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Myotonia may be a sign that prompts genetic testing for myotonic dystrophy type 1

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ABSTRACT

Objectives: Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder characterized by myotonia, atrophy, and muscle weakness. Even though myotonic discharges can be shown on electromyography in DM1 instances, it is still difficult to distinguish DM1 clinically from other myotonic disorders. We aimed to examine the relationship between DM1 and myotonia.

Methods: Data from 22 patients who had myotonia were analyzed retrospectively. Patients with DM1 (n=9) and non-DM1 myotonia (n=13) were categorized by genetic testing.

Results: There was a significant difference in the incidence rates of myotonia (P=0.0001) between 8 out of 9 DM1 patients (89%) and 1 out of 13 non-DM1 (8%). Myotonic discharges were seen in 7 of the 9 DM1 (78%) patients. On the other hand, only 2 out of 13 (15%) patients with non-DM1 had myotonic discharges. There was a statistically significant difference in the incidence rates across the groups (P=0.003). In this study, we also encountered three siblings with DM1. We observed myotonia and muscle weakness in the twins, indicating there is a possibility of anticipation being seen in their grandchildren in the future.

Conclusion: Genetic counseling is crucial for understanding disease variability, aiding in better DM1 management.

Keywords: Myotonic dystrophy type 1 (DM1), DM1 protein kinase gene, myotonia, CTG repeats

Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder characterized by myotonia, atrophy, and muscle weakness [1]. An increase in CTG causes this condition repeats in the 3′ untranslated region (3′UTR) of the DM1 Protein Kinase (DMPK) gene. This microsatellite typically has 5-37 repeats, but at 50 repetitions or more, it becomes pathogenic because the enlarged CUG repeats (CUGexp) generate ribonuclear foci, which make the resultant RNA poisonous. RNA-binding proteins are bound and sequestered by hairpin structures seen in these foci [2]. DM1 patients frequently have myotonia, which is defined as having trouble releasing a contracted muscle [3, 4].

Myotonia is a unique electrodiagnostic result that is seen during the needle section of an electromyography (EMG) investigation. The structure and sound of EMG myotonia are distinctive, and when the recording needle is introduced into a muscle that is at rest, the usual dive bombardment sound is audible

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rather than electrical silence [5, 6]. In both the upper and lower extremities, myotonia is evident in the proximal and distal muscle groups [7]. Myotonia is a disease-specific clinical and electrodiagnostic indication that arises from skeletal muscle chloride channel failure [8]. Myotonia is associated with myotonic discharges on EMG. Myotonic discharges are easily recognized on electrodiagnostic testing because of the waxing and waning discharges [7].

Myotonic dystrophy is now classified as type 1 DM1 for the classic form and type 2 DM2 for the more recently discovered variation. Myotonia (present in 90% of affected individuals) and muscle dysfunction (weakness, pain, and stiffness) account for 82% of the symptoms of myotonic dystrophy type 2 DM2. Less frequently occurring conditions include subcapsular cataracts, cardiac conduction defects, and insulin-insensitive type 2 diabetes mellitus. While myotonia has been documented in the first ten years of life, it usually manifests in the third decade, usually with episodic or fluctuating excruciating muscular pain and weakening of the flexors in the neck and fingers. Rarely does myotonia result in severe symptoms [9]. DM2 is the sole additional hereditary form of multisystem myotonic dystrophy that has been discovered thus far. DM2 is caused by pathogenic polymorphisms in CNBP. CNBP intron 1 may include a complex repeat motif, (TG)n(TCTG)n(CCTG)n. DM2 is brought on by the CCTG repetition expanding. Counting the CTG repeats in myotonic dystrophy protein kinase (DMPK) allows one to distinguish between DM1, DM2, and other hereditary myopathies. To rule out other causes of muscle disease, further testing with EMG, serum CK levels, and/or muscle biopsy is frequently necessary if the DMPK CTG repeat length is within the normal range and DM2 has been ruled out by molecular genetic testing of CNBP.

During both meiosis and mitosis, the mutant DMPK CTG is unstable. Somatic mosaicism frequently results from the instability during mitosis. The afflicted people may exhibit mutations with varying sizes within a single tissue type or mutations with varying sizes across many tissue types. The patient population's diverse spectrum of clinical characteristics might perhaps be attributed to somatic mosaicism arising from mitotic instability [10, 11]. In the meanwhile, during the allele's transmission to the following generation, meiotic instability may cause changes in the CTG's size. In most cases, the size will increase, leading to more severe clinical symptoms known as anticipation and an earlier age of start. An individual with severe, early-onset diabetes usually gets the enlarged mutant allele from the mother [12, 13].

