Spinocerebellar ataxia type 28 (SCA28) is an uncommon cause of dominant ataxia among Chinese kindreds

Dandan Jia, Beisha Tang, Zhao Chen, Yuting Shi, Zhanfang Sun, Li Zhang, Junling Wang, Kun Xia, Hong Jiang

Abstract

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily affecting the cerebellum. Nearly 33 genetically distinct subtypes have been defined, and 19 seemingly unrelated disease genes have been identified so far. Recently, mutations in the ATPase family gene 3-like 2 (AFG3L2) gene were presented to cause SCA28 subtype. In order to define the frequency of SCA28 mutation in Chinese mainland, we performed molecular genetic analysis in 67 unrelated affected individuals with ADCA. At last, we did not find AFG3L2 gene mutation, except for three known SNPs. It suggests that SCA28 subtype is very rare in Chinese mainland.
Spinocerebellar ataxia type 28 (SCA28) is an uncommon cause of dominant ataxia among Chinese kindreds

Dandan Jia¹, Beisha Tang¹,²,³, Zhao Chen¹, Yuting Shi¹, Zhanfang Sun¹, Li Zhang¹, Junling Wang¹, Kun Xia², Hong Jiang¹,²,³*

¹ Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China
² National Key Lab of Medical Genetics of China, Changsha, Hunan, China
³ Neurodegenerative Disorders Research Center, Central South University, Changsha, Hunan, China

*Corresponding author should be addressed to: Hong Jiang, Department of neurology, Xiangya Hospital, Central South University, 87# Xiangya Road, Changsha, Hunan 410008, P. R. China.
E-mail: jianghong73868@yahoo.com.cn
Tel:+86-731-84327216 (office)
Cell:+86-13975806840
Fax:+86-731-84327332

Running head: Spinocerebellar ataxia type 28 is rare in China.
Abstract

Autosomal dominant cerebellar ataxias (ADCAs) are a clinically and genetically heterogeneous group of neurodegenerative disorders primarily affecting the cerebellum. Nearly 33 genetically distinct subtypes have been defined, and 19 seemingly unrelated disease genes have been identified so far. Recently, mutations in the ATPase family gene 3-like 2 (AFG3L2) gene were presented to cause SCA28 subtype. In order to define the frequency of SCA28 mutation in Chinese mainland, we performed molecular genetic analysis in 67 unrelated affected individuals with ADCA. At last, we did not find AFG3L2 gene mutation, except for three known SNPs. It suggests that SCA28 subtype is very rare in Chinese mainland.

Keywords: ADCA; SCA28; AFG3L2 gene; mutation analysis; SNPs

Introduction

Autosomal dominant cerebellar ataxias (ADCAs), alternatively called spinocerebellar ataxias (SCAs), are a group of clinically and genetically heterogeneous neurodegenerative disorders primarily characterized by imbalance, progressive gait and limb ataxia, and dysarthria \[^{1,2}\]. At present, 33 distinct genetic subtypes have been defined, and 19 seemingly unrelated disease genes have been identified (http://neuromuscular.wustl.edu/ataxia/domatax.html). In eleven types of ataxia, the disease is caused by trinucleotide CAG repeat expansion within the
coding region of the corresponding gene (SCA types 1, 2, 3, 6, 7 and 17, and dentatorubral-pallidoluysian atrophy [DRPLA]) or repeats falling outside the coding region (SCA types 8, 10, 12 and 31, 36) in genes whose function are still largely unknown\(^3\). In recent years, a group of SCAs have emerged that are caused by non-repeat mutations in specific genes (SCA types 5, 11, 13, 14, 15/16/29, 23, 27, 35). The distinct functions of these disease genes have revealed the complex heterogeneity of the pathogenic mechanisms leading to cerebellar degeneration and ataxia.

In 2006, the researchers of Milano, Italy had mapped a previously unidentified SCA locus (SCA28) on chromosome 18p11.22–q11.2 in a four-generation Italian family with a novel form of juvenile-onset, slowly progressive, autosomal dominant cerebellar ataxia, and after 2 years, they have found SCA28 is associated with mutations in the \(AFG3L2\) gene (ATPase family gene 3-like 2) which encodes the mitochondrial metalloprotease \(AFG3L2\). Overall, they have found at least six different missense mutations in the \(AFG3L2\) gene in eight families. Affected individuals show slowly progressive gait and limb ataxia, dysarthria, hyperreflexia at lower limbs, nystagmus and ophthalmoparesis. Onset was reported to start at juvenile age (mean age: 27 years), and no evidence of anticipation between generations was observed\(^4,5\). In 2010, a group of 140 unrelated patients with a familial history was screened in exons 15 and 16 of \(AFG3L2\) gene using an SSCP approach in Germany, a novel missense mutation of
p.E700K(c.2098G>A) was detected in a four-generation German family presented with early-onset dominant cerebellar ataxia and slowly progressive phenotype[6].

No dominant ataxia has thus far been associated with mitochondrial dysfunction except SCA28. The AFG3L2 gene is composed of 17 coding exons. The ubiquitously expressed AFG3L2 is highly homologous to paraplegin, a cognate mitochondrial protease, the loss of which causes a distinct neurodegenerative disorder, the recessively inherited form of hereditary spastic paraplegia SPG7. AFG3L2 and paraplegin form hetero-oligomeric paraplegin-AFG3L2 and homo-oligomeric AFG3L2 complexes in the inner mitochondrial membrane, named m-AAA proteases. These complexes ensure protein quality control in the inner membrane, jointly with a chaperone-like activity on the respiratory chain complexes [7,8].

