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SHORT COMMUNICATION

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Further evidence for a major ancient mutation underlying myotonic dystrophy from linkage disequilibrium studies in the Japanese population

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Abstract The myotonic dystrophy (DM) mutation is an unstable $(CTG)_n$ repeat, present at a copy number of 5–37 repeats on normal chromosomes but amplified to 50–3000 copies on DM chromosomes. Previous findings in Caucasian populations of a DM founder chromosome raise a question about the molecular events involved in the expansion mutation. To investigate whether a founder chromosome for the DM mutation exists in the Japanese population, we genotyped families using polymorphic markers near the $(CTG)_n$ repeat region and constructed haplotypes. Six different haplotypes were found and DM alleles were always haplotype A. To find an origin of the $(CTG)_n$ repeat mutation and to investigate the mechanism of the expansion mutation in the Japanese population we have studied 90 Japanese DM families comprising 190 affected and 130 unaffected members. The results suggest that a few common ancestral mutations in both Caucasian and Japanese populations have originated by expansion of an ancestral $n = 5$ repeat to $n = 19$–37 copies. These data support multistep models of triplet repeat expansion that have been proposed for both DM and Friedreich's ataxia.

Key words Myotonic dystrophy · CTG repeat · Haplotype A · Linkage disequilibrium · Multistep model

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Introduction

Myotonic dystrophy (DM) is the commonest adult muscular dystrophy in Europe, North America, India, and Northeastern Asia, with a reported prevalence of 0.2–9.9 per 100,000 individuals (Araki 1976; Harper 1989; Nakagawa et al. 1991). The mutation causing DM has been identified as an expansion of an unstable trinucleotide $(CTG)_n$ repeat in almost all ethnic populations (Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992).

Osame and Furusho (1983) carried out the first detailed epidemiological studies in Kagoshima prefecture and suggested the possibility of a DM founder chromosome in this geographically small region of Japan. Imbert et al. (1993) analyzed an Alu insertion/deletion polymorphism and a $(CA)_n$ repeat marker close to the DM mutation in 149 French families, and reported that most affected individuals surveyed had one haplotype, assumed to reflect the founder lineage in their study samples. They also proposed that alleles with 19–30 CTG repeats are always associated with the Alu insertion allele and these may serve as a reservoir for recurrent mutations to unstable alleles with 30–50 repeats. The situation is very similar to that proposed for Friedreich's ataxia (FRDA), where almost all large normal alleles (16–34 GAAs) in the Caucasian population stem from a single founder expansion (Cossée et al. 1997). The ancestral haplotype hypothesis is further supported by the findings of Neville et al. (1994), who performed a high-resolution genetic analysis of the locus using polymerase chain reaction (PCR)-based assays of nine polymorphisms immediately flanking the DM repeat. With the exception of one recently reported case from Africa (Krahe et al. 1995), all cases of DM in the world appear to share a single haplotype that contains these putative at-risk CTG alleles. However, Rubinsztein et al. (1994) have reported that some chromosomes with more than 19 CTG repeats were found to be associated with the deletion allele in non-Europeans, indicating that repeat expansions can occur on haplotypes other than type A in the nomenclature of Neville et al.

(1994). The present study is an extension of preliminary results previously reported using DM families from a different and diverse population. The purpose of this work was to find the origin of the DM mutant allele and to investigate the mechanism of the expansion mutation in DM patients in the Japanese population. We confirm that the expansions occur on chromosomes of haplotype A as described by Neville et al. (1994), but we find a different frequency distribution pattern of the six haplotypes in the Japanese population compared with the distribution pattern previously reported for Caucasians.

Subjects and methods

Subjects

We studied 90 Japanese families with DM comprising 190 affected and 130 unaffected individuals, and 12 non-DM families comprising 36 individuals. DM families were ascertained from geographic locations all over Japan, although the majority of the samples were collected from western areas of Japan. Informed consent was obtained from the families.

