Japanese Families with Autosomal Dominant Pure Cerebellar Ataxia Map to Chromosome 19p13.1-p13.2 and Are Strongly Associated with Mild CAG Expansions in the Spinocerebellar Ataxia Type 6 Gene in Chromosome 19p13.1


1Department of Neurology, Institute of Clinical Medicine, and 2Department of Biochemistry, Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Japan; 3Department of Neurology, Brain Research Institute, Niigata University, Niigata, Japan; 4Department of Neurology, Jichi Medical School, Yakushiji, Japan; 5Department of Gerontology, Kochi Medical School, Kochi, Japan; and 6Department of Neurology, Institute of Brain Research, University of Tokyo, and 7Department of Neurology, Tokyo Medical and Dental University, Tokyo

Summary

Autosomal dominant cerebellar ataxia is a group of clinically and genetically heterogeneous disorders. We carried out genomewide linkage analysis in 15 families with autosomal dominant pure cerebellar ataxia (ADPCA). Evidence for linkage to chromosome 19p markers was found in nine families, and combined multipoint analysis refined the candidate region to a 13.3-cM interval in 19p13.1-p13.2. The remaining six families were excluded for this region. Analysis of CAG-repeat expansion in the alpha1A-voltage-dependent calcium channel (CACNL1A4) gene lying in 19p13.1, recently identified among 8 small American kindreds with ADPCA (spinocerebellar ataxia type 6 [SCA6]), revealed that 8 of the 15 families studied had similar, very small expansion in this gene: all affected individuals had larger alleles (range of CAG repeats 21–25), compared with alleles observed in neurologically normal Japanese (range 5–20 repeats). Inverse correlation between the CAG-repeat number and the age at onset was found in affected individuals with expansion. The number of CAG repeats in expanded chromosomes was completely stable within each family, which was consistent with the fact that anticipation was not statistically proved in the SCA6 families that we studied. We conclude that more than half of Japanese cases of ADPCA map to 19p13.1-p13.2 and are strongly associated with the mild CAG expansion in the SCA6/CACNL1A4 gene.

Introduction

Autosomal dominant cerebellar ataxia (ADCA) is a group of heterogeneous conditions (Harding 1982). The cardinal clinical feature is progressive ataxia and other cerebellar signs, although extracerebellar signs such as pyramidal or extrapyramidal signs, ophthalmoparesis, amyotrophy, or peripheral neuropathy may be variably present. Clinical or neuropathological classifications of ADCA sometimes have been unreliable, but identification of several distinct gene loci and causative genes has made it possible to classify ADCA within molecular levels: spinocerebellar ataxia type 1 (SCA1) on chromosome 6p22-p23 (Yakura et al. 1974; Zoghbi et al. 1991; Kwiatkowski et al. 1993), SCA2 on 12q23-q24.1 (Gispert et al. 1993), Machado-Joseph disease (MJD)/SCA3 on 14q24.3-q32.1 (Takayama et al. 1993; Stevanin et al. 1994), SCA4 on 16q22.1 (Gardner et al. 1994; Flanigan et al. 1996), SCA5 on 11cen (Ranum et al. 1994), and SCA7 on 3p12-p13 (Benomar et al. 1995; Gouw et al. 1995; David et al. 1996). The genetic heterogeneity likely accounts for some clinical and pathological variations in ADCA. In addition, SCA1 (Orr et al. 1993), SCA2 (Imbert et al. 1996; Pulst et al. 1996; Sanpei et al. 1996), and MJD/SCA3 (Kawaguchi et al. 1994) have been demonstrated to be associated with unstable trinucleotide-CAG repeats, and the number of CAG repeats is found to correlate with the variable age at onset, with the phenomenon of anticipation (i.e., younger age at onset in successive generation), or with the progression in these diseases. Furthermore, CAG repeats in these genes are in their coding region and are shown to be translated into polyglutamine tracts that are suggested to exert neurotoxic effect (Servadio et al. 1995; Ikeda et al. 1996).

