Possible Reduced Penetrance of Expansion of 44 to 47 CAG/CAA Repeats in the TATA-Binding Protein Gene in Spinocerebellar Ataxia Type 17

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**Background:** Spinocerebellar ataxia type 17 (SCA17) is an autosomal dominant cerebellar ataxia caused by expansion of CAG/CAA trinucleotide repeats in the TATA-binding protein (TBP) gene. Because the number of triplets in patients with SCA17 in previous studies ranged from 43 to 63, the normal number of trinucleotide units has been considered to be 42 or less. However, some healthy subjects in SCA17 pedigrees carry alleles with the same number of expanded repeats as patients with SCA17.

**Objective:** To investigate the minimum number of CAG/CAA repeats in the TBP gene that causes SCA17.

**Design:** We amplified the region of the TBP gene containing the CAG/CAA repeat by means of polymerase chain reaction and performed fragment and sequence analyses.

**Patients:** The subjects included 734 patients with SCA (480 patients with sporadic SCA and 254 patients with familial SCA) without CAG repeat expansions at the SCA1, SCA2, Machado-Joseph disease, SCA6, SCA7, or dentatorubral-pallidolysian atrophy loci, with 162 healthy subjects, 216 patients with Parkinson disease, and 195 with Alzheimer disease as control subjects.

**Results:** Eight patients with SCA possessed an allele with more than 43 CAG/CAA repeats. Among the non-SCA groups, alleles with 43 to 45 repeats were seen in 3 healthy subjects and 2 with Parkinson disease. In 1 SCA pedigree, a patient with possible SCA17 and her healthy sister had alleles with 45 repeats. A 34-year-old man carrying alleles with 47 and 44 repeats (47/44) had developed progressive cerebellar ataxia and myoclonus at 25 years of age, and he exhibited dementia and pyramidal signs. He was the only affected person in his pedigree, although his father and mother carried alleles with mildly expanded repeats (44/36 and 47/36, respectively). In another pedigree, 1 patient carried a 43-repeat allele, whereas another patient had 2 normal alleles, indicating that the 43-repeat allele may not be pathologic in this family.

**Conclusions:** We estimate that 44 CAG/CAA repeats is the minimum number required to cause SCA17. However, the existence of unaffected subjects with mildly expanded triplets suggests that the TBP gene mutation may not penetrate fully. Homozygosity of alleles with mildly expanded triplet repeats in the TBP gene might contribute to the pathologic phenotype.

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**S**pinocerebellar ataxia type 17 (SCA17) is a new type of autosomal dominant cerebellar ataxia caused by an expanded CAG repeat in the TATA-binding protein (TBP) gene. Koide and colleagues initially described a patient with sporadic SCA17 who presented with cerebellar ataxia, mental disorder, and involuntary movement and who possessed an expanded triplet repeat in the TBP gene. Four reports of SCA pedigrees linked to a TBP gene mutation followed, and the minimum number of triplets in patients with SCA17 was 43 (Table 1). The TBP gene contains a polymorphic CAG/CAA repeat, which has been investigated in healthy subjects and in those with some psychiatric disorders. In those studies, the largest number of triplets in healthy control subjects was 42. In the present study, to determine the minimum number of triplets in the TBP gene associated with SCA17, we screened TBP gene mutations in patients with sporadic and familial SCA and no other trinucleotide repeat expansions, and we compared the allele distribution with that in healthy controls and patients with other neurodegenerative diseases.

**METHODS**

The subjects included 734 patients with SCA (480 patients with sporadic SCA and 254 patients with familial SCA) without CAG...
repeat expansions at the SCA1, SCA2, Machado-Joseph disease (MJD), SCA6, SCA7, or dentatorubral-pallidoluysian atrophy (DRPLA) loci (age range, 1-83 years; mean±SD age, 46±19 years) and some of their non-affected relatives, with 162 healthy subjects (age range, 56-94 years old; mean±SD age, 68±9.4 years), 216 patients with Parkinson disease (age range, 41-80 years old; mean±SD age, 62±8.9 years), and 195 patients with Alzheimer disease (age range, 45-93 years old; mean±SD age, 70±11 years) as controls. Informed consent for participation in the study was obtained from all subjects.