Detection of polymorphisms in the DM protein kinase (*DMPK*) gene

Genomic DNA was extracted from peripheral blood leukocytes. DNA extraction, restriction enzyme digestion, Southern transfer, and hybridization were performed by standard methods (Sambrook et al. 1989). DNA probes used in this study and their sources are previously reported: D19S63 (Brook et al. 1991), p37.1 (Yamagata et al. 1992), and p5B1.4 (Shelbourne et al. 1992). We determined the length of the CTG repeat using previously described methods involving PCR (Davies et al. 1992) and Southern hybridization (Shelbourne et al. 1992). PCR conditions used for pCN400, intron11, exon 10, intron 9, intron 8, intron 5, intron 4 and N9 were as detailed in Neville et al. (1994) with the following modifications: the restriction enzymes for PCR–restriction fragment length polymorphism were modified from Fnu4HI to BbvI (intron 11), from BpmI to BsrI (exon 10), and from HphI to BanI (intron 4).

Genotyping in families and linkage disequilibrium analysis

We carried out haplotype segregation analysis for each family. Haplotypes were determined with a method previously described by Neville et al. (1994). A pCN400-TaqI polymorphism is located approximately 15 kb centromeric to the *DMPK* gene. An N9-DraIII polymorphism is located within 1 kb telomeric to exon 1 of the *DMPK* gene. The CTG repeat and eight polymorphic markers are all within a 30 kb region, within and flanking the *DMPK* gene on chromosome 19q13.3. We genotyped family members and produced haplotypes in the *DMPK* gene. We then determined the haplotype frequency in DM and non-DM Japanese individuals using eight polymorphisms, and then compared the frequency with the report by Neville et al. (1994). Due to the lack of DNA for Southern hybridization or limited numbers of informative family members, we could not determine all the haplotypes. Seventy families were successfully haplotyped and 20 families failed complete haplotyping, although several family members could be haplotyped. The exact chromosome numbers counted for haplotyping were 230 for non-DM and 90 for DM patients. Only normal chromosomes for which the genotype could unequivocally be determined were included in the disequilibrium analysis. The χ^2 test was used to compare allele frequencies between DM patients and each group. A value of $P < 0.05$ was considered significant.

Results

A–K haplotypes

To find a founder chromosome of the mutated DM gene, we genotyped the families intensively using polymorphic markers close to the repeat and constructed haplotypes. The three basic ancestral lineages detected by Neville et al. (1994) in Canadians are characterized by haplotype A (2,2,1,2/TaqI, BsrI, 1 kb I, BanI), haplotype C (1,1,2,1/TaqI, BsrI, 1 kb D, BanI), and haplotype D (1,2, 2,2/TaqI, BsrI, 1 kb D, BanI). Table 1 shows the frequency of haplotypes A–D in the Japanese population. The two distinct ethnic groups have the same single DM haplotype, equivalent to haplotype A. Ninety-three percent of individuals showed one of the three founding haplotypes, while 4.8% of the sample showed haplotype J, and 1.3% and less than 1% of the samples showed haplotypes K and G, respectively. Haplotypes B, E, F, H, and I were not found in this study. However, haplotypes J and K in Table 1 are not found in the Canadian population. Haplotype A is very frequent in the Canadian population (49%), but less so in the Japanese (30%), where haplotype D is the commonest (53%).

As we have reported before (Davies et al. 1992), it seems evident that there is a different distribution of repeat length alleles at the DM locus when comparing Japanese and Caucasian populations. Nevertheless, haplotype A is the commonest DM haplotype in both populations. Haplotype K is almost the same as haplotype A (differing only at the pCN400 site), but DM has not been reported associated with haplotype K. From these results, we conclude that an ancestral mutation occurred once or extremely rarely that gave rise to an expanded $(CTG)_n$ repeat associated with haplotype A which was subsequently unstable and predisposed to full mutation.

