Identification of Abnormal 51 CTA/CTG Expansion as Probably the Shortest Pathogenic Allele for Spinocerebellar Ataxia-8 in China

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Dear Editor,

Spinocerebellar ataxias (SCAs) are a group of genetic disorders characterized by slowly progressive incoordination of gait and are often associated with poor coordination of the hands, speech, and eye movements. Frequently, atrophy of the cerebellum occurs. The genetic forms of ataxia are diagnosed by family history, physical examination, neuroimaging, and molecular genetic testing. At present, 36 SCA subtypes including 27 pathogenic genes have been identified [1]. Different subtypes of SCAs have clear distribution differences among ethnic populations, and SCA8 is an infrequent entity worldwide, which has mostly been reported in Japanese, but has never been reported in Chinese [2]. SCA8 involves bidirectional expression based on the total number of both the (CTA)_n and (CTG)_n expansion transcripts in ATXN8OS. The pathogenesis of this disorder is complex and the spectrum of clinical presentations is broad. It is predominantly characterized by drawn-out slowness of speech and gait instability, followed by slowly progressive ataxia, with disease onset typically occurring in adulthood [3]. However, the lowest full-penetrance allele for SCA8 onset remains elusive and the current understanding of the phenotypic and genotypic features of SCA8 is limited. Since SCA8 has not yet been reported in the Chinese population and is scantily reported in a small proportion of

pedigrees so far, clinical knowledge is still developing. Moreover, the boundary between the normal and pathogenic alleles of SCA8 is uncertain. Here we report the clinical and molecular genetic characteristics of 3 Chinese SCA8 families and have identified 51 CTA/CTG repeats within ATXN8OS, probably the shortest pathogenic allele for SCA8.

In the current study, 144 unrelated patients and their family members were examined in the Department of Laboratory Medicine, West China Hospital, between October 2009 and June 2017. All patients had a clear family history and were diagnosed as having SCAs in accordance with Harding's criteria [4]. They were all eliminated from other general SCA subtypes including SCA1, SCA2, SCA3, SCA6, SCA7, SCA10, SCA12, SCA17, and Dentatorubral-pallidoluysian Atrophy (DRPLA) by mutation screening. The medical data of the SCA8 pedigrees were reviewed and are summarized in Table 1.

This study was approved by Ethics Committee of West China Hospital and informed consent was given by each participant.

After genetic screening of ATXN8OS, we identified 3 probands carrying 112, 51, and 127 discrete CTA/CTG repeats (Fig. 1 A–C, Families 1–3), while the other 141 cases had < 45 CTA/CTG repeats. We further investigated the affected family members with CTA/CTG repeats of ATXN8OS. In Family 1, the proband's father with similar symptoms had died before recruitment. In Family 2, the proband's older sister (II₂) carried the same 51 CTA/CTG repeats. In Family 3, the proband's uncle (II₁) and brother (III₁) carried 125 CTA/CTG repeats, indicating that the length of CTA/CTG expansion transcripts was unstable in this pedigree. Through comparative analysis, the 51 CTA/CTG repeats detected in Family 2 were identified as

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Table 1 Clinical characteristics of the three SCA8 pedigrees.

Affected subject	Family 1	Family 2		Family 3		
	II ₂	II ₂	II ₄	II ₁	III ₁	III ₄
Age at onset (years)	34	45	43	50	47	41
(CTA/CTG)n in ATXN8OS	22/112	31/51	31/51	27/125	32/125	22/127
Onset symptom	Gait disability	Gait disability	Gait disability	Gait disability	Gait disability	Gait disability
Dysarthria	Yes	Yes	Yes	No	No	No
Dysphagia	No	No	No	No	No	No
Muscle force	Normal	Normal	Normal	Normal	Normal	Normal
Muscular tension	Normal	Normal	Normal	Normal	Normal	Normal
Muscular reflex	Normal	Hyper-	Hyper-	Hypo-	Hypo-	Hypo-
Radius reflex	Hypo-	Normal	Hyper-	Normal	Normal	Hypo-
Knee reflex	Normal	Normal	Normal	Hypo-	Hypo-	Hypo-
Ankle reflex	Absence	Absence	Normal	Normal	Hypo-	Hypo-
Patella clonus	No	No	No	No	No	No
Ankle clonus	No	Yes	Yes	Yes	No	No
Babinski sign	Abnormal	Abnormal or Normal	Abnormal	Normal	Normal	Normal
Nystagmus	Yes	Yes	Yes	No	No	No
Cerebellar ataxia	Noted	Noted	Noted	Noted	Noted	Noted
Cognitive decline	Yes	Yes	Yes	Yes	Yes	Yes
Action tremor	No	No	No	Yes	Yes	Yes
MRI	Cerebellar and brainstem atrophy	Cerebellar atrophy	Cerebellar, pontine and oblongata atrophy	Not available	Cerebellar atrophy and leukodystrophy	Leukodystrophic demyelination change

