Spinocerebellar ataxia type 27 (SCA27) is an uncommon cause of dominant ataxia among Chinese Han population

Zhao Chen,1 Xiaohui Li,1 Beisha Tang,1 Junling Wang,2 Yuting Shi,2 Zhanfang Sun,2 Li Zhang,3 Qian Pan,2 Kun Xia,2 Hong Jiang1,2,∗

1 Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, PR China
2 Neurodegenerative Disorders Research Center, Central South University, Changsha, Hunan 410008, PR China
3 State Key Laboratory of Medical Genetics, Central South University, Changsha, Hunan 410078, PR China

HIGHLIGHTS
▶ Mutations in FGF14 gene were screened in 67 unrelated Chinese Han probands with ADCA by DHPLC and DNA direct sequencing.
▶ We found a pair of siblings carried the same heterozygous variation (c.-10delC) characterized by different clinical features.
▶ A probable novel insertion/deletion (I/D) polymorphism (c.-10delC) was found.
▶ It suggests that SCA27 is a rare subtype in China.

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ABSTRACT
Autosomal dominant cerebellar ataxias (ADCA), genetically classified into spinocerebellar ataxias (SCAs), are a highly heterogeneous group of neurodegenerative disorders. Recently, mutations in the fibroblast growth factor 14 gene (FGF14) have been reported to cause SCA27 subtype. To evaluate the frequency of FGF14 mutations in mainland China, we performed molecular genetic analysis in 67 unrelated familial ataxia cases and 500 normal controls by denaturing high-performance liquid chromatography (DHPLC) and DNA direct sequencing. Interestingly, we found a pair of siblings carried the same heterozygous variation (c.-10delC) characterized by different clinical features, which is probably a novel insertion/deletion (I/D) polymorphism in the 5′ UTR region of the exon 1b. It suggests that SCA27 is a rare subtype in China.

1. Introduction

Autosomal dominant cerebellar ataxias (ADCA), genetically classified into spinocerebellar ataxias (SCA), are a group of highly heterogeneous neurodegenerative disorders characterized by progressive cerebellar symptoms of imbalance, progressive gait and limb ataxia, and dysarthria [3,6]. Till now, 33 distinct genetic subtypes have been defined, and 19 seemingly unrelated disease genes have been identified (http://neuromuscular.wustl.edu/ataxia/domatax.html). The disease is caused by trinucleotide or polynucleotide repeat expansions within the coding region or non-coding region of the corresponding gene (SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36 and dentatorubral-pallidoluysian atrophy [DRPLA]), and non-repeat mutations (SCA types 5, 11, 13, 14, 15/16/29, 23, 27, 28, 35), with the heterogeneity of the pathogenic mechanisms leading to cerebellar degeneration and ataxia [12].

Among nine SCA causative genes involved in non-repeat mutations, FGF14 is responsible for SCA27, which encodes a member of a subclass of fibroblast growth factors (FGF), and was reported to be expressed in developing and adult central nervous system [7]. The FGF14 gene is located at chromosome 13q34 and composed of five exons, including alternatively spliced transcript variants differing in exon 1 [11]. In 2003, van Swieten described a large three-generation Dutch family with a novel form of early-onset tremor, dyskinesia and slowly progressive cerebellar ataxia (assigned as SCA27) (OMIM ID: 609307), associated with a novel mutation of the FGF14 gene [10]. Till now, only a heterozygous mutation (F1455 mutation) and a frameshift mutation (p.Asp163fsX12) in the FGF14 gene have been reported in Dutch and Germany, while no other pathogenic DNA variations were identified worldwide [2,8,10,14].

To further define the frequency of FGF14 mutations in Chinese mainland patients, we examined the coding region of FGF14 in 67 unrelated probands with familial history diagnosed as ADCA by means of DHPLC and DNA direct sequencing.
2. Materials and methods

2.1. Subjects

67 unrelated affected individuals with ADCA enrolled from the outpatient neurology clinics of Xiangya Hospital, Central South University from January 1995 to March 2011, were previously excluded for mutations on SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 35 and DRPLA gene. The clinical diagnosis of SCA was made based on the criteria proposed by Harding [4]. The subjects consisted of 39 men and 28 women, for which the clinical data could be procured as follows: mean age: 39.08 ± 14.14 years (range 12–71 years); mean age at onset of the first neurological symptoms related to ataxia: 34.17 ± 13.66 years (range 12–69 years); mean course: 4.61 ± 5.54 (range 4 months–40 years). A total of 500 healthy Chinese individuals were recruited as a control group. Informed consent was obtained from all patients and controls for participation in the study.