Our study aimed to investigate the relationship between myotonia in patients with DM1 by retrospectively analyzing their genetic report. Additionally, we sought to highlight the importance of genetic counseling for patients with myotonic dystrophy and their family members.

METHODS

We examined patients treated at Umraniye Training and Research Hospital from 2020 to 2024 for electrodiagnostic testing. Cases having verified gene test findings and myotonic discharges on the EMG were chosen. The research excluded patients who were on cholinesterase inhibitors or any other drug that might reduce myotonic discharges. The results of gene tests were used to divide the patients into two groups: the DM1 group and the non-DM1 myotonia group. Patients in the DM1 group had amplifications of the DMPK gene including more than 50 CTG trinucleotide sequence repeats. Patients without DM1 validated by gene testing made up the non-DM1 myotonia group. Both percussion and requesting the patient to carry out bodily actions like opening their eyes or squeezing their hand were used to demonstrate clinical myotonia.

Peripheral blood samples were taken after written consent was obtained from the patients. Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using standard methods. DNA extraction from the blood sample was performed using a semi-automatic robot as recommended by the manufacturer (Qiagen). Concentration and quality control of DNA samples (260/280 nm and 260/230 nm values) were determined by fluorometric (Qubit v3.0) and UV spectrophotometry. We brought the reagents to room temperature. Vortex Primer Mix and PCR Mix were then spun briefly to remove debris from the cover. We mixed the enzyme gently by inverting or pipetting. Following the recipe given above, we prepared a Master Mix that calculates the number of samples and controls. We mixed briefly by pipetting or Vortex Master

Mix. For each sample, we transferred 21.5 µL of Master Mix into each 0.2 ml PCR tube. We added 1 sample of DNA (1-5 ng per reaction) to each PCR tube. We prepared a positive control PCR tube using the DNA provided in the kit and added 1 µl of sterile Direct Q dd H2O instead of DNA for the negative control. We vortexed each PCR tube. We made sure that no drops remained on the tube wall or lid. We placed the tubes in the thermal cycler. We used the following PCR program for amplification of all markers. The PCR products were incubated for three minutes at −20°C on ice or in a cold box. We centrifuged the plate for 10 seconds at 1000xg to get bubbles out of the wells. We placed the plate in the Genetic Analyzer and started the run. Samples were analyzed using Applied Biosystems® fragment analysis software compatible with the Genetic Analyzer for GT DM1 Detector. The program of PCR reaction was designed as follows: Initial Denaturation, 95°C 5 min; Denaturation, 95°C 1 min; Primer Annealing, 63°C 70 sec; Extension, 72°C 80 sec; Final Extension, 72°C 10 min; 30 cycles.

Statistical Analysis

The two-sample Pearson's chi-square test was used to assess the myotonia occurrence rate between the myotonic DM1 and non-DM1 groups. The statistical analyses were performed using SPSS version 21 at a significance level of $P<0.05$. Patients in the second group did not have DM1 validated by gene testing.

RESULTS

Individuals were divided into the DM1 and non-DM1 groups after being analyzed using Applied Biosystems® fragment analysis software compatible with the Genetic Analyzer for GT DM1 Detector (Fig. 1).