To evaluate the frequency of AFG3L2 mutations in patients in mainland China, we examined the coding sequence and intron–exon boundaries of AFG3L2 in 67 unrelated familial cases (probandz) diagnosed with ADCA using PCR and direct sequencing. With respect to the clustering of the described missense mutations of AFG3L2, we analyzed the cases in exons 15 and 16 especially.

Subjects and materials

Mutation analysis of the AFG3L2 gene was carried out in 67 unrelated affected individuals with ADCA who were previously excluded for mutations
on SCA1, 2, 3, 6, 7, 8, 10, 12, 17 and DRPLA gene. All of them were Chinese Han, recruited from the outpatient neurology clinics of Xiangya Hospital, Central South University from January 1995 to March 2011. The clinical diagnosis of SCA was made based on the criteria proposed by Harding and Deufel [9]. The subjects consisted of 38 men and 29 women, for which the clinical data could be obtained as follows: mean age: 39.07 ± 14.12 years (range 12–71 years); mean age at onset of the first neurological symptoms related to ataxia: 34.45 ± 13.12 years (range 12–69 years); mean course: 4.61 ± 5.56 years (range 4 months–40 years). Informed consent was obtained from all subjects before participation in the study. This study also got prior approval by the institutional review board and ethics committee of Xiangya Hospital affiliated to Central South University.

Methods

Genomic DNA was extracted from peripheral blood leukocytes by standard extraction methods. The 17 coding exons of the AFG3L2 gene were PCR amplified using primer and conditions reported by Taroni[10]. Amplification products were purified and subjected to sequence analysis on an automated DNA sequencer (Applied Biosystems) and compared to the normal control sequences.

Results

We screened for the AFG3L2 mutations in 67 unrelated Chinese Han patients who were clinically diagnosed as ADCA (The mutations in SCA1, 2, 3,
6, 7, 8, 10, 12, 17 and DRPLA had been excluded). As SCA28 was described as a pure cerebellar syndrome and classified as ADCAIII, 36 out of 67 probands in our study showed pure cerebellar syndrome, while the rest had additional extra-cerebellar symptoms.

There was no disease-related mutation identified in \textit{AFG3L2} in all analyzed DNA samples, except for three SNPs including NM\_006796.2:c.1389G>A, NM\_006796.2:c.1650A>G and NM\_006796.2:c.*2G>T, which had previously been enrolled in dbSNP database. (Table 1).

**Discussions**

Several reasons may lead to the result of our study, suggesting as follows: (1) SCA28 caused by \textit{AFG3L2} mutations is rare in Chinese, as none of the 67 patients analyzed in our present study belongs to this disease while these 67 patients were from 430 unrelated probands with ADCA. (We will further screen unassigned SCA patients for abnormalities in the \textit{AFG3L2} gene in our future work); (2) We might leave out the mutations that existed in introns of the gene or regulatory regions; (3) The possible mutation might be from gene rearrangement (But we think the possibilities of (2) and (3) are relatively small); (4) The differences of race and geography lead to the differences of phenotype distribution.

In summary, while SCA3 is the most common type of SCA in mainland China as we reported before\cite{11}, we provided the evidence through our present study that SCA28 is a rather rare genotype in mainland Chinese patients with
SCA. We suggest that an increasing number of patients with dominantly
inherited ataxia in China, especially with the similar features with SCA28 and
excluded from the common gene mutations, are potential candidates for
screening mutation of AFG3L2 gene by means of direct sequencing. Moreover,
the whole-exome sequencing may be used to identify novel causative genes or
novel mutations of known genes in future, which gain new insights into
identification of unknown form of SCA subtypes [12].

Conclusions:

We did not find AFG3L2 gene mutation in 67 unrelated affected individuals
with ADCA, except for three known SNPs. It suggests that SCA28 subtype is
very rare in Chinese mainland.

Acknowledgments

We are grateful to the participating patients for their involvement. This study was
Supported by National Basic Research Program (973 Program) (No.
2012CB944600, 2012CB517902, 2011CB510002, to Hong Jiang), New Century
Excellent Talents in University (No. NCET-10-0836, to Hong Jiang), National
Natural Science Foundation of China (No. 30971585, 30871354, 30710303061,
30400262, to Hong Jiang), and the Key Project of Hunan Natural Science
Foundation (No. 08JJ3048, to Hong Jiang).

Reference


Table 1  Three SNPs identified by direct sequencing of AFG3L2 gene
<table>
<thead>
<tr>
<th>HGVS Names</th>
<th>Position in chromosome</th>
<th>Position in exon</th>
<th>refSNP ID</th>
<th>Rate of SNPs in our study Hete (%)</th>
<th>Homo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM_006796.2:c.1389G&gt;A</td>
<td>chr18:12351342</td>
<td>exon 11</td>
<td>rs11080572</td>
<td>59.70</td>
<td>22.39</td>
</tr>
<tr>
<td>NM_006796.2:c.1650A&gt;G</td>
<td>chr18:12348285</td>
<td>exon 13</td>
<td>rs11553521</td>
<td>53.73</td>
<td>32.84</td>
</tr>
<tr>
<td>NM_006796.2:c.*2G&gt;T</td>
<td>chr18:12329562</td>
<td>3’-UTR</td>
<td>rs113981080</td>
<td>56.72</td>
<td>0</td>
</tr>
</tbody>
</table>

a: Rate of heterozygous changes in our study (Hete)

b: Rate of homozygous changes in our study (Homo)