CTG Repeat Number at the Myotonic Dystrophy Locus in Healthy Kuwaiti Individuals

Possible Explanation of Why Myotonic Dystrophy Is Rare in Kuwait

Suad Alfadhli, PhD; Alaa E. Elshafey, MD, PhD; Laila Bastaki, MD; Sadeqa Al-Awadi, MD

Background: Myotonic dystrophy is caused by unstable (CTG)n repeat expansion. On normal chromosomes, this repeat is highly polymorphic, with a copy number ranging from 4 to 38. Myotonic dystrophy is considered more prevalent in Western European and Japanese populations but less prevalent, rare, or even absent in others. It has been proposed that the expanded (CTG)n alleles originated from the group of the large normal alleles.

Objective: To determine whether there is a lower prevalence of the large alleles in the Kuwaiti population.

Design and Participants: We determined the size distribution of the CTG repeats by means of polymerase chain reaction in blood DNA derived from 185 healthy Kuwaiti individuals representing the 5 Kuwaiti provinces.

Results: We found a total of 17 (CTG)n alleles, with a range of 5 to 37 repeats. The (CTG)5 allele was the most frequent single allele (100/370 [27.0%]), whereas the (CTG)10-13 was the most frequent class of alleles (161/370 [43.5%]). Using 18 repeats as the cutoff point, χ² analysis showed a statistically significant lower frequency of greater than 18 alleles in the Kuwaiti population compared with the European population (χ²=12.7; P<.001).

Conclusions: These data may explain the rare occurrence of myotonic dystrophy in the Kuwaiti population. Further study of healthy families within the high-normal repeat range is in progress to investigate the possible instability of the (CTG)≥18 alleles in our area.

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Myotonic dystrophy (DM) is an autosomal dominant disorder with high penetrance and rare new mutations.1,2 It is the most common adult form of muscular dystrophy. The clinical features of this disease are myotonia, weakness, muscle wasting, frontal baldness, cataract, hypogonadism, and electrocardiographic changes.3 The causative mutation in DM is an expansion of an unstable tandem repeat of the CTG sequence located in the 3′ untranslated region of a gene, with strong homology to the protein kinase family, on chromosome 19q13.3.4-9 In unaffected individuals, the (CTG)n repeat number is polymorphic, ranges from 5 to 38 repeats, and is stably inherited.7,10 In DM, at least 50 copies are present in the minimally affected patients’2 and dramatically increase to an estimated 2000 copies in severely affected individuals.8,9

Factors that affect trinucleotide repeat stability, normal allelic variation, and the generation of new disease alleles are not fully understood. It has been proposed that expanded alleles originated from the group of large normal alleles.11,12 The heterogeneous class of (CTG)≥19 alleles may constitute a reservoir for recurrent DM mutations. This model has been supported by the finding that differences between the frequency of large alleles in the high-normal range of allele size distribution ([CTG]≥19) in the global human population are congruent with observed variation in the prevalence rate of the disease.13 Moreover, at the higher allele range, loss of interrupting motifs within tracts of trinucleotide repeats leads to greater instability and predisposes alleles to expansion.14,16

At present, disease incidence is globally quite variable. Myotonic dystrophy is considered to be most prevalent in western European and Japanese populations,
less prevalent in Southeast Asian populations, and rare or absent in southern and central African populations.3,17-19 In the state of Kuwait, the Ministry of Planning estimates the population at 2243 million, of which about 38% are Kuwaitis. Because DM is less frequently observed in native Kuwaitis (only 2 families have been identified with the disease [unpublished data, S.A., L.B., and S. Al-Awadi, MD, January 2004]), we conducted a study of CTG–repeat length polymorphism in healthy Kuwaiti individuals in an attempt to explain the paucity of DM-affected families in this population.

