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AUTOSOMAL DOMINANT CEREBELLAR ATAXIAS IN THE KINKI AREA OF JAPAN

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Summary The autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders characterized by slowly progressive cerebellar ataxia. Recently, among the ataxias, spinocerebellar ataxia type 1 (SCA1), Machado-Joseph disease (MJD) and dentatorubral-pallidoluysian atrophy have been found to be caused by expansion of a CAG trinucleotide repeat in the coding region of the disease genes. We have analyzed the CAG repeats of 67 patients from 47 families with dominantly inherited ataxia who lived in the Kinki area of Japan. The following results were obtained. First, 31 patients from 22 families were found to be positive for the MJD repeat expansion, indicating that MJD is the most common dominantly inherited ataxia in the Kinki area of Japan. Second, no SCA1 repeat expansion was found among the families studied. This presents a striking contrast to the fact that there are many families with SCA1 in Hokkaido and the Tohoku area of Japan. These findings suggest geographic variation in autosomal dominant cerebellar ataxias in Japan.

Key Words trinucleotide repeat, CAG repeat, spinocerebellar ataxia type 1, Machado-Joseph disease, dentatorubral-pallidoluysian atrophy

INTRODUCTION

The autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders characterized by variable combinations of cerebellar ataxia, ophthalmoplegia, pyramidal signs, extrapyramidal signs and peripheral neuropathy (Harding, 1993). The ataxias typically have onset in adult life, though there are exceptions. The clinical classification of dominantly inherited ataxias has proved difficult and unreliable due to variation and overlapping of clinical features both between and within families. In recent years, however, much progress

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has been made toward the development of a genetic classification for the ataxias. To date, seven different gene loci causing dominantly inherited ataxia have been mapped: spinocerebellar ataxia type 1 (SCA1) on chromosome 6p22-23 (Orr *et al.*, 1993), SCA2 on 12q23-24.1 (Gispert *et al.*, 1993), Machado-Joseph disease (MJD)/SCA3 on 14q32.1 (Kawaguchi *et al.*, 1994; Twist *et al.*, 1995), SCA4 on 16q24-ter (Gardner *et al.*, 1994), SCA5 on the centromeric region of chromosome 11 (Ranum *et al.*, 1994), SCA7 on 3p14-21 (Gouw *et al.*, 1995), dentatorubral-pallidoluysian atrophy (DRPLA) on 12p12-ter (Nagafuchi *et al.*, 1994). Among these disorders, the mutations responsible for SCA1, MJD and DRPLA have been identified as expansion of a CAG trinucleotide repeat in the coding region of the disease genes (Orr *et al.*, 1993; Kawaguchi *et al.*, 1994; Koide *et al.*, 1994; Nagafuchi *et al.*, 1994). The mechanism by which CAG repeat expansion causes neurodegeneration is unknown.

The identification of the SCA1, MJD and DRPLA mutations provided us with the means for accurate classification and diagnosis of these disorders. To elucidate the characteristics of the autosomal dominant cerebellar ataxias in the Kinki area of Japan, we have collected blood samples from 47 families with dominant ataxia and determined the frequency of the SCA1, MJD and DRPLA mutations in this area.

MATERIALS AND METHODS

Families. A total of 67 patients from 47 families were neurologically examined by the authors and considered to have an autosomal dominant cerebellar ataxia. All the families lived in the Kinki area which is located in the western part of the main island of Japan and consists of Nara, Osaka, Hyogo, Kyoto, Shiga and Wakayama Prefectures. In this study, there were no patients who lived in Wakayama Prefecture. As far as could be traced, the original residence of the 47 families was as listed in Table 1.

DNA analysis. Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures. Polymerase chain reaction (PCR) for detection of

Table 1. Original residence of the 47 families with autosomal dominant cerebellar ataxia in the Kinki area.

