Original Article

Genotype –Phenotype Correlations in Iranian Myotonic Dystrophy type I patients

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Objectives: Myotonic Dystrophy type I (DM1) is a dominantly inherited disorder with a multisystemic pattern affecting skeletal muscle, heart, eye, endocrine and central nervous system. DM1 is associated with the expansion and instability of CTG repeat in the 3' untranslated region of the myotonic dystrophy protein kinase (DMPK) gene located on chromosome 19q13.3. The aim of this study was to determine clinical and genetic characteristic of DM1 in Iranian patients. Genotype-phenotype correlation was also assessed in a small group of studied patients.

Method: Twenty six DM1 patients belonging to seventeen families were analyzed. Clinical assessment was based on the muscular disability rating scale (MDRS) and a sum of symptoms score (SSS). Molecular analysis (PCR and Southern blot) was used to clarify uncertain clinical diagnosis and in order to confirm clinical findings.

Results: There was an inverse and significant correlation between age of onset and expanded allele length ($p=0.026$, tau-b=-0.360) based on Kendall's tau-b correlation coefficient, while there was no significant correlation between age of onset and severity of the clinical symptoms ($p<0.05$). Also no significant correlation was observed between the two severity scales of the disease (MDRS and SSS) and expanded allele length ($p<0.05$). Expanded allele length was correlated with hypogonadism ($p=0.007$) and cognitive impairment ($p=0.034$).

Conclusion: There was no correlation between cataract and endocrine dysfunction with the expansion size in DM1 patients. Generally it seems there is discordant correlation between clinical symptoms and expanded allele length.

Key Words: CTG Repeat Expansion, Iran, MDRS, Myotonic Dystrophy, SSS

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Introduction

Myotonic dystrophy type 1 (DM1) is a multisystem disorder that characterized by muscle wasting and weakness, ptosis, myotonia, cataract, cardiomyopathy, gonadal atrophy and mental deficiency (1). The clinical findings, which span a continuum from mild to severe, have been categorized into three somewhat overlapping phenotypes: mild, classic, and congenital. Mild DM1 is characterized by mild myotonia (sustained muscle contraction); life span is normal. Classic DM1 is characterized by muscle weakness and wasting, myotonia, cataract, and often by cardiac conduction abnormalities; adults may become physically disabled and may have a shortened life span. Congenital DM1 is characterized by hypotonia and severe generalized weakness at birth, often with respiratory insufficiency and early death; mental retardation is common. DM1 with autosomal dominant inheritance (1) identified as a $(CTG)_n$ repeat in the 3'-UTR of a myotonic dystrophy protein kinase gene (DMPK) on chromosome 19q13.3 (2). The diagnosis of DM1 is suspected in individuals with characteristic muscle weakness and confirmed by molecular genetic testing of DMPK. Disease-causing alleles may expand in length during gametogenesis resulting in the transmission of longer trinucleotide repeat alleles that may be associated with earlier onset and

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increase in severity in successive generation that exhibiting genetic anticipation. The trinucleotide repeat regions in DMPK gene are highly polymorphic, not only in affected individuals but also in the normal population (3, 4). The DM locus shows considerable variability: over 75% of normal individuals are heterozygous (5). Up to 36 repeats of CTG considered as normal (6), while in DM patients extreme expansions ≥2000 have been observed (7-14). Molecular analysis and non molecular testing such as Electromyography (EMG), serum CK concentration and muscle biopsy are used to diagnose the disease. The aim of our study was to analyze the genotype-phenotype relationship in a group of Iranian DM1 patients via clinical phenotype as molecular assessment of the $(CTG)_n$ repeat number in the DM1 gene.

Materials and Methods

Patients:

Forty six DM1 patients, aged 14-60 years, from twenty five families registered at the Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences and Department of Neurology, Shareeati Hospital, Tehran Iran. Primary clinical diagnosis of DM1 patients was based on clinical symptoms, laboratory findings (such as CPK concentration) and Electromyographic (EMG) findings. Molecular analysis was accessed to confirm the diagnosis. The degree of muscle impairment was assessed using the Muscular Disability Rating Scale (MDRS) and the Sum of Symptoms Score (SSS). The SSS was described based on six-point scale (5), which was determined according to sum of six clinical symptoms including cognitive impairment, cataract, cardiomyopathy, diabetes, hypogonadism and motor impairment. Patients having one of these symptoms received score 1 and those with all of symptoms received score 6. In this scoring protocol, hypogonadism was described as gonadal atrophy and/or increasing in Follicular Stimulating Hormone (FSH). In order to screen diabetes, FBS was first measured and in cases with more than 110 mg/dl, 2 hours post prandial blood sugar (2hpp BS) measurement was done. Thyroid dysfunction was investigated by measuring TSH (Thyroid Stimulating Hormone) and if necessary further investigation (such as Electrocardiography, Ecocardiography, Ophthalmologic and neurologic exams) was performed**.** The MDRS is based on five-point scale (15); Scale 1: There is no muscular weakness. In this group diagnosis was assessed via EMG and DNA analysis. Scale 2: There are mild clinical symptoms such as myotonia, tempro-mandibular and sternocleidomastoid muscular atrophy, facial muscle weakness, ptosis and nasal speech. There is no distal muscular weakness expect in flexor muscles. Scale 3: There is distal muscular weakness. Proximal muscular weakness has just been observed in triceps' muscle. Scale 4: There is mild proximal muscular weakness. Scale 5: There is sever proximal muscular weakness; the patients are wheelchair dependent.

