**Triplet repeat expansion in 3 untranslated region of myotonic dystrophy protein kinase (DMPK) gene has been implicated as causative in myotonic dystrophy (DM). In cases of DM, high levels of somatic instability have been reported, in which inter-tissue repeat length differences as large as 3000 repeats have been observed. This study highlights the inter-tissue (CTG)n expansion variability at the DMPK locus. Molecular analysis of DMPK gene, encompassing the triplet repeat expansion, was carried out in 31 individuals (11 clinically identified DM patients, 20 controls). All controls showed a 2.1 kb band (upto 35 CTG repeats), while four cases exhibited an expansion (>50 repeats). A novel observation was made in one case, wherein the DNA from lymphocytes showed a normal 2.1 kb band while the muscle tissue DNA from the same patient was heterozygous for normal and 4.3 kb band (>700 repeats). Our results suggested that because inter-tissue variability existed in the (CTG)n repeat number at DMPK locus, an attempt should be made to evaluate affected tissue along with blood wherever possible prior to making a final diagnosis. This is important not only for diagnosis and prenatal analysis, but also while providing genetic counseling to families.**

**Keyword:** (CTG)n expansion, DMPK locus, Muscle tissue

Myotonic dystrophy (DM) is the most common form of inherited muscular dystrophy in adults with an incidence of 1 in 8000 in the Caucasians. The autosomal disorder affects multiple organ systems and it is characterized by myopathy, dystrophy, myotonia of skeletal muscles and altered creatinine phospho kinase levels. Myotonia, an abnormality in relaxation after muscle contraction is the primary feature of the disease.

Fourteen diseases including DM are caused by an unstable expansion of gene specific repeat sequences.

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(CTG)_n Expansion at DMPK locus seen only in muscle tissue: A novel case

Qurratulain Hasan 1,2,*, Vasavi Mohan 1,2, Y R Ahuja 1,3

1Department of Genetics, Bhagwan Mahavir Medical Research Centre, Hyderabad 500 004, India.
2Department of Genetics and Molecular Medicine, Kamineni Hospitals, Hyderabad 500 068, India.
3Department of Genetics, Vasavi Hospital and Research Centre, Hyderabad 500 004, India.

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*Correspondent author
Phone: 23396324, 24022272-76 X210
Fax: 24022277
E-mail: qhasan2000@yahoo.com

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(CTG)_n repeats in 3' untranslated region of DMPK gene on the chromosomal region 19q13.3 are associated with DM. The array length of tandemly repeated CTGs in normal alleles is variable with a copy number of 5-35, mild to classical symptoms patients, 50-1000 repeats are observed while more than 1000 repeats are seen in severely affected individuals. The disease shows anticipation in families i.e. an increase in clinical severity and an earlier age of onset in successive generations, due to intergenerational expansion of CTG repeats.

This study was carried out to evaluate (CTG)_n repeat expansion at myotonic dystrophy protein kinase (DMPK) locus in individuals with clinical symptoms of myotonic dystrophy. Controls from normal population were also included in the analysis.

Sampling—Heparinised peripheral blood samples (1 ml) were collected from individuals by the method approved by the Bhagwan Mahavir Medical Research Centre (BMMRC) Ethical Committee. All controls included in the study were enrolled after obtaining informed consent and the investigation conforms to the principles outlined in the Declaration of Helsinki.

Samples were collected from eleven individuals suspected with DM and controls taken from random population (n=20). Detailed information about the cases was recorded in a well-designed proforma; salient findings are given in Table 1.

Molecular analysis—Genomic DNA isolation from 31 individuals was carried out using a Mini Blood DNA isolation Kit (Pharmacia, Sweden) according to the manufacture's instructions. Good quality genomic DNA was isolated and stored appropriately. The amount of DNA obtained was ~10 μg per sample. The quality of DNA was checked by carrying out a polymerase chain reaction (PCR) for the housekeeping gene, β-actin, as control by the method described earlier.