Place of original residence	Total	MJD	DRPLA	SCA1
Kinki area	37	17	5	0
Shikoku area	3	1	1	0
Kyushu area	3	2	0	0
Kanto area	2	2	0	0
Toukai area	1	0	0	0
Korea	1	0	0	0
Total	47	22	6	0

the SCA1, MJD and DRPLA mutations was carried out using the following published primers: Rep1 and Rep2 for SCA1 (Orr *et al.*, 1993), MJD52 and MJD70 for MJD (Kawaguchi *et al.*, 1994) and CTG-B37 primers for DRPLA (Li *et al.*, 1993; Koide *et al.*, 1994; Nagafuchi *et al.*, 1994). For determination of the CAG repeat length in the SCA1 and DRPLA genes, the PCR products were electrophoresed on 6% denaturing acrylamide gels and compared with an M13 sequencing ladder according to the previous methods (Orr *et al.*, 1993; Koide *et al.*, 1994; Nagafuchi *et al.*, 1994). For determination of the CAG repeat length in the MJD gene, the PCR products were directly sequenced using an automated DNA sequencer (Applied Biosystems, model 373A) as described (Matsumura *et al.*, 1996).

RESULTS

MJD repeat expansion

Of the 47 families with autosomal dominant cerebellar ataxia, 31 patients from 22 families (47%) were found to have expanded CAG repeats in the MJD gene. Figure 1a shows the distribution of CAG repeat length in normal and expanded alleles of the 31 patients. Normal alleles ranged from 14 to 37 repeats, with a peak at 14 repeats in 15 (48%) of the patients. Expanded alleles ranged from 64 to 84 repeats (mean $\pm$ SD $=$ 74.7 $\pm$ 4.2). All the patients were heterozygous for the MJD mutation, with one allele in the mutated range and the other in the normal range. The mean age of onset of the 31 patients was 38.6 $\pm$ 11.4 years, with a range of 13 to 60. Figure 2a shows the relationship between the age of onset and the repeat length in the MJD gene. We found a significant inverse correlation between these two factors ($r = -0.830$, $p < 0.0001$). Juvenile onset MJD individuals (age $<$ 20) had 81 to 84 repeats, while late onset (age $>$ 50) showed 69 to 70 repeats.

DRPLA repeat expansion

Ten patients from six families (13%) were positive for the DRPLA trinucleotide repeat expansion. Figure 1b shows the distribution of CAG repeat length in normal and expanded alleles of the 10 patients. Expanded alleles had 57 to 72 repeats, whereas normal alleles had 10 to 19 repeats. All the patients were heterozygous for the DRPLA mutation. As is the case with MJD, a significant inverse correlation ($r = -0.840$, $p = 0.0024$) was observed between the age of onset and the repeat length in the DRPLA gene (Fig. 2b).

SCA1 repeat expansion

No SCA1 repeat expansion was found among the families studied. Every subject had two normal alleles with less than 37 repeats in the SCA1 gene. Sequence analysis of the largest allele (36 repeats) revealed that the CAG repeat tract was interrupted by two CAT trinucleotides (data not shown). This finding is