MOLECULAR STUDIES

Genomic DNA was extracted from peripheral blood by standard methods. The region of the TBP gene containing the CAG/CAA repeats was amplified by means of polymerase chain reaction (PCR) using TaKaRa Ex Taq (Takara Bio Inc, Otsu City, Japan). The primers for the PCR were 5’-(6FAM)CCTTATGGCACTGGACTGAC-3’ and 5’-GTTCCCTGTGTTGCCTGCTG-3’. Ten microliters of reaction mixture contained 10 to 20 ng of DNA, 5 pmol of each primer, 200 µmol/L of each deoxynucleotide triphosphate, 1.5 mmol/L of magnesium chloride, and 0.5 U of DNA polymerase. The PCR cycle conditions were 5 minutes at 95°C followed by 40 cycles of 95°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, with a final extension of 10 minutes at 72°C. Fragment analysis was performed with a genetic analyzer and analysis software (ABI PRISM 3100 Genetic Analyzer and GeneScan Analysis software, version 3.7; Applied Biosystems, Foster City, Calif). The number of CAG/CAA repeats was confirmed by means of sequence analysis using a BigDye Terminator Cycle Ready Reaction Kit (Applied Biosystems).

RESULTS

ALLELE DISTRIBUTION OF TRIPLET REPEATS IN THE TBP GENE

The allele distribution of the CAG/CAA repeat polymorphism in the TBP gene in all groups is shown in the Figure. An allele with 36 repeats was the most frequent in every group, followed by an allele with 37 repeats. In previous studies, the most frequent alleles had 35 or 36 repeats in Japanese subjects, but 38 in other ethnic groups (Table 1), suggesting that an ethnic difference exists.

ANALYSIS OF THE SCA GROUP

Eight patients with SCA (range of age at onset, 25-60 years) possessed an allele with more than 43 CAG/CAA repeats. Their neurological symptoms other than cerebellar ataxia were dementia, seizure, involuntary movement (myoclonus and choreoathetosis), pyramidal signs, and parkinsonism (Table 2).

Patient 1 (alleles with 56 and 36 repeats [56/36]) was a 34-year-old woman in whom upper limb myoclonus and progressive ataxia developed with dementia at 30 years of age. Her father had died at 42 years of age of unknown causes, and there was no other affected person in the pedigree.

Patient 2 (48/36) was a 47-year-old woman in whom ataxic gait and dementia developed at 27 years of age. Mutism and partial seizure of the right hand and face with marching had appeared from 37 years of age. Her parents and 2 sisters were healthy, but her older brother with SCA had died at 48 years of age.

Patient 3 was homozygous for expanded triplets (47/44) and was the only affected person in his pedigree. He was a 34-year-old man in whom progressive cerebellar

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Table 1. CAG/CAA Repeats in the TBP Gene in Previously Reported Cases of SCA17

<table>
<thead>
<tr>
<th>Source</th>
<th>Ethnic Group</th>
<th>Patients</th>
<th>Healthy Subjects</th>
<th>Most Frequent Alleles in Healthy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koide et al1</td>
<td>Japanese</td>
<td>63</td>
<td>31-42</td>
<td>35</td>
</tr>
<tr>
<td>Zühlke et al2</td>
<td>German</td>
<td>50-55</td>
<td>29-40</td>
<td>38</td>
</tr>
<tr>
<td>Fujisaki et al3</td>
<td>Belgian</td>
<td>46</td>
<td>29-40</td>
<td>38 (French)</td>
</tr>
<tr>
<td>Nakamura et al4</td>
<td>Japanese</td>
<td>47-55</td>
<td>29-42</td>
<td>36</td>
</tr>
<tr>
<td>Silveira et al5</td>
<td>Portuguese and Brazilian</td>
<td>43</td>
<td>29-40</td>
<td>38</td>
</tr>
</tbody>
</table>