DNA (~100 ng) from each sample was then amplified by PCR using specific primers for DMPK gene encompassing the triplet repeat expansion. Forward and reverse primers DMK 1111 (5'-GGTCGGGGTCTCAGTGCATCCA-3') and DMK 9003 (5'-CACAGGGCTGAAGTGGCAGTTCCA-3')

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CAGGGCTGAAGTGACGTTCCA-3') amplifying a 2.1 kb target were used for PCR.

Each 50 μl of reaction mix consisted of 1x PCR buffer (QIAGEN), 1x Q-solution (QIAGEN), 1μl MgCl₂ (25 mM) (Applied Biosystems), 0.2 mM each of dNTPs, 0.1 μM of each primer, and 2.5U of Taq DNA polymerase (QIAGEN, Cat.No.201203) which can amplify targets upto 12 kb. The PCR protocol used was initial denaturation at 95°C for 5 min followed by 94°C for 30 sec, 66°C for 2 min; 72°C for 2 min, run for 35 cycles with a final extension of 72°C for 7 min. PCR reaction mix (9 μl) was electrophoresed on agarose gels (0.8%) stained with ethidium bromide. A 1kb DNA ladder was used to identify the molecular weight of the bands visualized on the gel. The image was captured on an Alphatec Gel Electromager System (USA) and analysed using the Chemilmage software version 5.5.

All the 20 control samples and 6/11 cases clinically considered as DM showed a PCR product of 2.1 kb corresponding to the normal allelic band (Fig.1a). The 2.1kb target encompasses upto 35 CTG repeat sequences.

The DNA from blood of case no: 4 and 11 showed a normal allelic band and an expansion yielding larger band of 8 kb and 3.2 kb respectively confirming the diagnosis of DM. Band density analysis, computed by the Chemilimage software, showed 4'

Table I — Details of patients included in this study

<table>
<thead>
<tr>
<th>Case no</th>
<th>Age/Sex</th>
<th>CPK Levels/Biopsy</th>
<th>Symptoms/Clinical diagnosis</th>
<th>Any affected relatives</th>
<th>DM status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38y/M</td>
<td>Not available</td>
<td>Muscle weakness, change of voice, problem in walking and holding</td>
<td>None</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>2</td>
<td>62y/M</td>
<td>Not available</td>
<td>Repeated falling due to weak muscles, unable to carry weight, Myopathy?</td>
<td>Father, Aunt, Cousin.</td>
<td>Mild Expansion (2.3kb)</td>
</tr>
<tr>
<td>3</td>
<td>27y/F</td>
<td>62U/L Biopsy normal</td>
<td>Gradual muscle weakness in both upper and lower limbs, Fatigue</td>
<td>None</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>4</td>
<td>1.5y/M</td>
<td>190U/L pseudomuscular hypertrophy</td>
<td>Repeated falling, calf muscle bulky, Myotonia.</td>
<td>None</td>
<td>Positive (8kb)</td>
</tr>
<tr>
<td>5</td>
<td>18y/M</td>
<td>767U/L Biopsy inconclusive</td>
<td>Unable to run, Bulky calf, Myotonia, Weakness in both upper and lower limbs</td>
<td>None</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>6</td>
<td>6y/M</td>
<td>Not available</td>
<td>Gradual weakness, unable to walk</td>
<td>None</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>7</td>
<td>10y/M</td>
<td>264U/L Dystrophic features</td>
<td>Gradual muscle weakness, myotonia.</td>
<td>None</td>
<td>Normal (2.1kb) **Positive (4.3kb)</td>
</tr>
<tr>
<td>8</td>
<td>33y/M</td>
<td>Not available</td>
<td>Difficulty in grasping, tremors in hand, speech change, slow eye movements.</td>
<td>Mother, Brother.</td>
<td>Mild Expansion (2.3kb)</td>
</tr>
<tr>
<td>9</td>
<td>49y/M</td>
<td>Not available</td>
<td>Unable to walk, mild spondylosis, Osteoporosis.</td>
<td>None</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>10</td>
<td>46y/F</td>
<td>1686U/L</td>
<td>Muscle weakness in both lower limbs.</td>
<td>Not sure</td>
<td>Normal (2.1kb)</td>
</tr>
<tr>
<td>11</td>
<td>5y/M</td>
<td>15015U/L Dystrophin changes, Limbs weak, Falling frequent, muscle tenderness.</td>
<td>None</td>
<td>Positive (~3.2kb)</td>
<td></td>
</tr>
</tbody>
</table>

*Proband’s sample degraded, repeat sample not available.