Following gender and age-based case matching

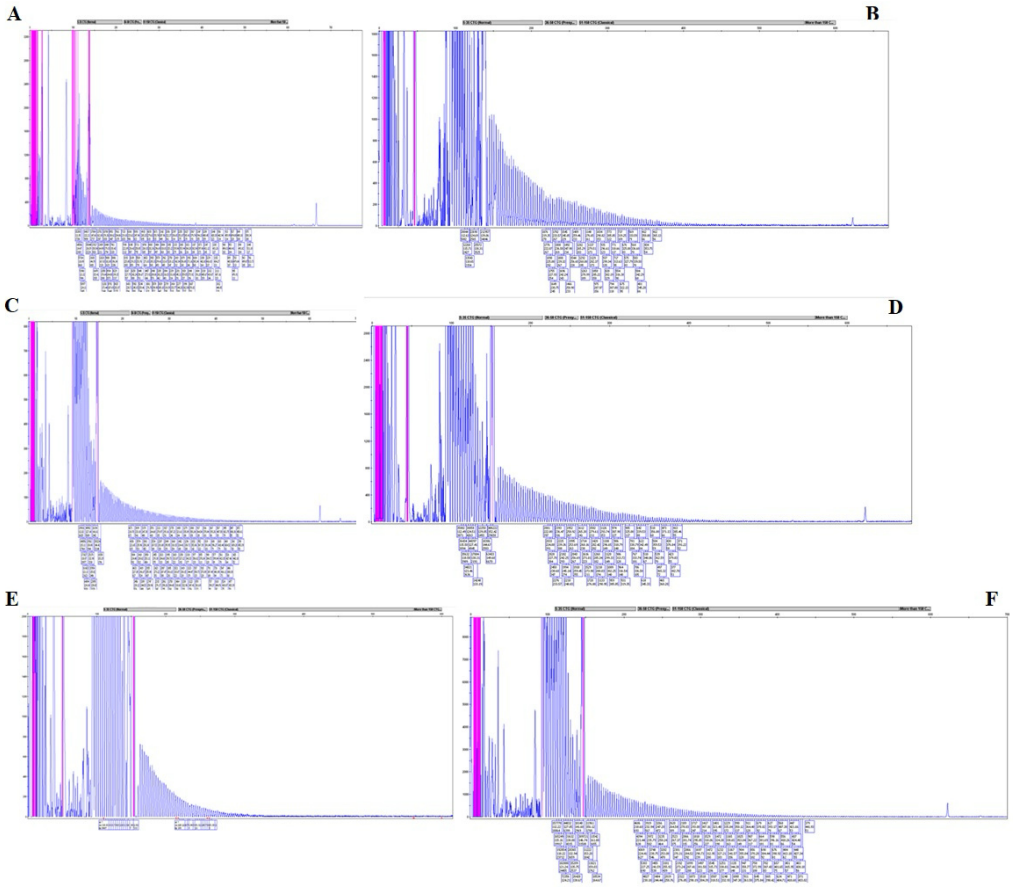

Fig. 1. DMPK gene 3'UTR region CTG repeat MLPA fragment analysis images. MLPA fragment analysis images of DM1 cases are shown here (A-F). The CTG repeat was over 50, and it has been genetically proven that the diagnosis is DM 1. MLPA=multiplex ligation-dependent probe amplification, DM1=muscular dystrophy type 1.

Table 1. Clinical features of the patients

No	Age/Sex	Eyes/Heart/ Gastrointestinal/Biliary tract/Genitourinary	Hair	Muscle/Soft tissue	Neurologic	Gene Transcript	The number of repetitions
1	39/F	No/ Not available		Myotonia, Myotonic discharge, Muscle weakness		NM_004409.5	**9/>50**
2	56/M	No/ Not available	Frontal balding	Myotonia, Myotonic discharge, Muscle weakness		NM_004409.5	**7/>182**
3	35/M	No/ Not available		Muscle weakness		NM_004409.5	**12/>111**
4	19/M	No/ Not available		Myotonia, Myotonic discharge, Muscle weakness	Speech disability	NM_004409.5	**16/>72**
5	39/M	No/ Not available		Mild muscle weakness		NM_004409.5	13/15
6	33/F	No/ Not available	Frontal balding	Mild muscle weakness		NM_004409.5	7/13
7	33/F	No/ Not available	Frontal balding	Mild muscle weakness		NM_004409.5	7/7
8	58/F	No/ Not available		Mild Muscle Weakness		NM_004409.5	7/7
9	52/M	No/ Not available		Myotonic discharge, Muscle weakness		NM_004409.5	7/21
10	14/M	No/ Not available		Mild muscle weakness		NM_004409.5	7/13
11	15/F	No/ Not available		Myotonic discharge, Muscle weakness		NM_004409.5	12/14
12	4/M	No/ Not available		Mild muscle weakness		NM_004409.5	11/14
13	43/F	No/ Not available		Myotonia, Muscle weakness		NM_004409.5	**14/>50**
14	42/F	No/ Not available		Myotonia		NM_004409.5	13/13
15	30/F	No/ Not available		Myotonia, Muscle weakness		NM_004409.5	**15/>50**
16	19/F	No/ Not available		Myotonia, Muscle weakness	Speech disability	NM_004409.5	**13/>50**
17	29/F	No/ Not available		Mild muscle weakness		NM_004409.5	4/22
18	26/F	No/ Not available		Mild muscle weakness		NM_004409.5	12/20
19	19/F	No/ Not available		Myotonia, Myotonic discharge, Muscle weakness		NM_004409.5	**13/>50**
20	12/F	No/ Not available		Mild Muscle Weakness		NM_004409.5	13/13
21	39/F	No/ Not available		Myotonia, Myotonic discharge		NM_004409.5	**10/>50**
22	51/F	No/ Not available		Mild muscle weakness		NM_004409.5	4/21