Abbreviations: SCA17, spinocerebellar ataxia type 17; TBP, TATA-binding protein.
ataxia and myoclonus developed at 25 years of age. He showed dementia and pyramidal signs. His father (age, 63 years) and mother (age, 58 years) also had slightly expanded triplet repeats (44/36 and 47/36, respectively) but were free of neurological symptoms. There was no consanguinity in the parents.

Patient 4 (47/36) was a 43-year-old man in whom slurred speech, ataxic gait, and dementia developed at 42 years of age. No other person had a neurological disorder in the pedigree.

Patient 5 (45/37) was a 58-year-old woman in whom cerebellar ataxia and pyramidal signs developed at 52 years of age. Her father had exhibited ataxia and parkinsonism and had been diagnosed as having striatoniogral degeneration. Her sister (age, 55 years) also carried a 45-repeat allele but had been healthy.

Patient 6 (45/36) was a 70-year-old woman in whom dysarthria, ataxic gait, and memory disturbance developed at 60 years of age. Oral dyskinesia, choreoathetosis, and parkinsonism had also been seen. No other member in the pedigree had the same symptoms except her younger sister.

Patient 7 (44/36) was a 58-year-old man in whom progressive ataxic gait and dysarthria developed at 29 years of age. Dementia, tonic seizure, choreoathetosis, rigidity, and pyramidal signs appeared after 51 years of age. There was no other affected person in the pedigree.

Patient 8 (43/33) was a 47-year-old woman in whom mild ataxia and spasticity developed. She had a brother with cerebellar ataxia who possessed 2 normal alleles (36/33).

We found no difference in the structure of the triplet repeat, (CAG)triplet(CAA)x(CAG), in SCA and non-SCA subjects. The proportions of (CAG), and (CAG), varied with number of repeats.

**ANALYSIS IN THE NON-SCA GROUP**

Among the non-SCA groups, the number of triplet units ranged from 29 to 45 in the healthy control group, 29 to 42 in the Alzheimer disease group, and 29 to 43 in the Parkinson disease group. Alleles with 43 or more repeats were seen in 3 healthy controls (a 63-year-old man [43/36], a 66-year-old woman [44/33], and a 66-year-old man [45/37]) and 2 patients with Parkinson disease (a 50-year-old woman [43/38] and a 67-year-old man [43/37]). The structure of the triplet in the non-SCA groups was not different from that in the SCA groups.

**COMMENT**

The initially reported case of SCA17 had an allele with a partially duplicated segment involving triplet repeats that encoded 63 glutamines. Her nonaffected parents had normal alleles. Zuhlke and colleagues described 2 families with expanded triplet repeats in the TBP gene and suggested that an expansion to 50 or more repeats was pathologic. Nakamura and colleagues performed an immunocytochemical study of a postmortem brain that carried 48 CAG/CAA units in the TBP gene, and they detected neuronal intranuclear inclusion bodies that were stained with antiubiquitin, anti-TBP, and anti-1C2 antibodies. In a Belgian family with a CAG/CAA repeat expansion, 6 members were affected in 4 successive generations. Two of them had an allele with 40 repeats, and polyglutamine deposition was immunocytochemically detected in the postmortem brain. However, 6 nonaffected members also carried the 46-repeat allele. In previous studies on TBP gene mutation, the range of triplets in healthy subjects was 29 to 42, and SCA17 alleles except for that in the initially reported case contained 43 to 55 repeats (the allele in the initially reported case contained 63 partially duplicated repeats). The TBP gene triplet is thought to show stable transmission differing from the MJD and DRPLA genes, and the CAA interruptions may contribute to the stability as SCA1 and SCA2 genes.