We as well as others have elsewhere reported that there is a distinct group of ADCA with only cerebellar symptoms (autosomal dominant pure cerebellar ataxia...
ADPCA] (Harding 1982; Frontali et al. 1992; Ishikawa et al. 1996). Neuropathologically, ADPCA shows cerebellar cortical atrophy often with degeneration in the inferior olivary nucleus (Hoffman et al. 1971; Eadie 1991). Although ADPCA has been well recognized, the SCA5 locus is so far the only gene locus identified for this subtype (Ranum et al. 1994). However, our previous study on eight Japanese families with ADPCA excluded the SCA5 locus, demonstrating that different genetic mutations cause a similar phenotype (Ishikawa et al. 1996).

To identify the gene(s) for ADPCA, we collected 15 families with ADPCA and initiated a genomewide search for linkage. After screening all autosomes with 243 microsatellite markers spaced in 15-cM intervals, we found evidence that in some of our families ADPCA was linked to chromosome 19p13.1-p13.2. Very recently, small expansions of trinucleotide (CAG) repeats in the alpha1A-voltage-dependent calcium channel (CACNL1A4) gene (Ophoff et al. 1996) were identified in eight small American kindreds and were designated “SCA6” (Zhuchenko et al. 1997). The chromosomal location of the CACNL1A4 gene had been identified in the 19p13.1 region (Diriong et al. 1995). We analyzed this CAG repeat in the SCA6/CACNL1A4 gene and found that Japanese families in which ADPCA was linked to chromosome 19p also had mild CAG expansion. Here we describe our linkage mapping to 19p13.1-p13.2, the mutational profile of the SCA6/CACNL1A4 gene, and correlations between the CAG expansion and clinical features in the ADPCA families that we studied.

Subjects and Methods

ADPCA Families

Fifteen Japanese families were investigated (fig. 1). Ten of these 15 families were the original residents of Ibaraki Prefecture, and 2 other families (P13 and P14) were from nearby prefectures located in the center of Honshu, the main island of Japan. The remaining three families were from distant areas: two families (P2 and P10) were from Kyushu and Shikoku, the islands in the southwestern area of Japan, and one family (P8) was from Aomori Prefecture, the northernmost prefecture of Honshu. All families were unrelated, and thus our cohort could be considered to represent a certain proportion of ADPCA in Japan.

Clinical features of the 68 affected individuals were summarized as “pure” cerebellar ataxia and fulfilled the diagnostic criteria of ADPCA (Harding 1982; Frontali et al. 1992; Ishikawa et al. 1996). Gait ataxia was invariably the initial symptom and was the chief symptom throughout the clinical course. Other symptoms were cerebellar speech (93.8%), limb ataxia (92.2%), decreased muscle tonus (90.0%), and horizontal gaze nystagmus (62.5%). Tendon reflexes were normal or slightly increased. Extracerebellar symptoms, such as pyramidal or extrapyramidal tract signs, ophthalmoparesis, or decreased sensation, were not seen. None of our patients complained of migraine. Magnetic-resonance imaging demonstrated restricted atrophy in the cerebellum in all individuals examined (n = 35).

Reliable information on the age at onset was obtained for 65 individuals, including 5 deceased subjects. The age-at-onset range was 20–72 years, and the average ± SD age at onset was 49.5 ± 10.7 years. Analysis of difference in the age at onset in 28 parent-offspring pairs revealed that the phenomenon of anticipation was mild but statistically significant (age at onset was 2.8 ± 7.7 years earlier in offspring; P = .042 by Wilcoxon’s test). The most striking anticipation in our families was seen in family P3 (age at onset 13.8 ± 9.8 years earlier in offspring). In this family, two offspring (P3-8 and P3-13) noticed gait ataxia during their 20s, whereas the mother developed ataxia during her mid 40s. Anticipation was also suggested by the observation of an obligate asymptomatic carrier (P1-15), who was not included in the parent-offspring analysis. This individual was a 60-year-old woman without apparent cerebellar signs. Her 31-year-old son (P1-22) already showed marked gait ataxia only 2 years after the onset.

After informed consent was obtained, blood samples were collected from 133 individuals, consisting of 68 affected individuals, 57 at-risk family members, and 8 individuals married into these families. The genes for SCA1–SCA5 and SCA7 were excluded for these families, either by the analysis of the CAG repeats in the causative genes or by linkage analysis (data not shown).