We analyzed 370 chromosomes for CTG repeat variability. The frequencies of each (CTG)n allele size in Kuwaiti population are shown in Figure 1. A total of 17 (CTG)n alleles were found, with a range of 5 to 37 repeats. A bimodal distribution of allele sizes with peaks at 5 and at 10 to 13 repeats is shown. The (CTG)5 allele was the most frequent single allele (100 of 370 [27.0%]), whereas the (CTG)10-13 was the most frequent class of alleles (161 [43.5%]). No chromosome was found with 9, 18, or 19 repeats. Except for the occurrence of 1 chromosome with 37 repeats, very few chromosomes (13 [3.5%]) were found with a repeat size of greater than 18.

Figure 2 shows the frequency of various (CTG)n repeats of this study compared with that of European11 and black South African populations.20 Results of χ² analysis of the overall distribution of the alleles showed highly significant differences between a Kuwaiti population and black South African (P<.01) and European populations (P<.001).

With 18 repeats designated as the cutoff point, the (CTG)18 repeats were grouped into 3 classes (5, 10-18, and >18) (Table). Results of χ² analysis of these data showed a statistically significant lower frequency of greater than 18 alleles in the Kuwaiti compared with the European population (χ²=12.7; P<.001). The (CTG)5,18 alleles were significantly more frequent in the Kuwaiti compared with the black South African population (χ²=8.8; P=.003). Regarding the (CTG)18 alleles, no significant difference was observed between the Kuwaiti and European populations (χ²=0.83; P=.36), whereas a statistically significant lower frequency of this allele group was seen in the Kuwaiti population compared with the black South African (χ²=8.2; P=.004). In the studied Kuwaiti population, the prevalence of the (CTG)18 allele was shown to be not statistically different from that seen in the black South African population (χ²=0.01;

POLYMERASE CHAIN REACTION ANALYSIS

We extracted DNA from peripheral blood leukocytes and used polymerase chain reaction (PCR), with the primer set 409/406,2 to amplify a DNA segment that contains the CTG repeats at the 3’ end of the DM-kinase gene. Nested PCR using the primer set 409/410 was performed to create a shorter product for proper sizing. The PCR analyses were performed in a 30-µL PCR mix containing 1× GeneAmp PCR buffer (Applied Biosystems, Foster City, Calif), 200 µmol/L of each dNTP (deoxyribonucleic acid), 25 pmol of each primer, 1 µg of genomic DNA, and 2 U of AmpliTaq DNA polymerase (Applied Biosystems). Tubes were heated to 95°C for 5 minutes, and 32 PCR cycles were started as follows: 95°C for 1 minute, 64°C for 1 minute, and 72°C for 1.5 minutes, followed by a final extension step of 72°C for 5 minutes. For nested PCR, we used 1 µL of the first-round PCR product in a reaction with the same previous conditions. The nested PCR products were resolved on 2% Nusieve (Karlan Research Products Corp, Santa Rosa, Calif) agarose gel electrophoresis alongside a 25–base pair ladder and PCR products that contain known CTG repeat lengths detected by sequencing. For proper sizing of the PCR products, we analyzed gel photographs by means of the GeneLine GeneTools software program (version 3.00.22; Spectronics Corporation, Westbury, NY).

RESULTS

METHODS

SUBJECTS

One hundred eighty-five healthy native Kuwaiti individuals participated in this study. Samples were collected from Kuwaiti individuals coming to the major hospitals in the 5 provinces of Kuwait for checkup. All individuals belonged to known Kuwaiti tribes with minimal non-Kuwaiti admixture. Consent agreement was obtained from all volunteer subjects in this study.

Figure 1. CTG allele distribution at the myotonic dystrophy protein kinase gene in 370 chromosomes in a healthy Kuwaiti population.