In our study, the 56 and 48 repeats of CAG/CAA in patients 1 and 2, which were not seen in the unaffected subjects, were thought to be fully penetrant. Patient 8, who carried a 43-repeat allele, had a brother with cerebellar ataxia who possessed 2 normal alleles, indicating that the 43-repeat allele may not be pathologic in this family. On the other hand, the range of 44 to 47 repeats partially overlapped with the number of triplets in the healthy. Alzheimer disease, and Parkinson disease control groups and were carried by the affected and unaffected subjects in the SCA pedigrees with no difference in triplet structure among the affected and unaffected subjects. Although these unaffected subjects might have cerebellar ataxia and other neurological disorders in the future, the 44 to 47 repeats were thought to be in the border zone. In Huntington disease, an allele with an expanded CAG triplet of more than 40 repeats leads to a pathologic phenotype; an allele with fewer than 30 repeats represents a normal phenotype; and there is a region of overlap between unaffected and affected alleles. Some patients with Huntington disease have 36 repeats, whereas some individuals with 36 to 39 repeats have survived in an apparently healthy state into old age. We suspect that mildly expanded triplet repeats (44-47 repeats) in the TBP gene may not be fully penetrant, as seen in Huntington disease. We speculate that patients with

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age at Onset, y</th>
<th>Triplet Unit</th>
<th>Other Persons With SCA in the Pedigree</th>
<th>Neurological Symptoms Other Than Cerebellar Ataxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/30</td>
<td>56/36</td>
<td>Sporadic</td>
<td>Dementia, myoclonus, pyramidal signs</td>
</tr>
<tr>
<td>2/F/27</td>
<td>48/36</td>
<td>1 Brother</td>
<td>Dementia, seizure</td>
</tr>
<tr>
<td>3/M/25</td>
<td>47/44</td>
<td>Sporadic</td>
<td>Dementia, myoclonus, pyramidal signs</td>
</tr>
<tr>
<td>4/M/42</td>
<td>47/36</td>
<td>Sporadic</td>
<td>Dementia</td>
</tr>
<tr>
<td>5/F/52</td>
<td>45/37</td>
<td>Father</td>
<td>Pyramidal signs</td>
</tr>
<tr>
<td>6/F/60</td>
<td>45/36</td>
<td>1 Sister</td>
<td>Dementia, choreoathetosis, parkinsonism</td>
</tr>
<tr>
<td>7/M/29</td>
<td>44/36</td>
<td>Sporadic</td>
<td>Dementia, myoclonus, pyramidal signs</td>
</tr>
<tr>
<td>8/F/47</td>
<td>43/33</td>
<td>1 Brother</td>
<td>Pyramidal signs</td>
</tr>
</tbody>
</table>

Abbreviations: SCA, spinocerebellar ataxia; TBP, TATA-binding protein.
SCA17 have a lower limit of CAG/CAA repeats of 44 units, but diagnosis of SCA17 by means of genetic analysis should be made with care.

Patient 3 was homozygous for a mildly expanded allele, and his parents, who were free of neurological symptoms, also had slightly expanded triplet units but were heterozygous. Several studies have shown that homozygosity for the CAG expansion in triplet diseases enhances phenotypic severity or modulates the phenotype caused by a gene dosage effect. Therefore, we speculate that homozygosity in this patient might have contributed to his pathologic phenotype. His age at onset was the youngest in our subjects with possible SCA17, indicating that a gene dosage effect may contribute to the phenotype in TBP gene mutation.

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Author contributions: Study concept and design (Drs Oda and Kawakami); acquisition of data (Drs Komure, Imamura, Yasuda, Ichikawa, and Ogawa); analysis and interpretation of data (Drs Oda and Morino); drafting of the manuscript (Drs Oda, Maruyama, and Kawakami); critical revision of the manuscript for important intellectual content (Drs Komure and Matsumoto); administrative, technical, and material support (Drs Terasawa and Izumi); study supervision (Dr Kawakami).

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