Markers and Genotyping

Two hundred forty-three polymorphic microsatellite markers were selected from either the Génethon map (Gyapay et al. 1994) or the Cooperative Human Linkage Center (CHLC) database (http://www.chlc.org), to cover whole autosomes with 15-cM intervals. High molecular-weight genomic DNA was extracted and was amplified by PCR using fluorescein-labeled primers. In brief, 125 ng of genomic DNA was amplified in a 25-µl volume with 2.5 pmol of each primer, 100 µM each dNTP, 10 mM Tris-HCL (pH 8.4), 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, and 0.5 units of Taq polymerase (Takara). Samples were processed at 94°C for 3 min; for 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 3 min; and finally at 72°C for 5 min. Genotyping was performed by use of an Automated Laser Fluorescence (A.L.F.™) DNA Sequencer II (Pharmacia Biotech) and was analyzed with Fragment Manager (Pharmacia Biotech), as described elsewhere (Ishikawa et al. 1996).
Figure 1  Fifteen families with ADPCA. Circles and squares denote females and males, respectively; and blackened symbols denote affected individuals. Deceased individuals are denoted by diagonal lines through the symbols. Subjects who were sampled for genetic analysis are indicated by numbers.
Linkage Analysis

Pairwise and multipoint LOD scores (Z) were calculated by use of MLINK, ILINK, and LINKMAP in the computer software LINKAGE, version 5.1 (Lathrop et al. 1984; Terwilliger and Ott 1994). The disease was considered to be autosomal dominant with a gene frequency of .00001. Recombination fractions (θ) in men and women were assumed to be equal. Since penetrance of the disease is a function of age, five age-dependent penetrance classes were established on the basis of the cumulative age-at-onset profile of the pedigrees: class 1, 0–29 years (3.4%); class 2, 30–39 years (16.9%); class 3, 40–49 years (49.2%); class 4, 50–59 years (86.4%); and class 5, age >60 years (95%). For multipoint analysis, the method of maximum Z (Z_{max}) minus 3 (Z_{max} − 3) was used to determine the support interval, and the distance was calculated by use of the Haldane mapping function (Terwilliger and Ott 1994). The heterogeneity analysis within the families was performed by use of the program HOMOG, version 3.1 (Ott 1991).

Analysis of CAG Repeats in the SCA6/CACNL1A4 Gene

CAG length polymorphism in the SCA6/CACNL1A4 gene was determined by use of the 5-5 primers described elsewhere (Zhuchenko et al. 1997). PCR was performed in a similar manner, with microsatellite markers, except that 250 μm of dimethyl sulfoxide (DMSO) was added in each reaction, and the annealing temperature was raised to 62°C. Direct nucleotide-sequence analysis was also performed, to determine the accurate number of CAG repeats and to determine the presence or absence of any interrupting sequences within the CAG repeat. CAG-repeat polymorphism in the Japanese population was determined by examination of 151 healthy volunteers who had neither neurological symptoms nor family history of ataxia. The relationship between the number of CAG repeats and the age at onset was examined by a simple linear-regression analysis.

Clinical Comparison between Families with Expansions and Families without Expansions

Clinical features of the SCA6 families were compared with those of the non-SCA6 families. Statistical analysis of anticipation was performed by Wilcoxon’s test, and statistical analysis comparing SCA6 and non-SCA6 families was performed by the Mann-Whitney U-test or by the χ² test.

