Spinocerebellar ataxia type 23 is an uncommon SCA subtype in the Chinese Han population

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HIGHLIGHTS

► Mutations in \textit{PDYN} gene were firstly screened in 305 unrelated Chinese Han ataxia patients by PCR and DNA direct sequencing.
► A probable novel single nucleotide polymorphism (c.255G>A, p.Lys85Lys) was found.
► It suggests that SCA23 is a rare subtype in mainland China.

ABSTRACT

The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of neurodegenerative diseases. In 2010, four missense mutations in the \textit{prodymorphin} (\textit{PDYN}) gene were found in two families and two sporadic cases of SCA type 23 (SCA23) from the Netherlands. In addition, one missense mutation in \textit{PDYN} was also found in one sporadic SCA23 case in America in 2012. To date, there have been no reports of \textit{PDYN} gene mutations in mainland China. To investigate the frequency of SCA23 among the Chinese Han population, we performed polymerase chain reaction (PCR) and DNA direct sequencing of the \textit{PDYN} gene in 305 unrelated ataxia patients. Although no SCA23 mutation was identified, one novel single nucleotide polymorphism (c.255G>A, p.Lys85Lys) in exon 4 of the \textit{PDYN} gene was found. This suggests that SCA23 is a rare form of dominant ataxia in the Chinese Han population.

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1. Introduction

The spinocerebellar ataxias (SCAs) are a heterogeneous group of inherited neurodegenerative disorders that affect the cerebellum and its afferent and efferent pathways. They are characterized by progressive cerebellar ataxia of gait and limbs, variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, and many additional symptoms [5]. To date, classical genetic studies have revealed 33 distinct genetic forms of spinocerebellar ataxias, but only 20 causative genes have been identified (http://neuromuscular.wustl.edu/ataxia/aindex.html). SCA3/MJD is the most common SCA subtype, with an approximate frequency of 62.08% [25]. The existing mutation patterns of SCA include both trinucleotide and polynucleotide repeat expansions, including SCA1-3, 6-8, 10, 12, 17, 31, 36 and dentatorubral-pallidoluysian atrophy (DRPLA), and conventional mutations such as deletions, duplication, missense, nonsense or frameshift mutations in the corresponding genes, including SCAs 5, 11, 13, 14, 15/16/29, 23, 27, 28 and 35 [5,14,4,12,15,25,23,16,27].

Among the nine SCA-causative genes that exhibit conventional mutations, \textit{PDYN} is responsible for SCA23. Verbeek et al. described a three-generation Dutch SCA pedigree in 2004. Through linkage analysis, these authors mapped the disease gene to chromosome 20p13-p12.3 and designated this locus as SCA23 [21]. In 2010, Bakalkin et al. identified \textit{PDYN} (GenBank ID: NM024411.3) as the causative gene of SCA23 and found four missense mutations in two ADCA families and two sporadic ataxia cases from the Netherlands [1]. In addition, one sporadic case of SCA23 with a \textit{PDYN} mutation was found in America [6].

Aside from the above reports, there have been no positive findings for SCA23 in other countries, ethnic groups, or populations. In our previous work, we performed series of genes mutation detection studies to evaluate SCA subtypes in 709 unrelated Chinese SCA patients (457 probands with familial history and 252 patients with...
and sporadic ataxia) [19,8–10,25,22–24,29,30,31,2], which indicated an unidentified genetic etiology for approximately 43% (305/709) of our SCA patients, which comprised 26.7% (122/457) familial cases and 72.6% (183/252) seemingly sporadic cases. To explore whether SCA23 mutation is present in mainland Chinese SCA patients, we analyzed the coding sequence of PDYN gene in these 305 probands by PCR and DNA direct sequencing.

