CHARACTERISTICS OF DYNAMIC MUTATION IN JAPANESE MYOTONIC DYSTROPHY

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Summary To study the characteristics, if any, of unstable CTG repeat sequence in Japanese myotonic dystrophy (DM), we analyzed DNA from 351 at risk individuals (including affected and non-affected carriers and their descendants) from 105 families in Japan. A total of 93 DM families (196 affected and 116 unaffected individuals), including 84 DM parent-child pairs (44 father-child and 40 mother-child pairs), were examined, many of which had been previously tested by linkage analysis. We detected unstable CTG repeat mutations between 0.15 kb and 8.7 kb in size. The size of the mutation correlated with the age of onset of symptoms. There was a significant difference in DM allele size among the four groups (congenital, juvenile onset, classical, and minimal). Congenital DM had on average the largest repeat sizes. Comparison of parent-child pairs showed that most offspring had a larger repeat size than their parents, with only 2 of 84 showing a definite decrease in repeat size. The correlation coefficients for maternal and paternal transmission were 0.41 and 0.15, respectively. The parental age (maternal and paternal) did not correlate with intergenerational change of repeat. These observations are similar to those reported in Caucasians.

Key Words myotonic dystrophy, unstable CTG repeat sequence, anticipation, dynamic mutation, contraction

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INTRODUCTION

Myotonic dystrophy (DM) is an autosomal dominant, multisystemic disease characterized by highly variable expression in both age at onset and clinical severity. The manifestations include myotonia, muscle weakness, cataracts, cardiac arrhythmias, frontal baldness, impaired glucose tolerance, and mental retardation (Harper, 1989). DM is the most prevalent adult muscular dystrophy in Europe, North America, India, and North Eastern Asia, with prevalence of 0.2–9.9 per 100,000 individuals (Araki, 1976; Harper, 1989; Nakagawa *et al.*, 1991). The mutation causing DM has been characterized as an expansion of the unstable CTG trinucleotide repeat in the 3'-untranslated region (UTR) of the DM protein kinase (DM-PK) gene located in chromosome 19q13.3 (Brook *et al.*, 1992; Buxton *et al.*, 1992; Fu *et al.*, 1992; Harley *et al.*, 1992a; Mahadevan *et al.*, 1992). Statistical data on DM mutation are essential for genetic counseling. The characteristics of DM dynamic mutation have been reported in English (Harley *et al.*, 1992b, 1993), French (Lavedan *et al.*, 1993), Spanish (Cobo *et al.*, 1993), Canadian (Hunter *et al.*, 1992; Tsilfidis *et al.*, 1992; Barcelo *et al.*, 1993), Australian (Mulley *et al.*, 1993), and American people (Ashizawa *et al.*, 1992, 1993, 1994a; Redman *et al.*, 1993). We previously showed that CTG expansion was also detected in Japanese DM (Yamagata *et al.*, 1992).

In the present study, we analyzed a large sample of Japanese DM families to further appreciate the characteristics of Japanese DM mutation.

MATERIALS AND METHODS

Families. Blood samples of 351 individuals from 105 Japanese families including 93 DM families (196 affected, 116 unaffected) and 12 muscular disease families (with floppy infants of unknown cause, Duchenne muscular dystrophy, parkinsonism, and amyotrophic lateral sclerosis, *etc.*) were collected from all over Japan. In our DM families, there were 84 DM parent-child pairs (44 father-child and 40 mother-child). All were Japanese. The clinical diagnosis of DM was based on the diagnostic criteria (Griggs *et al.*, 1989), and we obtained careful histories from patients and their families to document the age of onset. Muscle weakness, myotonia or cataract as first noted by the patient, the parents or the doctor defined the onset of the disease. Other symptoms, such as school difficulties or poor performance in sports, were recorded but were not considered specific enough on retrospective evaluation to mark the onset of DM.

All subjects were referred to us for molecular analysis in a context of presymptomatic, prenatal or confirmative diagnosis. According to severity and the age of onset, we grouped the patients into four different classes: (1) congenital DM—onset between 0–1 year (facial weakness, hypotonia, respiratory distress, and hydram-

nios, *etc.*), (2) juvenile onset DM (1–19 year), (3) classical DM (onset ≥ 20 year), (4) minimal or subclinical DM—no noted symptoms other than cataracts (onset > 50 year) and asymptomatic obligate carriers.

DNA analysis. High molecular weight DNA was extracted from whole blood, lymphoblastoid cell lines, or chorionic villus samples (CVS) according to standard procedures. The DNA was analyzed with the p5B1.4 probe after restriction enzyme digestion with *EcoRI* or *BglII* as previously reported (Shelbourne *et al.*, 1992). PCR analysis was also performed to study both the non-DM individuals and the DM patients showing a small increase of the CTG repeat (< 0.5 kb) on Southern blots (Davies *et al.*, 1992).

Statistical methods. All statistical analyses were carried out with the program package, Stat View (Abacus Concepts, Inc.). It consisted of Wilcoxon rank sums test in a non-parametric analysis, ANOVA (one-way analysis of variance), Bonferroni *t*-test, Pearson's coefficient, and chi-square test with Yates' correction.

RESULTS

Relationship of CTG repeat size to phenotype of the disease

As was expected, DM-specific CTG repeat expansion was observed. All definite DM patients had repeat expansions, and both healthy individuals and non-DM patients had no expansions. The normal stable upper limit was 35 repeats. Information on the clinical onset of features of DM and CTG mutation was available in a total of 93 affected individuals, from 93 unrelated DM families. Figure 1 shows the relationship of repeat length to age at onset, which indicated strong negative correlation ($r = -0.587$, $p = 0.0001$). No significant difference was seen when the data were divided by sex (data not shown).