Results

Linkage Mapping to Chromosome 19p13.1-p13.2

We screened all autosomes and found a possibility for linkage to D19S432 in chromosome 19p (combined Z_{max} of 1.65 at θ = .20). Eight nearby markers (D19S1034–D19S586–D19S394–D19S221–D19S432–D19S410–D19S434–D19S433–D19S178) were then analyzed. In the most informative family, P1, significant Z values were obtained for D19S394 (Z_{max} = 4.92 at θ = .00) and D19S433 (Z_{max} = 3.03 at θ = .00), and positive LOD scores (Z > Z_{max} > 2.5) also were obtained for D19S586, D19S221, and D19S410. Three smaller families (P3, P5, and P10) also showed supporting data (Z > 1.0) for D19S394, D19S221 and D19S432, whereas six others (P2, P4, P6, P12, P14, and P15) showed significantly negative Z values (i.e., <−2.0). Genetic heterogeneity was proved in D19S394 and D19S221, with significant odds against the “absence of linkage.” The conditional probabilities of linkage suggested this linkage in nine families (P1, P3, P5, P7–P11, and P13). The combined pairwise LOD scores in these nine families reached Z_{max} values of 9.41 for D19S394, 6.62 for D19S221, and 3.97 for D19S432, all at θ = .00.

Haplotype reconstruction placed the candidate region within a 16-cM region flanked by D19S586 and D19S410: the centromeric boundary was placed at D19S410 by two recombinations in P5 and P10, and the telomeric boundary was placed at D19S586 by three recombinations in P1, P7, and P10. However, haplotypes for the most tightly linked markers (D19S394–D19S221–D19S432) were different in all families. The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.

Analysis of CAG-Repeat Polymorphism in the Japanese Population

The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.

Analysis of CAG-Repeat Polymorphism in the SCA6/CACNL1A4 Gene in 19p13.1

The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.

Analysis of CAG-Repeat Polymorphism in the SCA6/CACNL1A4 Gene in 19p13.1

The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.

Analysis of CAG-Repeat Polymorphism in the SCA6/CACNL1A4 Gene in 19p13.1

The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.

Analysis of CAG-Repeat Polymorphism in the SCA6/CACNL1A4 Gene in 19p13.1

The analysis of CAG-repeat length in neurologically normal Japanese individuals revealed that the length range was 5–20 repeats (fig. 3). The distribution pattern showed two major peaks; the largest peak was at 13 repeats (allele frequency 42.1%), and the second peak was at 11 repeats (allele frequency 23.2%). There were no sequences interrupting the CAG repeat in the normal chromosomes. Two individuals harboring larger alleles (18 and 20 CAG repeats) both 30 years of age, did not have any neurological symptoms or family history of ataxia.
lies (P5, P8, P9, and P10) had 22 repeats, two families
(P1 and P7) had 24 repeats, and 1 family (P3) had 25
repeats. The range in the number of CAG repeats in
normal chromosomes in SCA6 families was 7–19. Four
individuals with 19 repeats who were 70–75 years of
age were neurologically normal on examination.

One of the affected individuals (P13-4) was homozy-
gous for CAG expansions (both alleles 21 repeats).
This individual developed ataxic gait at the age of 58 years
and showed very mild ataxic symptoms at our examina-
tion of him 4 years after onset of his symptoms. His
clinical course was indistinguishable from that of his
mother (P13-1), who was heterozygous for CAG repeats
(18 and 21 repeats). Five asymptomatic individuals
(P1-15, P3-9, P8-4, P8-5, and P13-2) were also found
to carry the mild expansions. On the basis of haplotype
analysis, these individuals were predicted to carry the
disease genes.

The remaining seven families did not have expanded
alleles (range 7–16 repeats). In all of these families link-
age to 19p13.1-p13.2 had been excluded, except for a
small family (P11) that showed inconclusive data for
linkage (by multipoint analysis $Z_{max} = 10.71$ was
obtained at the same position. D19S221).

The precise locus of the SCA6/CACNL1A4 gene is also indicated.

Figure 2  Combined multipoint LOD scores for nine families
(P1, P3, P5, P7–P11, and P13) with linkage to chromosome 19p. The
combined $Z_{max}$ of 10.94 was obtained at the location of D19S221,
supporting linkage. The support interval, for the $Z_{max} = 3$ method,
revealed that the candidate locus lies in a 13.3-cM interval between
0.4 CM centromeric to D19S586 and the location of D19S432. Even
when family P11, later excluded for SCA6 expansion, was excluded
from this analysis, $Z_{max} = 10.71$ was obtained at the same position.
The precise locus of the SCA6/CACNL1A4 gene is also indicated.