2. Materials and methods

2.1. Human subjects

The diagnoses in all 305 SCA patients were determined based on the Harding diagnostic criteria [7]. All patients were Chinese Han, recruited from the outpatient neurology clinics of Xiangya Hospital of Central South University from April 1994 to March 2011. Mutation analysis of the PDYN gene was carried out in patients who were previously found not to have causative gene mutations in SCA1-3, 5-8, 10-14, 15/16/29, 17, 27, 28, 31, 35 or DRPLA. This cohort consisted of 122 (40%) familial cases and 183 (60%) seemingly sporadic cases, including 180 men and 125 women, with clinical data as follows: mean age: 41.39 ± 14.89 (range 4–72 years); mean age at onset of the first neurological symptoms related to ataxia: 36.50 ± 15.74 (range 2–69 years); mean course: 4.76 ± 5.31 (range 1 month–40 years). A total of 500 healthy Chinese Han individuals were recruited as a control group. Informed consent was obtained from all participants in the study, as approved by the Ethical Committee of Xiangya Hospital of the Central South University in China.

2.2. PCR and PDYN sequencing

DNA was extracted from peripheral blood leukocytes using standard extraction methods. The genomic DNA of all participating subjects in this study was used to amplify coding exons 3 and 4 of the PDYN gene by PCR. PCR primers were designed as previously described by Bakalkin et al. [1]. The primer sequences and PCR conditions are shown in Table 1. The PCR products were screened for mutations via Sanger sequencing on an ABI 3730xl DNA Analyzer (Applied Biosystems).

3. Results

We screened for PDYN mutations in 305 unrelated affected Chinese Han individuals who were clinically diagnosed with SCAs. None of the analyzed samples carried mutations in the PDYN coding region. However, we found a synonymous nucleotide polymorphism (c.255G>A) in exon 4 of the PDYN gene (p.Lys85Lys) (Figs. 1 and 2). This SNP is not reported in the 1000 genomes database (http://www.1000genomes.org/ensemble-browser) and was not found in 500 healthy controls, suggesting that it is a novel SNP. In the two-generation family, the proband (II-2) was a 56-year-old male who carried the single nucleotide polymorphism (c.255G>A) in exon 4 of the PDYN gene and suffered from progressive gait unsteadiness accompanied by occasional dizziness since age 53 with marked cerebellar signs. Upon neurological examination at age 56, he showed tendon hyperreflexia and was positive for Romberg’s sign and Babinski’s sign. The patient’s International Ataxia Cooperative Rating Scale (ICARS) and Scale for the Assessment and Rating of Ataxia (SARA) scores were 7 and 3, respectively. Brain MRI revealed moderate atrophy in the cerebellum. Moreover, the patient’s mother (I-2), presented with similar clinical features at age 50 and died at age 70. No other members of the family showed clinical symptoms of ataxia (Table 2).

4. Discussion

The PDYN gene (OMIM ID: 131340) is located on chromosome 20p13, and PDYN is the precursor protein for the opioid neuropeptides α- and δ-endorphin, Dyn A, and Dyn B, which are ligands of the κ-opioid receptor [1]. PDYN in humans contains four exons (1–4) and three introns. However, the entire coding sequence is contained in exons 3 and 4 [18]. Genetic studies have analyzed associations between polymorphisms in PDYN and both cocaine and opioid addiction [3], heroin dependence [26], episodic memory in the elderly [13], temporal lobe epilepsy [11], and alcohol dependence [20]. Numerous studies have shown that the functions of dynorphins are related to learning and memory, emotional control, stress response and pain. Dynorphins and kappa opioid receptor-related pathophysiological mechanisms may be involved in diseases including epilepsy, addiction, depression and schizophrenia [18].

Table 1

<table>
<thead>
<tr>
<th>Exons</th>
<th>Sequence (5’→3’)</th>
<th>T_m (°C)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Forward: CCTGTCGGCAGGAGTTAGAG&lt;br&gt;Reverse: GCCTCTATAGGCGAGGACA</td>
<td>60</td>
<td>397</td>
</tr>
<tr>
<td>4-1</td>
<td>Forward: TACGCTTCCTCATTTTG&lt;br&gt;Reverse: TCTGGCTTCTTGGGTGA</td>
<td>56</td>
<td>545</td>
</tr>
<tr>
<td>4-2</td>
<td>Forward: GGGCATCTTACCTCTGCTGA&lt;br&gt;Reverse: ACCCTTCCCCATCATCACAC</td>
<td>56</td>
<td>484</td>
</tr>
</tbody>
</table>

T_m: annealing temperature.