**Positive in muscle tissue DNA.
of the patient's blood lymphocytes with the expanded 1.2 kb fragment (corresponding to an increase in ~2000 repeats) and 37% of the blood lymphocytes with the 1.2 kb fragment (corresponding to an expansion of ~300 repeats). Case no: 2 and 8 exhibited a 2.3 kb band indicating an expansion of ~66 repeats (Fig. 1a).

The DNA isolated from the blood of patient no: 7, gave a homozygous normal 2.1 kb band. Muscle biopsy material with dystrophic changes (Fig. 2) that was fortunately available to us (see acknowledgement) was used for molecular analysis. DNA isolated from it showed a normal 2.1 kb band along with an expanded 4.3 kb fragment of lesser intensity when compared to normal allele indicating a lower proportion of the somatic cells with the expanded allele (Fig. 1b).

Expansion of gene-specific triplet repeats cause many diseases including DM. Both meiotic and mitotic tissue instability of these repeats has been reported. Phenotypic manifestation of the disease varies due to somatic heterogeneity at the non-coding repeat tract.

In the present study, it was seen that all controls had a repeat size in the normal range, while five of the eleven cases clinically identified as DM exhibited an expansion (Fig. 1a). Case no. 7 was unique as it had only the normal allele in the blood DNA while the DNA from muscle tissue had an expanded allele along with the normal one (Fig. 1b). The decreased intensity of the expanded allele was indicative of a low proportion of cells with the disease allele when compared to the normal allele. Tract length heterogeneity within a tissue has been reported and is known to correlate with the progression process and disease severity. Considerable greater expansions in muscle, fibroblasts and skin than in blood in the same individual with DM have been observed. However, it is for the first time to our knowledge that a triplet repeat expansion has been observed only in the affected tissue, while the lymphocytes had the normal allele.

The mechanism(s) by which these triplet repeat expansions occur and lead to the complex symptomatology in DM is still unclear. Trinucleotide repeats are generally thought to expand during DNA replication in multiplying cells, however, an alternate mechanism where expansion occurs during DNA repair in non-multiplying cells has also been proposed and may be responsible for our observation.

Hence, (CTG)n expansion at the DMPK locus may be systemic or associated with specific tissues, which

Fig. 1 — (a) Ethidium bromide stained agarose gel (0.8%) showing: [Lane 1 - 1 kb DNA ladder, lanes 2.3.4.9 - normal 2.1 kb band, lane 5 - normal and 8 kb expanded band (case no.4, Table 1), lanes 6.7 - 2.3 kb band indicating mild expansion (case no.2 & 8, Table 1), and lane 8 - normal and an expanded band of 3.2 kb (case no.11, Table 1)]. (b) Inverted Image of ethidium bromide stained agarose gel (0.8%) showing: [Lane 1 - 1 kb DNA ladder, lane 2 - normal 2.1 kb band seen in the blood DNA of the patient no.7, and lane 3 - normal 2.1 kb band and expanded 4.3 kb band seen in the muscle biopsy DNA of the patient no.7].

Fig. 2 — Muscle tissue biopsy showing dystrophic features. (a) Section showing variation in fibre size; and (b) Occasional degenerating basophilic fibres seen in the section. [Transverse sections of muscle tissue stained in H & E x 100].

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are clinically affected. This is an important fact to be kept in mind while carrying out molecular diagnosis, doing prenatal analysis and providing genetic counseling to families with DM.

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