possibly the shortest pathogenic allele for SCA8. The proband (II₂) of Family 1 displayed gait disability and weakness of the lower limbs when she was 34, and her symptoms gradually increased over the past four years with repeated attacks of dizziness and visual rotation, which resolved by themselves. Neurological examinations revealed reduced deep tendon reflexes and the Babinski sign was abnormal bilaterally. A brain MRI performed when the proband was 37 showed cerebellar and brainstem atrophy. Her father, with similar symptoms, passed away ~ 15 years after onset. In Family 2, the 44-year-old proband (II₄) showed evident cerebellar ataxia-like dysarthria and gait instability more than a year ago. Since then, the symptoms have gradually increased; his ankle reflex and Babinski sign were abnormal bilaterally. Moreover, both eyes had nystagmus and limb tendon reflex symmetry was hyperactive on recent inspection. The brain MRI showed prototypical cerebellar, pontine, and

oblongata atrophy. His mother (I₂) with similar symptoms died 7 years after onset, and his older sister (II₂) had been paralyzed for 3 years because of cerebellar ataxia and hyperactive muscular reflexes. However, unlike the proband, II₂ had a normal radius reflex and no ankle reflex, while her brain MRI showed cerebellar atrophy. In Family 3, the 43-year-old proband (III₄) displayed a 7-month history of limb tremor and intention tremor but a normal gait. Further neurological examination showed an attenuated tendon reflex, hypalgesia and thermohypesthesia, and loss of vibratory sense at the ankle and knee, as well as lower-limb numbness. These electrophysiological manifestations indicated peripheral nerve injury and damage to motor sensory fibers. The brain MRI showed severe leukodystrophic demyelination changes without distinct cerebellar atrophy. His mother (II₄) had died before abnormal pathological manifestations appeared, but his uncle (II₁) and brother (III₁) had analogous neuromuscular

Fig. 1 **A–C** Pedigrees of 3 SCA8 families. Squares, males; circles, females; filled squares, affected males; filled circles, affected females; diagonal lines across symbols, deceased; triangles, propends. Numbers under the symbols indicate the number of CTA/CTG repeats within ATXN8OS. **D** Brain MRI images of the proband of Family 3. Axial and coronal T2-weighted MRIs showing fractional leukodystrophic demyelination changes.

symptoms. Especially II₁ developed gait ataxia at age 52 and also had segmental hypofunction of deep tendon reflexes, while III₁ had intermittent gait disability but his symptoms developed slowly in the past 5 years. The brain MRI showed slight cerebellar atrophy and leukodystrophy.

Considering the rarity of SCA8, corroboration of CTA/CTG repeats within ATXN8OS has only been implemented in a tiny minority of cases. The lowest full-penetrance allele of CTA/CTG repeats for SCA8 awaits to be determined. It is universally recognized that the pathological mutation alleles for SCA8 are > 80 CTA/CTG repeats within ATXN8OS [5, 6]. It is not yet clear whether repeat sizes ranging from 50 to 70 repeats are pathogenic. Moreover, repeat lengths ranging from 71 to > 1300 repeats have been found both in individuals who develop ataxia and in those who do not [7]. A recent report has revealed that 71 triplets is the shortest pathogenic-expanded allele of ATXN8OS [8]. In the current study, we reported a pedigree (Family 2) who presented with typical symptoms of SCA8 and carried 51 CTA/CTG repeats within ATXN8OS. This pathological mutation

allele of 51 CTA/CTG repeats is compactly relevant to the phenotypes of patients, suggesting that this is responsible for the disease onset in this pedigree. Therefore, we concluded that 51 CTA/CTG repeats of ATXN8OS is probably the shortest pathogenic allele for SCA8. In general, the common initial symptoms of SCA8 are scanning dysarthria with a characteristic drawn-out slowness of speech and gait instability. In our study, Family 1 and Family 2 initially exhibited gait ataxia and explicit dysarthria, but only Family 3 initially presented with typical symptoms of action tremor without dysarthria and the Babinski sign. In addition to these, compared to the late development of action nystagmus in Families 1 and 2, Family 3 have not yet presented the typical symptoms during the disease course. Notably, the proband of Family 3 and his brother both had fractional leukodystrophic demyelination changes (Fig. 1D), which differs significantly from other types of SCA. In conclusion, the initial symptoms were not universal among all SCA8 patients, signifying that SCA8 phenotypes can be heterogeneous, and inter-familial diversity should be taken into