P7–P10, and P13) had at least one allele with very mild
expansion. The range in the number of CAG repeats in
the expanded (i.e., SCA6) chromosomes was 21–25 (fig.
3). The expanded alleles were completely stable within
each family: one family (P13) had 21 repeats, four fami-
lies (P5, P8, P9, and P10) had 22 repeats, two families
(P1 and P7) had 24 repeats, and 1 family (P3) had 25
repeats. The range in the number of CAG repeats in
normal chromosomes in SCA6 families was 7–19. Four
individuals with 19 repeats who were 70–75 years of
age were neurologically normal on examination.

One of the affected individuals (P13-4) was homozy-
gous for CAG expansions (both alleles 21 repeats).
This individual developed ataxic gait at the age of 58 years
and showed very mild ataxic symptoms at our examina-
tion of him 4 years after onset of his symptoms. His
clinical course was indistinguishable from that of his
mother (P13-1), who was heterozygous for CAG repeats
(18 and 21 repeats). Five asymptomatic individuals
(P1-15, P3-9, P8-4, P8-5, and P13-2) were also found
to carry the mild expansions. On the basis of haplotype
analysis, these individuals were predicted to carry the
disease genes.

The remaining seven families did not have expanded
alleles (range 7–16 repeats). In all of these families link-
age to 19p13.1-p13.2 had been excluded, except for a
small family (P11) that showed inconclusive data for
linkage (by multipoint analysis $Z_{max} = 0.23$ for
D19S221).

Correlation between CAG-Repeat Length and Clinical
Features in the Eight Families with Expansions

The relationship between the length of the expanded
CAG repeat of affected individuals ($n = 37$) and their
ages at onset is shown in figure 4. A significant correla-
tion coefficient ($r$) of $-0.712$ ($r^2 = .507; P < .001$) was

Figure 3  Allele-frequency distributions in 302 normal chromo-
somes and in 44 SCA6 chromosomes, including those from 5 presymp-
tomatic individuals and 1 homozygous individual. The range in the
number of CAG repeats in normal chromosomes was 5–20. The range
in the number of repeats in expanded SCA6 chromosomes was 21–25.
Note that there was no repeat-number gap between the two groups.

Figure 4  Scattergram of number of CAG repeats in SCA6 chro-
mosomes, versus age at onset in 37 affected individuals. An individual
(P13-4) homozygous for expansion (21 repeats) was excluded from
this calculation. A simple linear-regression analysis yielded a signifi-
cant $r$ of $-0.712$ ($r^2 = .507; P < .001$).
obtained for the age at onset and the repeat length in SCA6 chromosomes. No significant correlation was observed between the repeat length in the normal chromosomes in affected individuals and their ages at onset (data not shown).

Clinical Comparison between Families with Expansions and Families without Expansions

Clinical features of eight families with CAG expansion in the SCA6/CACNL1A4 gene (termed the “SCA6” families) were compared with those of seven families without such expansion (termed the “non-SCA6” families) (table 1). Between the two groups, there were no statistically significant differences in either the age at onset or the duration of illness at examination, and the clinical features in both groups were almost identical and were summarized as ADPCA. However, the age at onset was significantly younger in the SCA6 families than in the non-SCA6 families (SCA6, 45.0 ± 10.0 years; non-SCA6, 55.9 ± 8.2 years; P < .0001). Analysis of anticipation did not prove significant anticipation in SCA6 families (n = 21 pairs; 2.1 years younger age at onset in offspring; P = .31), although great variability was noted: the changes in offspring ranged from a 23-years-younger age at onset (i.e., anticipation) to an 11-years-older age at onset. Anticipation in the non-SCA6 families was statistically significant (n = 7 pairs; 4.9 years younger age at onset in offspring; P = .046).

The difference in anticipation between the two groups (SCA6 vs. non-SCA6) was not statistically significant (P = .41). No other features were found that could differentiate the two groups.

Discussion

We performed a genomewide search for linkage and found strong evidence for linkage to chromosome 19p13 in the ADPCA families that we studied. This is the first successful linkage study in ADPCA other than SCA5 (Ranum et al. 1994). Haplotype and multipoint analyses further refined the candidate locus to a 13.3-cM region around D19S221 in 19p13.1-p13.2. Exactly within the candidate locus that we defined, the mild CAG expansion was identified in the CACNL1A4 gene.

