penetrance. Further investigation is needed to determine whether alleles with 400 to 760 ATTCT repeats are full penetrance (Alonso *et al*. 2006). Thus, the SCA10 repeat expansion is one of the largest known microsatellite repeat expansion existing in the human genome.

In our research, no obvious abnormal change in *ATXN10* gene was observed in 136 unassigned SCA patients and 264 healthy controls. From capillary electrophoresis analysis, we detected 31 (22.8%) and 70 (26.5%) homozygotes in 136 SCA patients and 264 healthy controls, respectively, which was a few more than that of the former report (Matsuura *et al*. 2000), but was similar with that of some other reports (Matsuura *et al*. 2000, 2002; Sulek *et al*. 2004; Alonso *et al*. 2006).

The ATTCT repeat in our study ranged from 9 to 32 in healthy controls, which is the smallest and the largest normal number of ATTCT repeats ever reported, comparing with the 10–22 normal repeats from other research in Caucasian, Japanese and Mexican populations, which were 11–22, 11–20 and 10–20, respectively (Matsuura *et al*. 2000). There was a reductive tendency in the mean ATTCT repeat number of 13.96 in SCA patients compared with 14.51 in controls, for which no other report was similar. Therefore, this result suggests that there may be potentially reductive tendencies of ATTCT repeat in SCA patients excluding SCA10.

The most frequent ATTCT repeat number in our research was 14 in both healthy controls and SCA patients, which accounted for 69.8% and 65.5%, respectively, while most alleles were carrying 12–17 repeats. In contrast, these results

were extremely similar to the distribution tendency of previous reports in SCA10, but markedly different from SCA8 which had two distribution peaks (Sulek *et al*. 2004).

Neither SCA10 expansion was identified from the Southern blot results. In sum, it has been confirmed that SCA10 is a rare subtype, and the normal reference standard for *ATXN10* gene's ATTCT pentanucleotide repeat of 9–32 is verified in the Chinese Han ethnic group.

Acknowledgements

This work was supported by the Major State Basic Research Development Program of China (grant no. 2006cb500700), the National High-Tech Research and Development Program of China (grant no. 2004AA227040), the National Key Technologies Research and Development Program of China (grant no. 2004BA720A03) and National Natural Science Foundation of China (30400262, 30671151) . The authors thank all the SCA patients and healthy controls for participating in this study.

References

Alonso I., Jardim L. B., Artigalas O., Saraiva-Pereira M. L., Matsuura T., Ashizawa T. *et al*. 2006 Reduced penetrance of intermediate size alleles in spinocerebellar ataxia type 10. *Neurology* **66**, 1602–1604.

Tang B., Liu C., Shen L., Dai H., Pan Q, Jin L. *et al*. 2000 Frequency of SCA1, SCA2, SCA3, MJD, SCA6, SCA7, and DRPLA CAG trinucleotide repeat expansion in patients with hereditary spinocerebellar ataxia from Chinese Kindreds. *Arch. Neurol*. **57**, 540–544.

Soong B. and Paulson H. L. B. 2007 Spinocerebellar ataxias: an update (movement disorders). *Curr. Opin. Neurol*. **20**, 438–446.

Brusco A., Gellera C., Cagnoli C., Saluto A., Castucci A., Michielotto C. *et al*. 2004 Molecular genetics of hereditary spinocerebellar ataxia: mutation analysis of spinocerebellar ataxia genes and CAG/CTG repeat expansion detection in 225 Italian families. *Arch. Neurol*. **61**, 727–733.

Chakravarty A. and Mukherjee S. C. 2002 Autosomal dominant cerebellar ataxias in ethnic Bengalees in West Bengal-an eastern Indian state. *Acta Neurol. Scand.* **105**, 202–208.

Fujigasaki H., Tardieu S., Camuzat A., Stevanin G., LeGuern E., Matsuura T. *et al*. 2002 Spinocerebellar ataxia type 10 in the French population. *Ann. Neurol*. **51**, 408–409.

Harding A. E. 1993 Clinical features and classification of inherited ataxias. *Adv. Neurol*. **61**, 1–14.

Jiang H., Tang B., Xia K., Zhou Y., Xu B., Zhao G., Li H. *et al*. 2005 Spinocerebellar ataxia type 6 in Mainland China: molecular and clinical features in four families. *J. Neurol. Sci*. **236**, 25–29.

Matsuura T., Fang P., Pearson C. E., Jayakar P., Ashizawa T., Roa B. B. *et al*. 2006 Interruptions in the expanded ATTCT repeat of spinocerebellar ataxia type 10: repeat purity as a disease modifier? *Am. J. Hum. Genet*. **78**, 125–129.

Matsuura T., Ranum L. P. W., Volpini V., Pandolfo M., Sasaki H., Tashiro K. *et al*. 2002 Spinocerebellar ataxia type 10 is rare in populations other than Mexicans. *Neurology* **58**, 983–984.

Matsuura T., Yamagata T., Burgess D. L., Rasmussen A., Grewal R. P., Watase K. *et al*. 2000 Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. *Nat. Genet*. **26**, 191–194.

Rasmussen A., Matsuura T., Ruano L., Rasmussen A., Matsuura T., Ruano L. *et al*. 2001 Clinical and genetic analysis of four Mexican families with spinocerebellar ataxia type 10. *Ann. Neurol*. **50**, 234–239.

Sasaki H., Yabe I. and Tashiro K. 2003 The hereditary spinocerebellar ataxias in Japan. *Cytogenet. Genome Res*. **100**, 198–205.

Seixas A. I., Maurer M. H., Lin M., Callahan C., Ahuja A., Matsuura T. *et al*. 2005 FXTAS, SCA10, and SCA17 in American patients with movement disorders. *Am. J. Med. Genet*. **136**, 87–89.

Sulek A., Hoffman-Zacharska D., Bednarska-Makaruk M., Szirkowiec W. and Zaremba J. 2004 Polymorphism of trinucleotide repeats in non-translated regions of SCA8 and SCA12 genes: allele distribution in a Polish control group. *J. Appl. Genet*. **45**, 101–105.

Teive H. A., Roa B. B., Raskin S., Fang P., Arruda W. O., Neto Y. C. *et al*. 2004 Clinical phenotype of Brazilian families with spinocerebellar ataxia 10. *Neurology* **63**, 1509–1512.

Xi L. and Ashizawa T. 2005 Recent progress in spinocerebellar ataxia type-10. *Cerebellum* **4**, 37–42.

Zu L., Figueroa K. P., Grewal R. and Pulst S. M. 1999 Mapping of a new autosomal dominant spinocerebellar ataxia to chromosome 22. *Am. J. Hum. Genet*. **64**, 594–599.

Received 21 March 2008, in final revised form 10 June 2008; accepted 17 June 2008
Published on the Web: 15 October 2008