consideration. This phenomenon further implied that a lack of dysarthria, nystagmus and the Babinski sign is insufficient to exclude a diagnosis of SCA8. In addition, all the patients in this study suffered from cognitive decline during the course of the disease, but none experienced dysphagia and abnormal muscular tension, suggesting that dysphagia and muscular tension are rare signs of SCA8. These clinical signs differ to some degree compared with the previous reports of SCA8 patients from Japan, Brazil, and Finland [9–11].

SCA8 is a relatively uncommon, slowly progressive ataxia with an estimated worldwide prevalence of 1:100 000 and only a few SCA8 pedigrees have been specifically documented. In addition to several Japanese cases on account of a founder effect [2, 9], SCA8 cases have only been detected in 2 Brazilian [10, 11], 15 Finnish [8], and 8 Czech pedigrees [5]. Our study is the first report of SCA8 in the Han Chinese population, presenting clinical and genetic analysis of 3 Chinese SCA8 pedigrees. This implies that the incidence of SCA8 might not be as rare in China as previously assumed. In addition, our results indicated that 51 CTA/CTG repeats within ATXN8OS is probably the shortest pathogenic allele for SCA8. Therefore, the trinucleotide repeat expansion mutation of ATXN8OS is worthy of investigation in those who have been excluded from other general SCA subtypes including SCA1, 2, 3, 6, 7, and DRPLA.

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References

1. Muso Z, Sedlacek Z, Mazanec R, Klempir J, Roth J, Plevova P, *et al.* Spinocerebellar ataxias type 8, 12, and 17 and

dentatorubro–pallidoluysian atrophy in Czech ataxic patients. *Cerebellum* 2013, 12:155–161.

2. Ikeda Y, Dalton JC, Moseley ML, Gardner KL, Bird TD, Ashizawa T, *et al.* Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. *Am J Hum Genet* 2004, 75: 3–16.
3. Mariotti C, Di Donato S. Cerebellar/spinocerebellar syndromes. *Neurol Sci* 2001, 22 Suppl 2: S88–S92.
4. Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, *et al.* An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat Genet* 1999, 21: 379–384.
5. Silveira I, Alonso I, Guimaraes L, Mendonca P, Santos C, Maciel P, *et al.* High germinal instability of the (CTG)_n at the SCA8 locus of both expanded and normal alleles. *Am J Hum Genet* 2000, 66: 830–840.
6. Moseley ML, Zu T, Ikeda Y, Gao W, Mosemiller AK, Daughters RS, *et al.* Bidirectional expression of CUG and CAG expansion transcripts and intranuclear polyglutamine inclusions in spinocerebellar ataxia type 8. *Nat Genet* 2006, 38: 758–769.
7. Moseley ML, Schut LJ, Bird TD, Koob MD, Day JW, Ranum LP. SCA8 CTG repeat: en masse contractions in sperm and intergenerational sequence changes may play a role in reduced penetrance. *Hum Mol Genet* 2000, 9: 2125–2130.
8. Musova Z, Sedlacek Z, Mazanec R, Klempir J, Roth J, Plevova P, *et al.* Spinocerebellar ataxias type 8, 12, and 17 and dentatorubro–pallidoluysian atrophy in Czech ataxic patients. *Cerebellum* 2013, 12: 155–161.
9. Munhoz RP, Teive HA, Raskin S, Werneck LC. CTA/CTG expansions at the SCA 8 locus in multiple system atrophy. *Clin Neurol Neurosurg* 2009, 111: 208–210.
10. Cintra VP, Lourenço CM, Rocha MM, Tomaselli PJ, Marques W. Analysis of a fully penetrant spinocerebellar ataxia type 8 Brazilian family. *Acta Neurol Scand* 2017, 136: 541–545.
11. Juvonen V, Hietala M, Päiväranta M, Rantamäki M, Hakamies L, Kaakkola S, *et al.* Clinical and genetic findings in Finnish ataxia patients with the spinocerebellar ataxia 8 repeat expansion. *Ann Neurol* 2000, 48: 354–361.