The most significant point that we confirmed in this study is the pathogenic role of CAG-repeat expansions

---

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>SCA6 Families</th>
<th>Non-SCA6 Families</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 8)a</td>
<td>(n = 7)b</td>
</tr>
<tr>
<td>No. of patients examined</td>
<td>38</td>
<td>30</td>
</tr>
<tr>
<td>Age at examination (years)</td>
<td>61.2 ± 14.3</td>
<td>66.6 ± 10.8</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>16.5 ± 11.9</td>
<td>13.2 ± 9.3</td>
</tr>
<tr>
<td>Age at onset (years):c</td>
<td>20–66</td>
<td>45–72</td>
</tr>
<tr>
<td>Range</td>
<td>45.0 ± 10.0 (n = 38)</td>
<td>55.9 ± 8.2 (n = 27)</td>
</tr>
<tr>
<td>Averaged</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anticipation (years):e</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal transmission</td>
<td>−2.8 ± 9.8 (n = 14)f</td>
<td>−4.0 ± 4.6 (n = 6)g</td>
</tr>
<tr>
<td>Paternal transmission</td>
<td>−0.7 ± 5.5 (n = 7)g</td>
<td>−10 (n = 1)</td>
</tr>
<tr>
<td>Overall</td>
<td>−2.1 ± 8.5 (n = 21)f</td>
<td>−4.9 ± 4.8 (n = 7)g</td>
</tr>
<tr>
<td>Clinical features (%):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gait ataxia</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Limb ataxia</td>
<td>91.9</td>
<td>92.6</td>
</tr>
<tr>
<td>Cerebellar speech</td>
<td>94.6</td>
<td>92.6</td>
</tr>
<tr>
<td>Nystagmus</td>
<td>62.2</td>
<td>63.0</td>
</tr>
<tr>
<td>Extracebellar signs</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a Families P1, P3, P5, P7–P10, and P13.
b Families P2, P4, P6, P11, P12, P14, and P15.
c For 65 individuals, including 5 deceased subjects.
d Significantly younger in SCA6 families compared with non-SCA6 families (P < .0001).
e Calculated as age at onset in offspring minus age at onset in parent.
f Anticipation was not statistically significant.
g Anticipation was proved to be statistically significant (.01 < P < .05).
in the SCA6/CACNL1A4 gene. The CAG-repeat expansion in the SCA6/CACNL1A4 gene had three unique and important aspects, compared with other CAG expansions. First is that the size range of the expanded CAG allele was small (21–27 repeats [Zhuchenko et al. 1997] or 21–25 repeats [present study]) and was completely within the normal range of CAG-repeat number in other diseases associated with CAG-repeat expansions (La Spada et al. 1991; The Huntington’s Disease Collaborative Research Group 1993; Orr et al. 1993; Kawaguchi et al. 1994; Koide et al. 1994; Pulst et al. 1996). Second is that a single nucleotide deletion or the G→A nucleotide transition in the CACNL1A4 gene is identified as the cause of episodic ataxia (Ophoff et al. 1996), suggesting that mutation other than trinucleotide repeat could be responsible for ADPCA. Third is that the size of normal alleles was continuous up to 20 repeats and that no gap was observed between the distribution of CAG-repeat numbers on the normal and the SCA6 chromosomes. This is strikingly different from the distributions in SCA1 (Orr et al. 1993), SCA2 (Pulst et al. 1996), or MJD/SCA3 (Takiyama et al. 1995). No interrupting sequences were seen within the CAG-repeat sequences in the SCA6/CACNL1A4 gene, which was another difference from SCA1 (CAT interruption [Chung et al. 1993; Jodice et al. 1994]) and SCA2 (CAA interruption [Pulst et al. 1996]). At this moment, however, we cannot completely exclude the possibility that individuals with larger alleles, such as 20 repeats, might develop ataxic symptoms in the future or that such alleles are in the condition of premutation.