DNA analysis:

Genomic DNA was isolated from peripheral blood leukocytes by salting out according to standard procedures (16). Polymerase chain reaction (PCR) was carried out using primers 96 (GGT GCG TGG AGG ATG GAA CAC GGA A) and 102 (GAA CGG GGC TCG AAG GGT CCT TGT AGC) to amplify the CTG repeat region (2). PCR products were run on 8% polyacrylamide gels along side a 25-base pair standard DNA ladder. To estimate the size of the alleles, we used Lab Works TM Software (Version 4.0 for windows). Patients which showed only one band in PCR were also analyzed by Southern blotting. Digestion was performed with *BamHI* and *EcoRI* and hybridization with probe pGB2.6 (17, 18). Labeling was performed with ready prime II random prime labeling system by Amersham Biosciences. The probe was labeled with 5µl alpha $dCTP^{32}$ according to Feinberg and Vogelstein protocol (19, 20). To purify the probe after labeling and in order to remove unincorporated $dCTP^{32}$, we used Sephadex minicolumn G50 in SSC 3X (21). Sigma Ready-to- Use hybridization and prehybridisation buffer was used (hybridization buffer 1x REF H7033), membranes (Hybond N^+ Amersham) were washed with SSC 2X to 0.5X and SDS 1%. Bands were observed with autoradiography films. To size the fragments, a semi-log paper was used. We measured the molecular size of DNA Lambda Hind III of the 23 kb, 9.4 kb and 6.5 kb and draw the curve to estimate the expansion size.

Statistical Analysis:

Statistical analysis was performed via SPSS10 software. Correlation between age of onset and expanded allele length and scores related to severity of the disease was studied based on Kendall's tau-b correlation coefficient. This analysis was performed due to abnormal distribution of MDRS scores and

existence of conflicted numbers in most of variables. Comparison was performed between expanded allele lengths in patients with one of six symptoms and the ones with no symptoms via Mann-Whitney Test ($P<0.05$).

Result

We studied 25 DM1 families, a total of 46 individuals, among which 35 were diagnosed with a CTG repeat expansion. Clinical and molecular data were available on a total of 26 affected individuals for analysis of genotype and phenotype correlation.

Among 26 patients, 17 were males (65.4%) and 9 were females (34.6%). Mean age of the patients at the time of visit was 37.5±12.2 (±SD) years, aged between 18-60 years (median 37 years). Mean age at onset of symptoms was 24.4±15.5 (±SD) years, aged 1-60 years (median 22 years). For 3 patients, age of diagnosis was unknown. Normal allele length average was 9.2±3.1 (±SD) repeat (range 5 to 15, median 8.5) and mode was 8 repeat (5 cases, 19.2%). Expanded allele length average was considered 571.6±255.6 (± SD) repeat (range 97 to 833, median 567). In this case mode was 833 repeat which was observed in 10 patients (38.5%). All patients were heterozygous for CTG expansion and had motor impairment. Table 1 shows frequency of six symptoms which are used in SSS scoring beside thyroid dysfunction.

The average of SSS was 2.2±1.3 (±SD), range 1 to 6 and median 2. The average of MDRS was 3.1±0.8 (±SD), range 2 to 5 and median 3. Between these two scales significant correlation was observed ($p=0.004$, tau-b=0.504). None of the two disease severity scales had significant correlation with expanded allele length. Kendall's tau-b correlation coefficient between expanded allele length and SSS was 0.212 ($p=0.195$) and also between expanded allele length and MDRS was 0.199 ($p=0.233$). The only patient with SSS=6 had 833 repeat in expanded allele with onset age at 2. In this patient's family no other DM1 patient was diagnosed. In patients with hypogonadism and cognitive impairment, expanded allele length was higher than in patients without these symptoms. No such correlation was observed in patients with cataract and diabetes. No statistical analysis could be performed in the case of cardiac and thyroid dysfunction because of low frequency (table 2).

Disease onset showed an inverse and significant correlation with expanded allele length (tau-b=0.360, $p=0.026$, Fig. 1). There was no significant correlation between age of onset and SSS ($p=0.108$, tau-b=0.265) and also with MDRS ($p=0.749$, tau-b=-0.054). In two families we found the anticipation phenomenon with earlier onset of the disease accompanied by increasing symptom severity. In one family, father with 5 children were studied, among which 4 were DM1 patients. Trinucleotide expansion repeat in this family was between 333 and 833. All of patients in this family characterized by ptosis, temporo-mandibular joint dysfunction (TMJ), temporal muscle atrophy and frontal balding.