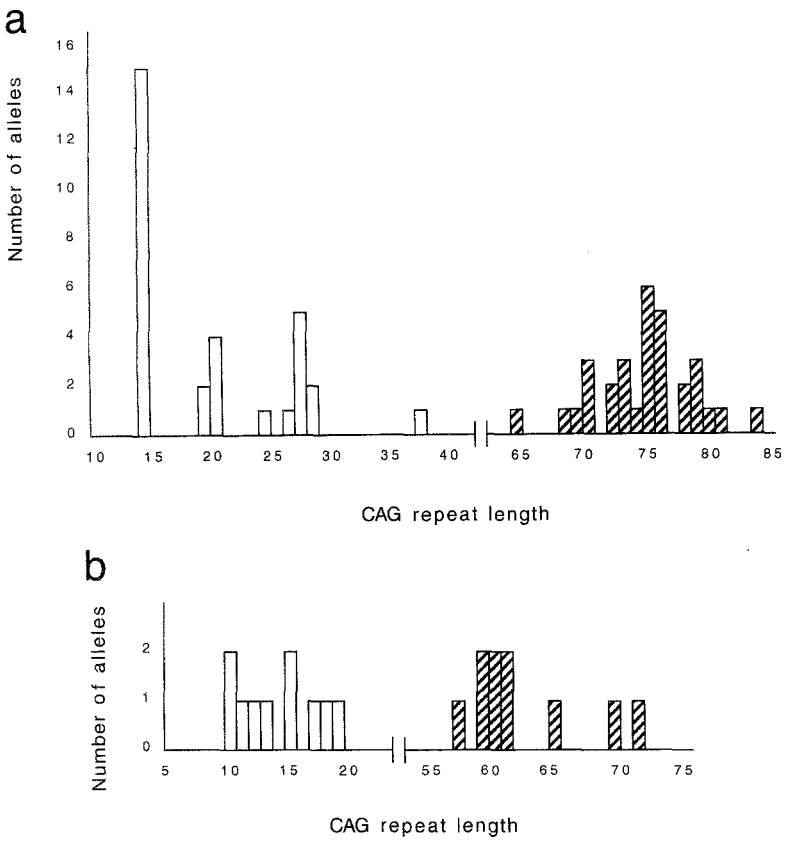

Fig. 1. Distribution of CAG repeat length in the MJD gene (a) and the DRPLA gene (b). Thirty-one MJD patients (a) and 10 DRPLA patients (b) were studied. Open bars represent normal alleles; hatched bars, expanded alleles.

consistent with the characteristics of normal alleles previously reported (Chung *et al.*, 1993).

The remaining 26 patients from 19 families were not classified as either MJD, DRPLA or SCA1. These families were too small to carry out linkage analysis for other disease loci such as SCA2, SCA4 and SCA5.

DISCUSSION

To assess the frequency of the MJD, DRPLA and SCA1 mutations in the Kinki area of Japan, we studied 47 families with dominant ataxia who lived in this area. The following results were obtained. First, approximately half of the families were found to be positive for the MJD repeat expansion, indicating that MJD is the most common dominantly inherited ataxia in the Kinki area of Japan. Second, no SCA1 repeat expansion was found among the families. This presents a striking

