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SHORT REPORT

Haplotype analysis of the myotonic dystrophy type 1 (DM1) locus in Taiwan: implications for low prevalence and founder mutations of Taiwanese myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder caused by a CTG trinucleotide expansion at the *DM1* locus. In this study, we investigated the frequency distribution of various CTG repeats in normal alleles and haplotyped the normal and expanded *DM1* locus in a group of Taiwanese people. In the 496 normal chromosomes examined, up to 18 alleles with different CTG lengths from 5 to 30 repeats were found and the frequency of $(CTG)_{>18}$ alleles was only 1.4% (7/496), predicting a low prevalence of DM1. In addition, there is no absolute association between $(CTG)_{5-19}$ alleles and *Alu* insertion/deletion polymorphism observed on normal chromosomes. All *DM1* alleles examined, however, were found to be associated with the *Alu* insertion. Further detailed genetic analysis demonstrated that at least eight haplotypes, including a new haplotype (L), were present in the Taiwanese population and that all *DM1* alleles were with the same haplotype (haplotype A) as that identified in Canadian and Japanese DM1 populations. These findings support the notion that the out-of-Africa *DM1* alleles were originated by stepwise expansion from a pool of large-sized normal chromosomes with haplotype A. *European Journal of Human Genetics* (2001) **9,** 638–641.

Keywords: CTG repeats; DM1; haplotype; founder effect; Taiwan

Introduction

Myotonic dystrophy type 1 (DM1; OMIN 160900) is an autosomal dominant disorder characterised by myotonia in conjunction with progressive weakening and wasting of skeletal muscle.[1] DM1 is one of the genetic disorders exhibiting anticipation in families, ie, an increase in clinical severity and an earlier age of onset in successive generations, due to the intergenerational expansion of (CTG)n in the 3′-untranslated region of the myotonic dystrophy type 1 protein kinase gene (*DM1PK*) on chromosome 19.[2] The array length of tandemly repeated CTGs in normal alleles is variable, with a copy-number range of 5–37, whereas, in *DM1* alleles, the copy number usually is greater than 50.

The prevalence of DM1 was found to be 2.2–5.5 in the Western European population[1] and 5.5 in the Japanese population.[3] However, a very low prevalence of DM1 in central and Southern Africans, Southern Chinese, and Thai was reported.[4] The prevalence of DM1 in the population appeared to correlate with the frequency of large CTG repeats (>18 repeats) within the normal range.[5–7] Therefore, in this study we investigated the frequency of those large-sized

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normal alleles in the Taiwanese population in an attempt to verify whether or not it correlates well with the low DM1 prevalence predicted.

It was also known that all *DM1* chromosomes in the Canadian and Japanese populations were exclusively associated with the *Alu* insertion and seven additional single base polymorphic markers (haplotype A),[8,9] suggesting a founder effect for the origin of myotonic dystrophy type 1 mutation. In the Africans, however, a reported *DM1* chromosome was found to be associated with the haplotype D allele.[10] In addition, a few normal chromosomes with more than 18 CTG repeats were also found to be associated with the *Alu* deletion allele in other diverse ethnic populations.[11,12] These findings raise the possibility that other non-A type *DM1* alleles may be present in non-African DM1 populations as well. To investigate the genesis of the expansion mutation of Taiwanese *DM1* chromosomes, we studied linkage disequilibrium between CTG repeats and the haplotypes. We confirm that the pathogenic expansions occur on chromosomes of haplotype A. However, we also find a different frequency distribution pattern of the haplotypes in the Taiwanese population compared with those defined in other ethnic populations.[8,9]

Subjects and methods

Subjects

The study of (CTG)n repeats frequency distribution and the *Alu* insertion/deletion polymorphism was performed using DNA samples from 248 unrelated, normal individuals and 17 DM1 patients. Haplotype analysis was performed in 42 non-DM1 and 12 DM1 Taiwanese families. The numbers of chromosome for which the haplotype could unequivocally be determined were 75 for non-DM1 and 13 for DM1 patients.

Determination of (CTG)n repeat size

Genomic DNA was isolated from peripheral blood leukocytes or lymphoblasts transformed with Epstein-Barr virus using Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). (CTG)n repeat size of the normal as well as *DM1* alleles was determined as described previously.[13] Statistical values were calculated using the χ^2 test in 2×2 tables with Yates' correction.