between the two groups, a total of 22 people were included in the study. Table 1 displays clinical symptoms, EMG readings, and gene test findings. Nine patients with mean age of 33.22±12.76 years comprised the DM1 group. They were all myotonic. This non-DM1 myotonia group consisted of thirteen patients with mean age of 31.38±16.89 years. Age and gender did not significantly differ across the groups (P=0.360 and P=0.899, respectively).

Seven of the nine patients in the DM1 group, or almost 78% of the total, had myotonic discharge. Myotonic discharge was seen in one patient out of the 13 in the non-DM1 myotonia group, representing an incidence rate of around 8%. There were notable variations in occurrence rates between the DM1 and non-DM1 myotonia groups (χ^2=8.56, P=0.003). Furthermore, there was a significant difference in the incidence rates of myotonia (P=0.0001) between 8 out of 9 DM1 patients (89%) and 1 out of 13 non-DM1 myotonia patients (8%).

In one household, we found three siblings. The younger sister, case 20, had a normal number of repetitions (13/13). Cases 19 and 16 are twin sisters, with DMPK 3'UTR CTG repeat counts of 13/>50. The mother of this family likewise had identical clinical signs but passed away, thus genetic testing could not be performed. The father was taken at an outside facility and genetic testing was verbally reported as normal.

DISCUSSION

We discovered a link between DM1 and myotonia. DM1 was significantly correlated with myotonia in the present research. This aligned with the findings of earlier investigations. Research revealed that distal motor weakness and related clinical and electrical myotonia are common in DM 1 patients [14, 15]. Myotonia might be a sign or symptom that leads people to inquire about genetic testing.

Moreover, we encountered three siblings including two twin patients in this study. The fact that their mother had the same symptoms and died at a young age suggests myotonic dystrophy, even if there is no opportunity for genetic testing. In addition, the fact that her father's genetic test was normal strengthens the possibility. We observed that muscle weakness and myotonia were prominent in the twin sisters. This is evidence of anticipation and demonstrates the necessity of genetic counseling for patients and family members. Genetic counseling provides critical information for family planning. Understanding the risks of passing on an expanded allele can help prospective parents make informed decisions. For instance, they might consider preimplantation genetic diagnosis or other reproductive options to reduce the risk of having a child with severe DM1 [16]. Counseling helps families understand the potential variability in disease presentation and progression due to genetic instability. This knowledge is crucial for preparing for the care needs of affected individuals and setting realistic expectations regarding the disease's impact on quality of life.

Because of the incredibly low incidence rate, this study's limited sample size is one of its limitations. In the future, larger sensitivity and specificity investigations will be required to validate these results. Moreover, our study examined the CTG repeat in the 3' UTR using classical PCR. However, while classical PCR can confirm whether the CTG repeat is over 50 or not, it is difficult to determine whether the CTG repeat is between 100-1000 or even over 1000, that is, it is difficult to decide which of the mild, classical and congenital subtypes it is. For this reason, it is necessary to use the Southern blotting. As we all know, having a Southern blotting device in every laboratory is impossible. For this reason, we are also unable to test the subtypes of DM1 in our study. We were unable to perform Southern blotting and prove that the patients' CTG repeat count was over 100.

CONCLUSION

Our study confirms that myotonia may be an important sign of DM1 associated with DMPK gene mutations. The strong correlation between DM1 and myotonia and the anticipation observed in familial cases emphasize the importance of genetic counseling. This counseling is crucial for family planning and understanding disease variability, aiding in better preparation and management of DM1's impact on quality of life.

Ethics Committee Approval

The participants have already given informed consent. This study was approved by the Ethical Committee of Umraniye Training and Research Hospital

(Ethics No: B.10.1.TKH.4.34. H.GP.0.01/143, 16/05/2024), School of Medicine, University of Health Sciences, Istanbul, Turkey.

Authors' Contribution

Study Conception: ME, GH, BK; Study Design: ME; Supervision: ME, GH; Funding: N/A; Materials: ME, BK; Data Collection and/or Processing: ME, BK; Statistical Analysis and/or Data Interpretation: ME, GH, BK; Literature Review: ME, GH; Manuscript Preparation: GH and Critical Review: ME, GH, BK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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