Linkage disequilibrium analysis

Allele typing for four polymorphic loci encompassing about 100 kb around the $(CTG)_n$ repeat was performed on unre-

Table 1 Haplotype frequency in DM and non-DM Japanese individuals

Haplotype	1 pCN400 (TaqI)	2 Intron 11 (BbvI)	3 Exon 10 (*Bsr*I)	4 Intron 9 (*Hin*fI)	5 Intron 8 (1 kb Δ)	6 Intron 5 (HhaI)	7 Intron 4 (BanI)	8 N9 (DraIII)	Frequency (%) Neville et al. (1994)	This study
DM	2	1	2	2	1	1	2	1	100	100
A	2	1	2	2	1	1	2	1	49	30
B	1	2	2	1	2	2	2	2	27	0
C	1	2	1	1	2	2	1	1	16	10
D	1	2	2	1	2	2	2	1	8	53
E	2	2	2	1	2	2	2	2	<1	0
F	1	2	2	1	2	2	1	1	<1	0
G	2	1	1	2	1	1	2	1	<1	<1
H	2	2	2	2	2	2	2	2	<1	0
I	1	1	2	2	2	2	1	1	<1	0
J	1	2	1	1	2	2	2	1	0	4.8
K	1	1	2	2	1	1	2	1	0	1.3
	1:676 bp 2:574 bp	1:282 bp 2:180 + 102 bp	1:350 bp 2:281 bp	1:253 bp 2:151 bp	1:1008 bp 2:494 bp	1:491 bp 2:419 bp	1:145 bp 2:124 bp	1:183 bp 2:158 bp		

Table 2 Analysis of linkage disequilibrium and relation to haplotype

		D19S63-HincII		*D19S63-PvuII*			*p37.1-BamHI*		*Alu 1 kb I/D*		
	$(CTG)_n$ repeat	1	2	1	2	3	1	2	Ins	Del	Haplotype
Normal	5	15*	8	2**	18	3	10**	17	50	0	A > K
	11–13	17	27	16*	24	12	44	16	4**	142	D > C > J > A
	14–18	6	6	3	1	5	7*	5	12**	17	A = J > D
	19–35	3	6	4	1	5	11	0	27	0	A ≫ G
DM	>50	9	18	9	4	13	32	3	90	0	A
		(1:20 kb	2:15 kb)	(1:7.1 kb	2:6.8 kb	3:6.5 kb)	(1:5.6 kb	2:5.3 kb)			

Asterisks show significant linkage disequilibrium observed between DM and normal repeat: * $P < 0.05$; ** $P < 0.01$

lated normal Japanese and DM chromosomes. The order of the markers is cen–D19S63–p37.1–Alu I/D–qter. Haplotype analysis between DM and normal chromosomes detected linkage disequilibrium similar to previously reported values. A striking level of linkage disequilibrium was observed when normal alleles were grouped according to repeat length (Table 2). Strong linkage disequilibrium was detected at the p37.1 locus between DM and $n = 5$ repeats. In the case of the D19S63 locus, which is located about 135 kb telomeric of the repeat, weak linkage disequilibrium was detected. These data further substantiate the notion of a limited number of predisposing normal alleles giving rise to present-day DM mutations and document that the Japanese population behaves in the same way as Caucasians in this respect.

Discussion

From these results we can surmise the following. (a) Little recombination has occurred between the $(CTG)_n$ repeat and the insertion/deletion polymorphism on chromosomes carrying normal alleles. If recombination had occurred, we would not detect linkage disequilibrium. (b) The $(CTG)_n$ repeat in the unaffected range is stably transmitted. If it were unstable, we would expect to see more insertion alleles in the $n = 6$–10 range or deletion alleles in the $n > 14$ range. We conclude from these data that few recombination events and stable transmission of repeat alleles has continued from the time when the Mongolian and the Caucasian populations separated. To confirm this, we need to analyze more DNA from different Mongolian peoples. Recently, a 1 kb deletion allele was reported in a Nigerian DM family (Krahe et al. 1995). This finding confirms that an Alu element insertion at the DM locus is not a necessary part of the predisposing haplotype and strongly suggests that the only critical component is the repeat expansion itself.

This is the first molecular evidence for a founder chromosome in Kagoshima prefecture, the location where Osame and Furusho (1983) carried out epidemiological studies of DM. Our data suggest that alleles with $n = 19$–37 may act as a reservoir responsible for successive premutation into the range $n = 40$–50. A reservoir pool with $n = 19$–37 might be derived from $n = 5$ alleles based on haplotypes. More recently, Cossée et al. (1997) reported the evolution hypothesis of the FRDA $(GAA)_n$ expansion similar to DM. The model that we have described promises to be invaluable in studying triplet repeat expansion mechanisms.

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