Haplotype analysis

The polymorphic markers used to construct haplotypes in this study were previously published.[8,14,15] In a total of 20 μl reaction, genomic DNA (100 ng) was PCR amplified with the appropriate primers (0.375 μM) in the presence of 200 μM each dNTP, 1× PCR buffer I, and 0.5 U FailSafe^{TM} PCR enzyme mix (Epicentre Technologies, Madison, WI, USA). For *Hinf*I-, *Bpm*I-, and *Fnu4*HI-polymorphism, amplification was conducted for 40 cycles at 96°C for 0.5 min, 55°C for 1 min, and 72°C for 1.5 min. For *Dra*III-, *Ban*I-, *Hha*I-, and *Taq*I-polymorphism, amplification was conducted for 40 cycles at 94°C for 0.5 min, 59°C for 1 min, and 72°C for 1.5 min. Amplified products were digested with appropriate restriction enzymes and were subsequently separated on 5% polyacrylamide gel for size determination.

Results

(CTG)n allele distribution

Frequency distribution of normal alleles with variable CTG repeat lengths in the *DM1* locus was analysed for 496 chromosomes and the result is shown in Figure 1. Heterozygote frequency in the studied population is 78%. A total of 18 alleles were found, ranging in size from 5 to 30 repeats. The most common allele had five CTG repeats (29% of chromosomes). Repeat sizes for the majority of the remaining alleles (60.3% of chromosomes) ranged from 11 to 14, with 12 repeats being most frequent (25.4%). In total, seven alleles (1.4%) were found to have a repeat size larger than 18 repeats which may have significant instability for further expansions towards becoming *DM1* alleles.[9]

Haplotype analysis

A total of 268 normal and 17 expanded *DM1* chromosomes were ascertained for the association of CTG repeats and the *Alu* insertion/deletion polymorphism (Table 1). Among the normal *DM1* chromosomes, 90% (182/202) of the $(CTG)_{6-17}$ alleles are associated with the *Alu* deletion polymorphism. On the other hand, $(CTG)_5$ alleles show a strong association with the *Alu* insertion polymorphism (92%, 48/52). Although there were only three samples included, no association between the insertion allele and the $(CTG)_{19}$ alleles was observed. However, all of the expanded alleles show the absolute linkage disequilibrium with *Alu* insertion polymorphism.

A more detailed genetic analysis consisting of (CTG)n as well as eight other polymorphic markers was then performed to investigate the association of certain (CTG)n repeat alleles and particular haplotypes. Our data demonstrate that all *DM1* and two $(CTG)_{25}$ alleles examined associate exclusively with haplotype A. A strong linkage disequilibrium between $(CTG)_5$ and haplotype A was also detected. Most of the $(CTG)_{8-19}$ alleles associate with haplotypes A–D, with a frequency of D > C > B > A. On normal *DM1* chromosomes, a total of eight different haplotypes, including a new haplotype (L) which has never been described in other populations, were identified. Table 2 shows the frequency of each haplotype in the Taiwanese population. Haplotype A is the most frequent one that appears in the Taiwanese people studied (41%). Haplotypes B and J, which were observed either only in the Canadians or in the Japanese, both were found in the Taiwanese. Haplotypes E, H, I, and K were not found in this study. Although the frequency distribution of haplotypes seems to be different in diverse ethnic groups, haplotypes A–D are the major ones and could have served as the founding haplotypes.

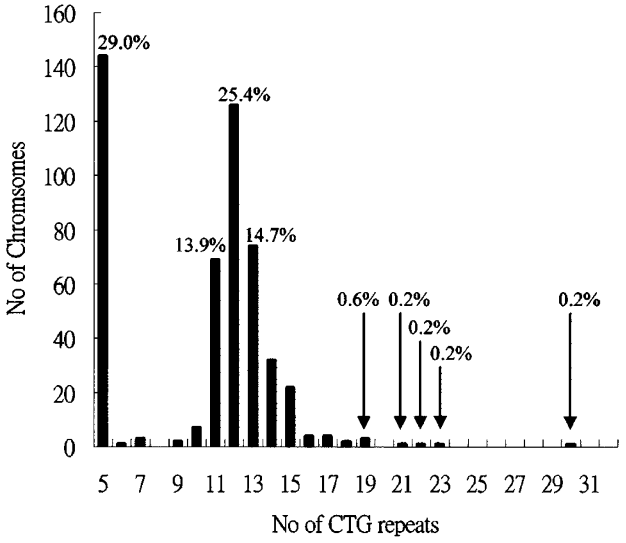

Figure 1 Distribution of CTG repeat sizes in the DM1PK gene in normal Taiwanese population. A total of 496 alleles were examined.