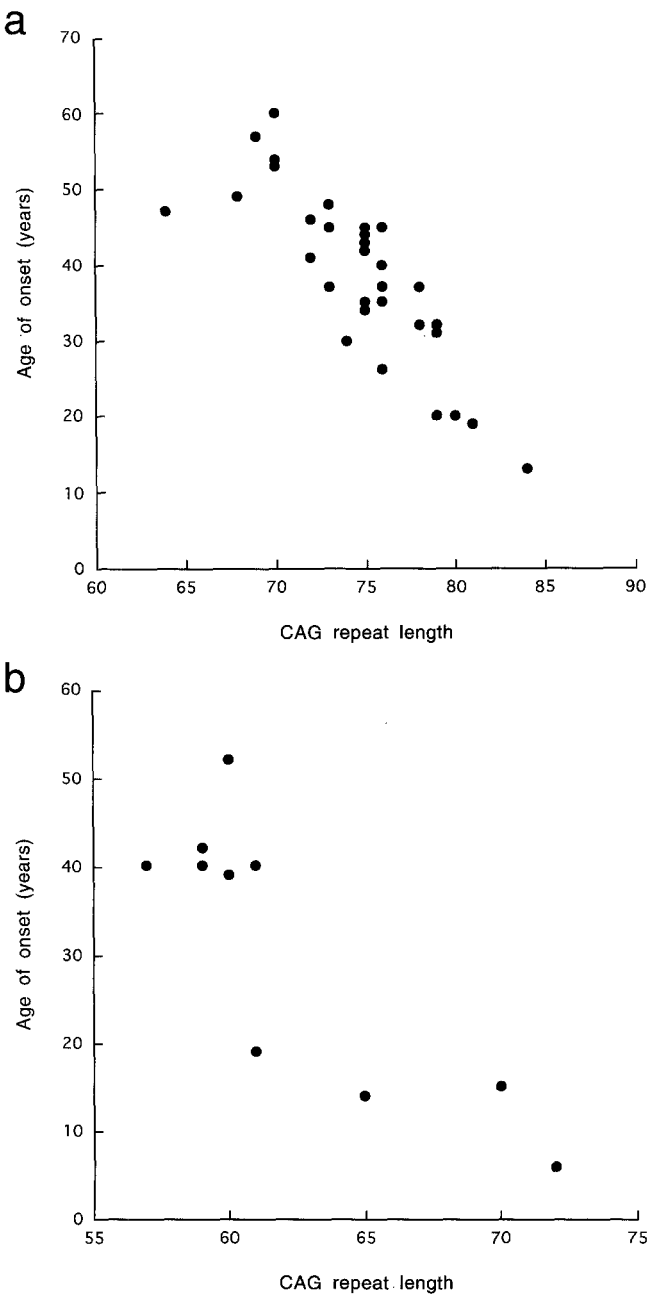

Fig. 2. Correlation between age of onset and CAG repeat length in MJD (a) and DRPLA (b).

contrast to the fact that there are many families with SCA1 in the Tohoku area and Hokkaido both of which are located in the northern part of Japan (Kameya *et al.*,

1995; Suzuki *et al.*, 1995). Among 25 families with dominant ataxia in the Tohoku area, 48% were positive for the SCA1 mutation, whereas 32% showed the MJD repeat expansion (Kameya *et al.*, 1995). This finding suggests that not MJD but SCA1 is the most common dominant ataxia in the Tohoku area. In Hokkaido, 10 SCA1 families have been reported. Interestingly, detailed haplotype analysis suggested that SCA1 in Hokkaido derives from a single common ancestry in the Tohoku area (Wakisaka *et al.*, 1995). In our study, no family migrated from the Tohoku area where the SCA1 mutation is frequent (Table 1). Most families originated from the Kinki area. Although our study did not contain all of the families with dominant ataxia in the Kinki area, we can conclude that the SCA1 mutation is rare in the Kinki area. Thus, these findings suggest geographic variation in autosomal dominant cerebellar ataxias in Japan. This variation may reflect the distribution of ancestral mutations of the diseases.

Ranum *et al.* (1995) studied the frequency of the SCA1 and MJD mutations in 149 dominant ataxia families with different ethnic backgrounds such as African-American, Caucasian-American and Asian. Of these, 3% had the SCA1 repeat expansion and 21% were positive for the MJD repeat expansion. Screening for the DRPLA mutation was not carried out. Similarly, Silveira *et al.* (1996) reported that among 29 families with dominant ataxia collected from North America, India and Brazil, the frequencies of the SCA1 and MJD mutations were 10% and 17%, respectively. The DRPLA mutation was found only in one family of Japanese origin. To date, only six families with DRPLA have been reported in non-Japanese populations (Burke *et al.*, 1994; Potter *et al.*, 1995; Warner *et al.*, 1995). In contrast, six families in the Kinki area (this study) and 40 families in other areas of Japan (Ikeuchi *et al.*, 1995; Komure *et al.*, 1995) were positive for the DRPLA repeat expansion. Thus, the DRPLA mutation seems to be common only in the Japanese. We could not compare the frequency of the DRPLA mutation in the Kinki area with that in other areas of Japan because we had little information on addresses of the 40 families with DRPLA reported elsewhere.

Distributions of CAG repeat length in expanded alleles from MJD and DRPLA patients in the Kinki area were similar to those previously reported (Ikeuchi *et al.*, 1995; Komure *et al.*, 1995; Maciel *et al.*, 1995; Maruyama *et al.*, 1995; Takiyama *et al.*, 1995). We also found a significant inverse correlation between the age of onset and the repeat length in both MJD and DRPLA. These findings indicate that the MJD and DRPLA repeat expansions in the Kinki area have the same molecular basis as those in other areas of Japan and foreign countries.

The cloning of the MJD, DRPLA and SCA1 genes allowed us to make an accurate diagnosis in families too small for linkage analysis. However, 40% of the families in our study were not classified as either of these diseases. Therefore, the genes responsible for SCA2, SCA4, SCA5 and SCA7 need to be identified.

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