Although the mild CAG expansion in SCA6 appeared to be pathogenic, the number of patients studied was small, and the genotype-phenotype correlation was not clear in the original study (Zhuchenko et al. 1997). In the present study, we confirmed that mild CAG expansions similar to those found in the American SCA6 kindred are present in Japanese families with ADPCA. Furthermore, an inverse correlation between the number of CAG repeats and age at onset was demonstrated, strongly suggesting that CAG expansion, even when mild, plays a pathogenic role in SCA6.

Another important fact that we observed is that the CAG expansion in the SCA6/CACNL1A4 gene was completely stable within the SCA6 families that we studied. This is strikingly different from the situation in other diseases, in which CAG-repeat expansions are often unstable and the transmission of unstable expansions is thought to be an important, although not the only, molecular basis for anticipation (La Spada et al. 1992; Chung et al. 1993; Duyao et al. 1993; Jodice et al. 1994; Maciel et al. 1995). In this context, highly “stable” transmission of the expanded CAG repeat appears to be consistent with the fact that the anticipation was not statistically proved in the SCA6 families that we studied. However, it should be noted that there was a substantial variation in the age at onset in the same family with the very same expansion: some showed striking “anticipation” whereas others exhibited the opposite. According to the statistical analysis (fig. 4), ~51% (r² = .507) of the variation in age at onset can be accounted for by the CAG-repeat number, indicating that the remainder of the variation remains to be explained. Although ascertainment bias should always be considered in the determination of the age at onset, some other factors—including environmental factors—could be present that influence the age at onset in individuals carrying the CAG expansion.

When clinical features of the American SCA6 kindred (Zhuchenko et al. 1997) are compared with those of the Japanese SCA6 families that we studied, several identical features are found. Both groups of families show predominantly cerebellar ataxia with slow disease progression (Ishikawa et al. 1996). The ages at onset are also similar in both groups; onset occurs mostly in the 40s and 50s in families with smaller expansions (21–24 repeats), whereas families with relatively larger expansions (25–27) show a tendency toward earlier onset (fig. 4). Restricted cerebellar atrophy, as diagnosed on the basis of magnetic-resonance imaging, is also a feature common to both groups (Ishikawa et al. 1996). These features are quite similar and could be considered as indicative of ADPCA (Harding 1982; Polo et al. 1991; Frontali et al. 1992). In addition to the cardinal cerebellar symptoms, mild vibratory and proprioceptive sensory loss, and choking were described in the American SCA6 families. In our series of patients, however, sensory dysfunction was not observed. Electrophysiological studies, such as nerve conduction studies or somatosensory evoked potentials, were examined in five patients with 22 or 24 repeats, but all patients were normal. Few subjects noticed dysphagia-like sensation. However, these patients were >70 years of age and had long (>20 years) durations of illness. It will be important to examine whether these “extracerebellar” signs develop in SCA6, particularly in patients with larger expansions of CAG repeats or with longer durations of illness.

Finally, our linkage data also showed that there are still several families with ADPCA in Japan that do not link to chromosome 19p13.1-p13.2. SCA4 and SCA5 were both excluded for these families. The clinical features of our non-SCA6 families are also those of pure cerebellar ataxia, which is hardly distinguished from that in SCA6 families, except for the average age at onset (table 1). Therefore, testing SCA6 mutation in patients clinically diagnosed as ADPCA would be highly important. The present study has indicated the presence of anticipation in non-SCA6 families as well, suggesting that similarly mild CAG expansion may also be present in this group. Further studies are needed to elucidate...
molecular bases of this heterogeneous group of diseases clinically showing pure cerebellar ataxia.

Acknowledgments

We would like to gratefully acknowledge Drs. T. Yoshizawa, N. Shiraiwa, T. Yamawaki, K. Fujimoto, M. Ishikawa, and K. Kawasaki for supporting clinical investigation, and we also thank Ms. S. Nissato and Drs. T. Arinami, H. Hamaguchi, J. D. Terwilliger, and J. Ott for supporting linkage analysis. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas, Ministry of Education, Science and Culture, Japan (to H.M.), by a grant from the Research Committee of Ataxic diseases, Ministry of Health and Welfare of Japan (to H.M.), and by a grant from Japan Society for the Promotion of Science (to K.I.).

References