Table 1 Association of CTG repeats and the *Alu* insertion/deletion polymorphism

CTG repeats	n	Alu *insertion*	Alu *deletion*
5	52	48	4
6	1	0	1
7	3	1	2
9	2	0	2
10	4	0	4
11	40	5	35
12	72	2	70
13	49	6	43
14	23	1	22
15	13	2	11
16	3	1	2
17	2	2	0
19	3	0	3
30	1	1	0
Expanded alleles	17	17	0

Discussion

In this study, we have demonstrated that the frequency of large CTG alleles in normal Taiwanese is low (Figure 1). This result suggests that the prevalence of DM1 in Taiwanese is much lower than those in the European and Japanese populations and may be close to that in the South African Negroids.[3,5]

The $(CTG)_{>16}$ alleles were previously shown to associate exclusively with the *Alu* insertion polymorphism and be passed on consistently in different ethnic populations.[7,16,17] In this study, however, three $(CTG)_{19}$ alleles examined were associated with *Alu* deletion alleles. This is in contrast to a generally accepted proposal that the $(CTG)_5$/*Alu* insertion evolved to $(CTG)_{19}$ in a unique event. Our data raises the possibility that the Taiwanese population was founded before the Japanese and Caucasian populations diverged from a common ancestry and the Taiwanese $(CTG)_{19}$ alleles evolved from $(CTG)_{11-14}$ alleles through a separate route. Alternatively, the $(CTG)_{19}$/*Alu* deletion simply arose from a *Alu* deletion event on a founder Taiwanese $(CTG)_{19}$ chromosomes. Furthermore, the identification of haplotype L, a new haplotype unique to the Taiwanese population, indicates that genetic recombination happened within the DM1 locus. This haplotype could be generated through a recombination event between chromosomes with haplotype A and D (Table 2). These results suggested the possibility that certain unique haplotypes could have been developed in different ethnic populations by recombination events in normal or *DM1* chromosomes.

We have demonstrated that all Taiwanese *DM1* chromosomes examined were of haplotype A. Our findings provide the first molecular evidence for the founder effect of DM1 mutations in Taiwan. It is likely that the Taiwanese, and maybe all non-African, *DM1* chromosomes could have originated from a pool of large-sized normal alleles with haplotype A which was generated after migration out of Africa. The only DM1 family observed in Nigerian people was probably derived from a very rare, individual mutation event.

Table 2 Haplotype frequency in DM1 and non-DM1 Taiwanese individuals

Haplotype	Dra*III* *(N9)*	Hph*I* *(Intron 4)*	Hha*I* *(Intron 5)*	△ *1kb* *(Intron 8)*	Hinf*I* *(Intron 9)*	Bpm*I* *(Exon 10)*	Fnu4*HI* *(Intron 11)*	Taq*I* *(D19S463)*	*Haplotype frequency (%)* *Canadian*[8]	*Japanese*[9]	*Taiwanese*
DM	1	2	1	1	2	2	1	2	100	100	100
A	1	2	1	1	2	2	1	2	49	30	41
B	2	2	2	2	1	2	2	1	27	0	11
C	1	1	2	2	1	1	2	1	16	10	15
D	1	2	2	2	1	2	2	1	8	53	21
E	2	2	2	2	1	2	2	2	<1	0	0
F	1	1	2	2	1	2	2	1	<1	0	2.6
G	1	2	1	1	2	1	1	2	<1	<1	4.0
H	2	2	2	2	2	2	2	2	<1	0	0
I	1	1	2	2	2	2	1	1	<1	0	0
J	1	2	2	2	1	1	2	1	0	4.8	4.0
K	1	2	1	1	2	2	1	1	0	1.3	0
L	1	2	2	2	2	2	1	2	0	0	1.3

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