Figure 2. CTG allele frequency in healthy (non–myotonic dystrophy) subjects from the following 3 populations: Kuwaiti (present study), black South African,20 and European.11 A low prevalence of (CTG)18 can be observed in Kuwaiti and black South African populations.
Study of Martorell et al. They concluded that premutations. This hypothesis was supported by the extensive
10%, may constitute a reservoir for recurrent DM mutations. This hypothesis was also supported by the finding of Meiner et al. who observed an increase of 11 repeats on a paternally transmitted 37 repeats in a non-DM family. The heterogeneous class of (CTG)19-30 alleles, which was found to have an overall frequency of about (CTG)10-13 constituted the most frequent class of alleles (43.5%). These data are in accordance with those observed in European, Japanese, black South African, and most human populations as concluded by Tishkoff et al. The factors that affect trinucleotide repeat stability, normal allelic variation, and the generation of new disease alleles are not fully understood. Haplotype analysis of DM chromosomes suggested a single origin (founder chromosome) for the trinucleotide repeat expansion. This finding was unexpected for DM, a dominant disease that in its severe forms diminishes or abolishes reproductive fitness. Such a disease in general would be characterized by a high level of new mutations that compensate for the loss of abnormal alleles due to the decreased fitness. The most accepted hypothesis to explain this finding was suggested by Imbert et al. They proposed that the initial predisposing event(s) leading to the formation of the DM chromosomes consisted of a limited number of duplication steps of a (CTG) allele that resulted in generation of (CTG)19. The heterogeneous class of (CTG)10-32 alleles, which was found to have an overall frequency of about 10%, may constitute a reservoir for recurrent DM mutations. This hypothesis was supported by the extensive study of Martorell et al. They concluded that premutation alleles could not be the long-term source of new DM families, which must ultimately arise from mutations of alleles within the upper-normal size range. The hypothesis was also supported by the finding of Meiner et al. who observed an increase of 11 repeats on a paternally transmitted 37 repeats in a non-DM family.

Using the 18 repeats as the cutoff point, our results showed a statistically significant lower frequency of (CTG)18 alleles in the Kuwaiti population compared with the European population ($\chi^2 = 12.7$, $P < .001$). This result agrees with those of previous studies in different ethnic groups, which demonstrated a correlation between the frequency of large normal CTG alleles (>18 repeats) and the prevalence of DM in a population.

In conclusion, the low frequency of DM among Kuwaiti individuals is mostly due to the lower prevalence of the (CTG)18 alleles in this population. Further study of healthy families within the high-normal repeat range is in progress to investigate the possible instability of the (CTG)18 alleles in our area.

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Author contributions: Study concept and design (Drs Alfadhli and Elshafey); acquisition of data (Drs Alfadhli and Al-Awadi); analysis and interpretation of data (Drs Alfadhli, Elshafey, and Bastaki); drafting of the manuscript (Dr Alfadhli); critical revision of the manuscript for important intellectual content (Drs Alfadhli, Elshafey, Bastaki, and Al-Awadi); statistical expertise (Drs Alfadhli and Elshafey); obtained funding (Dr Alfadhli); administrative, technical, and material support (Dr Alfadhli); study supervision (Drs Alfadhli and Al-Awadi).

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REFERENCES

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Comparison of CTG Allele Groups Among Non–Myotonic Dystrophy Subjects

<table>
<thead>
<tr>
<th>No. of CTG Repeats</th>
<th>No. of Chromosomes, Population</th>
<th>Kuwaiti vs Black South African*</th>
<th>Kuwaiti vs European†</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>100</td>
<td>112</td>
<td>104</td>
</tr>
<tr>
<td>10-18</td>
<td>210</td>
<td>280</td>
<td>157</td>
</tr>
<tr>
<td>&gt;18</td>
<td>14</td>
<td>3</td>
<td>32</td>
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*From Goldman et al.†From Imbert et al.

$P = .90$, but was significantly lower than that seen in the European population ($\chi^2 = 6.08$, $P = .01$).

**COMMENT**

Myotonic dystrophy is an autosomal dominant neurological disease with a globally variable disease incidence. This disease is very rare in the Kuwaiti population, and its clinical and genetic features have not yet been documented in this population. Therefore, we undertook a study of the CTG trinucleotide repeat in healthy individuals of the Kuwaiti population in an attempt to explain the apparently rare incidence of the disease and as a step toward more extensive study of the disease in our population.

The CTG allele distribution in the Kuwaiti population showed a bimodal distribution of allele sizes, with the (CTG)5 allele as the most frequent (27%), whereas (CTG)10-13 constituted the most frequent class of alleles (43.5%). These data are in accordance with those observed in European, Japanese, black South African, and most human populations as concluded by Tishkoff et al.21

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