Discussion

Myotonic dystrophy is the most frequent autosomal dominant myopathy in adults caused by a CTG trinucleotide repeat variation length variationlength. The aim of our study was to analyze the genotype-phenotype relationship in a group of Iranian DM1 patients using clinical phenotype as defined by clinical scores and molecular assessment of $(CTG)_n$ repeat number in the DM1 gene.

This is the first study for Genotype-Phenotype correlation of DM1 patients in Iranian population. The results of this study in Iranian DM1 patients confirm previous observations of the unstable CTG trinucleotide repeat in other populations (8, 10-12, 22).

In present study, all patients were heterozygous for the DM1 mutations and we did not observed homozygous cases as reported by Cobo et al. (23) and Martorell et al. (24).

In two families, contraction of the CTG repeat occurred in intergenerational transmission, however, this was not accompanied by increasing severity of the phenotype in the next generation.

We observed that the CTG repeat correlates positively with most of the clinical symptoms of the disease such as motor impairment (11) and gonadal dysfunction (15). Also no correlation was observed between muscle impairment (based on MDRS) and expanded allele length which is in controversy with other studies (10-12). Although this finding is partially depends on the limitation of our sample size, the definition of the variable also affects the quantity of the observed correlation. Alternatively, direct correlation was observed between expanded allele length with hypogonadism and cognitive impairment. This finding shows discordant correlation between expanded allele length and clinical symptoms and because of that no significant correlation was observed between SSS and expanded allele length. In addition most of DM1

clinical symptoms are multifactorial and their occurrence maybe influenced by other factors, independent of expanded allele length. Anyway, there is no exact explanation of the existence of correlation between expanded allele length and some symptoms and lack of correlation with other symptoms. Also, no correlation was reported by Jaspert et al. between symptoms such as cataract and myotonia, with CTG expansion size (11). In present study an inverse and significant correlation was observed between age of onset and expanded allele length which is in agreement with other studies (3, 13, 14).

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Table I. Frequency of clinical symptoms in 26 DM1 patients.

Symptoms	Number	Frequency (%)
Motor impairment	26	100
Hypogonadism	8	30.8
Cataract	8	30.8
Diabetes	6	23.1
Cognitive impairment	5	19.2
Cardiac dysfunction	3	11.5
Thyroid dysfunction	2	7.7

Table II. Correlation between clinical symptoms and expanded allele length based on Mann-Whitney Test.

Symptoms	Mean score (patients with symptoms)	Mean score (patients without symptoms)	Z	P
Hypogonadism	19.4	10.9	2.696	0.007
Cataract	13.4	13.6	0.057	0.954
Diabetes	14.8	13.1	0.471	0.637
Cognitive impairment	19.8	12.0	2.116	0.034

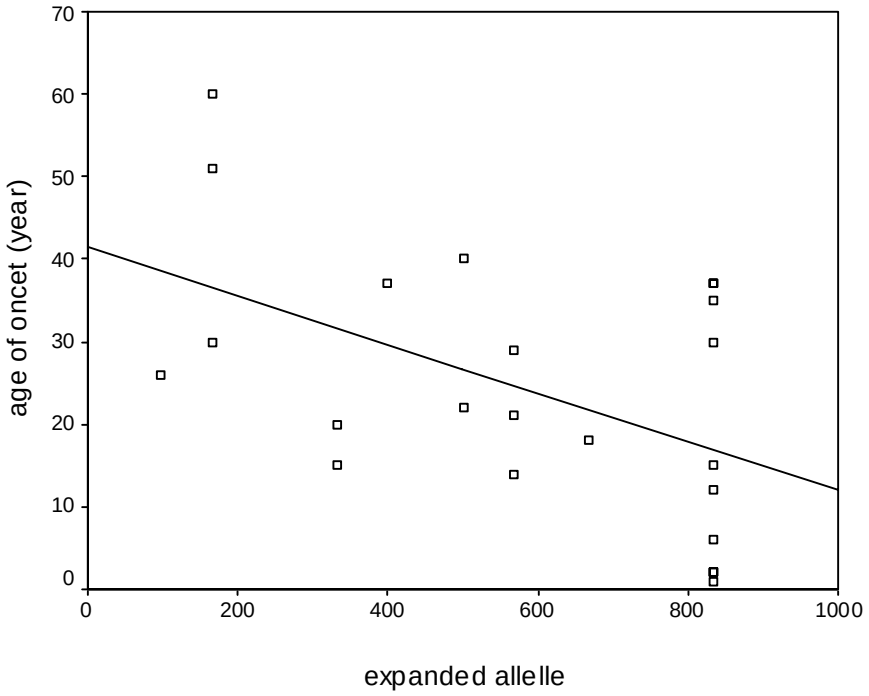

Fig 1. Correlation between age at onset of the disease and expanded allele length (p=0.026, tau-b=0.360).