Next, we grouped the patients into four classes—(1) congenital DM, (2) juvenile onset DM, (3) classical DM, and (4) minimal DM, and plotted the CTG repeat length, respectively (Fig. 2). Clinical and molecular data were available in 184 of the families studied. They included 27 congenital DM, 29 juvenile onset DM, 110 classical DM, and 18 minimal DM. An analysis of variance was performed (ANOVA and Bonferroni *t*-test). While there was overlap of repeat sizes between patient categories, there was nevertheless a correlation of increasing repeat size with disease severity. The largest repeat sizes on average belonged to congenital DM, and the smallest did to minimal DM. Figure 2 showed that there was a significant difference in repeat size among the four groups ($p < 0.001$).

Intergenerational differences in CTG repeat size

To investigate the relationship between CTG expansion and anticipation, we studied 84 parent-child pairs in which both parent and child had the expansion. In 76 (90%) of the 84 pairs, an increase in CTG repeat size was seen. In contrast, 2 (2%) of the 84 pairs showed a definite decrease between two generations, but

Fig. 1. Correlation of ages of onset with expansions of the CTG repeat. A Pearson's correlation coefficient of $r = -0.587$, $p = 0.0001$ ($n = 93$) was obtained. The figure displays a regression line and 95% confidence bands for the true mean of Y.

Fig. 2. Size of the CTG repeat and the age of onset of disease in 184 DM patients. The categories for age of onset are congenital for symptoms up to 1 year of life, juvenile for onset from 1 to less than 20 years, classical at 20 years or older, and minimal for no noted symptoms other than cataract.

Fig. 3. CTG repeat length for 84 parent-child pairs. (A) 44 father-child pairs. (B) 40 mother-child pairs. Points above the diagonal indicate anticipation (repeat size greater in child than in parent), and points below the diagonal indicate contraction. The figures display regression lines and 95% confidence bands for the true mean of Y.

in one of two cases, age of onset was earlier in the child than in the father. The exceptions in age of onset may be explained by bias in ascertainment, which was often based on the patient's recollection of his first awareness of symptoms.

For father-child pairs and mother-child pairs, repeat size is shown in Fig. 3 (A), and (B), respectively. Both analyses showed a weak positive correlation between parent and child (father-child: $r=0.154$, $p=0.32$; mother-child: $r=0.419$, $p=0.007$). The increase in size of repeat was greater in transmission from females than in one from males. Almost all cases of congenital DM were transmitted by affected mothers, except one pedigree (Ohya *et al.*, 1994).

Parental age effect on DM mutation

To examine whether older parental age influences upon fresh DM mutation, we compared CTG repeat length and parental age at birth. A Pearson's correlation coefficient of $r=0.095$, $p=0.61$ (Y: intergenerational variation (kb), X: father's age at birth (year)), and $r=0.178$, $p=0.32$ (Y: intergenerational variation (kb), X: mother's age at birth (year)) were obtained. Neither paternal nor maternal age effect was observed.

DISCUSSION

We investigated large DM families to obtain prognostic information. There were striking similarities between our findings in the Japanese population and those in Caucasians: there was a strong correlation between the size of the repeat and the age of onset. We confirmed previous reports for such a correlation. However, we also found clinical variation without a corresponding variation in repeat length, and *vice versa*. As Harley *et al.* (1993) has speculated, the overlap in the range of repeat sizes between congenital cases and others indicates that repeat size is not the only feature determining congenital DM. Factors, such as somatic mosaicism and/or environmental factors may contribute to the onset or severity of the phenotype. Thus, it is important to take into account that there are exceptions, especially in classical DM, when performing DNA analysis for evaluation of prognosis.

The tendency for anticipation is strikingly demonstrated in Fig. 3, with only two instances of contraction in repeat size in the offspring. However, one of the two revealed earlier onset age in the offspring regardless of contraction (father 3.2 kb, classical DM; daughter 2.2 kb, juvenile onset DM). Ashizawa *et al.* (1994b) examined 1,489 DM parent-offspring pairs and reported that the contraction occurred in 10% paternal transmissions, and 3% maternal transmissions. Surprisingly, 48% of such cases showed anticipation, earlier age of onset in successive generations. We confirmed such a discrepancy.

In the present study, DNA expansion was analyzed mostly in lymphocytes, and it is probable that different degrees of expansion may cause varying pheno-

typic effects in relevant tissues, such as muscle. For evaluation of severity or prognosis, it may be necessary to know the properties of the protein involved, which is predicted to belong the protein kinase family. To date, the effect of expansion of the repeat in the 3'-UTR of the gene has been suggested to be quantitatively related either to mRNA stability or to regulation of transcription of neighboring genes.

Jones *et al.* (1975) reported a paternal age effect in some autosomal dominant diseases. Brown (1979) reviewed autosomal dominant inheritance (most cases resulting from new mutation) because of the paternal age effect and the low frequency of parental consanguinity. To date, progeria has been considered to have parental age effect. DM is one of the progeroid syndromes. However, in the present study we did not detect a paternal or maternal age effect for intergenerational changes of CTG repeat size.

Finally, molecular data will allow improved diagnosis of the disease and it may also help with the evaluation of the risk of disease in the progeny of at risk individuals.

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