2.2. Methods

Genomic DNA was extracted from peripheral blood leukocytes by standard extraction methods. All exons (1a, 1b, 2, 3, 4 and 5) of FGF14 were amplified via primer and conditions published before [10]. PCR product screening was performed using a DHPLC method (WAVE DNA fragment analysis system, Transgenomic). Each PCR sample of the patients was mixed with normal control identified by direct sequencing. The mixture were denatured at 95 °C for 5 min and gradually re-annealed to 25 °C before DHPLC analysis. Two different melting temperatures were chosen by WaveMaker software for each PCR fragment in the process of DHPLC analysis (64.1 and 66.1 °C for exon 1a; 56.5 and 57.5 °C for exon 1b; 54.2 and 55.2 °C for exon 2; 54.6 and 55.6 °C for exon 3; 55.0 and 56.0 °C for exon 4; 58.4 and 59.4 °C for exon 5). Samples revealing abnormal peaks were sequenced directly.

3. Results

Mutations in FGF14 were screened in 67 unrelated Chinese Han patients who were clinically diagnosed as ADCA. A deletion variation (c.-10delC) in the 5′UTR region of the exon 1b was found in one proband using DHPLC analysis and direct sequencing. The sample of the patient showed an abnormal elution profile, with the further evidence supported by DNA sequencing (Figs. 1–3). In the two-generations family, the proband(II-1) was a 61-year-old male suffered from progressive gait unsteadiness accompanied by increasing muscle weakness in the upper limbs since 38 years old and presented with limb paralysis 23 years later with remarkable cerebellar symptoms. Upon neurological examination at age 61, he showed severe speech disturbance, dysarthria, oculomotor disorders and mental retardation, with muscle atrophy in thenars observed. Also, tendon hyperreflexia and Babinski's sign were found. The International Ataxia Cooperative Rating Scale (ICARS), Scale for the Assessment and Rating of Ataxia (SARA) and Mini-Mental State Examination (MMSE) were 90, 39 and 18 respectively. The MRI of the brain indicated the moderate and mild atrophy in the cerebellum. Moreover, his father(I-1), 50 years old at onset,
presented with similar clinical features and died at age 64. In addition, other members of the family, including the eldest brother at age 58 and the youngest sister at age 52, had no clinical symptoms of ataxia (Table 1). Mutations in FGF14 were also screened in other members of the family and 500 control individuals by direct sequencing. Interestingly, the younger healthy brother (II-2) of the proband carried the same heterozygotic variation of c.10delC as the proband, which was not found in 500 healthy controls (Fig. 3).

4. Discussion

The DHPLC analysis, based on chromatography and able to distinguish between homoduplexes and heteroduplexes [5,1], is increasingly used in mutation analyses of hereditary diseases. Compared with direct sequencing, it can be performed more easily and quickly, with the sensitivity of nearly 100%. Furthermore, the DHPLC technology can be automated and enables high-throughput analysis.

As we mentioned above, the variation of genotype is present in the proband as well as one of younger brother (II-2) without any clinical symptoms, which is probably a rare novel I/D polymorphism with low frequency rather than a pathogenic mutation. Although we did not find the same variation in 500 healthy controls, the heterozygote of c.10delC variation may be detected by further screening on more healthy individuals. Further functional studies on mutation spectrum will provide more evidence to define the variation as polymorphism or causative mutation. In addition, another reason accounting for the result of our research is that the younger brother of the proband (II-2) with c.10delC may be a patient on preclinical stage with incomplete penetrance. Follow-up clinical observations on the individual are necessary, though the latter condition we think is relative small.

In summary, compared to SCA3, the most common subtype of SCA in Chinese [9,13], the evidence through our present study demonstrates that SCA27 caused by FGF14 mutation is rare in Chinese SCA patients. We found a probable I/D polymorphism (c.10delC) in the 5‘UTR region of the exon 1b, which need evidence based on further functional study. It is suggested that patients with ADCAs in China, especially with the similar features of SCA27 such as tremor, dyskinesia, psychiatric episodes, cognitive defect and excluded from the routine gene mutation, are potential candidates for FGF14 screening by way of the DHPLC method and DNA sequencing. Moreover, a combined strategy of the whole-exome sequencing and linkage analysis can be used to identify novel causative genes in future [12], which gain new insights into identification of new form of SCA subtypes.

Conflicts of interest

The authors have no actual or potential conflicts of interest to report.

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