Table 2

<table>
<thead>
<tr>
<th>Family</th>
<th>I:1</th>
<th>I:2</th>
<th>II:1</th>
<th>II:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>73</td>
<td>Deceased</td>
<td>58</td>
<td>56</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>–</td>
<td>50</td>
<td>–</td>
<td>53</td>
</tr>
<tr>
<td>Gait ataxia</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>Limb ataxia</td>
<td>–</td>
<td>++</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Dysarthria</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Oculomotor impairment</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tremor</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Romberg test</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hyporeflexia</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hyperreflexia</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Extrapyramidal signs</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ICARS</td>
<td>1</td>
<td>ND</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>SARA</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Clinical signs are graded as follows: (−) absent; (+) mild; (++) moderate; (+++) severe; (ND) not determined.
The main clinical features of SCA23 were described as late onset, relatively slowly progression, and isolated cerebellar ataxia, which present as either dominantly inherited familial cases or sporadic cases. Bakalkin et al. identified four missense mutations in PDYN: R138S and R215C (OMIM ID: 131340.0001, 131340.0002) in two SCA23 families and L211S and R212W (OMIM ID: 131340.0003, 131340.0004) in two sporadic cases. These findings indicated that SCA23 is a rare cause of SCA (0.5%) in the Dutch population [1]. Moreover, Schicks et al. screened 314 German patients with dominant ataxia in 2011 and found no patients with PDYN mutations [17]. Furthermore, Fogel et al. reported a known mutation in the PDYN gene (p.R138S) from one America sporadic ataxia case in 2012 [6]. To date, there have been no additional reports of SCA23 in other countries, ethnic groups or populations, suggestive of an extremely low incidence of SCA23.

Compared to the highly variable clinical phenotype of SCA23 in Bakalkin’s study, where no common initial symptoms were present, the patient with a novel polymorphism in our study has similar clinical characteristics to patients in previous reports, which included late onset, a relatively slowly progression, and pure cerebellar ataxia, with exception of dysarthria, oculomotor impairment, polynuropathy and tremor.

All of the patients in our study were negative for SCA23 mutations. There are several possible reasons for this finding: (1) SCA23 is an extremely rare form of SCA. To date, three groups have screened for PDYN mutations in 1100 Dutch ataxia patients [1], 104 German ataxia patients [17] and 119 American ataxia cases, respectively [6], but only two SCA 23 families and three sporadically affected individuals were found. In our study, we screened 305 SCA patients but did not find any cases of SCA 23. We will expand the sample and further screen unassigned SCA patients for variants of the PDYN gene in our future work. (2) Differences in ethnicity and geography lead to differences in phenotype distribution. (3) Rare mutation types such as gene rearrangement or mutations in intron or UTR affecting the PDYN gene might be missed. Because the four mutations previously reported were missense mutations, we did not focus on the detection of other mutation types. We are now ready to apply new methods, such as RT-PCR or multiplex ligation-dependent probe amplification (MLPA) to screen possible rare PDYN mutations types in the future.

5. Conclusions

In summary, our study provided evidence that SCA23 is an uncommon SCA subtype within the Chinese Han population. A probable novel polymorphism (c.255G>A, p.Lys85Lys) in exon 4 of the PDYN gene was found, but further evidence based on functional studies will be required to determine its significance. Furthermore, a combination strategy involving whole-exome sequencing and linkage analysis may be used to identify novel causative genes in future research [23], which will yield new insights into the identification of new SCA subtypes.

Conflicts of interest

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

Acknowledgments

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