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Evidence for a relatively high proportion of DM2 mutations in a large group of Polish patients

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ABSTRACT

Introduction: Myotonic dystrophies (DMs) type 1 (DM1) and type 2 (DM2) are autosomal dominant, multisystem disorders, considered the most common dystrophies in adults. DM1 and DM2 are caused by dynamic mutations in the *DMPK* and *CNBP* genes, respectively.
Methods: Molecular analyses were performed by PCR and the modified RP-PCR in patients, in their at-risk relatives and prenatal cases.
Results: The analysis of Polish controls revealed the range of 5-31 CTG repeats for DM1 and 110-228 bp alleles for DM2. Among 318 confirmed probands - 196 (62%) were DM1 and 122 (38%) – DM2. Within DM1families, 10 subjects carried a low expanded CTG tract (< 100 repeats), which resulted in a full mutation in subsequent generations. Two related individuals had unstable alleles–188 bp and 196 bp without common interruptions.
Conclusion: The relative frequencies of DM1/DM2 among Polish patients were 68% and 32%, respectively, with a relatively high proportion of DM2 mutations (1.6:1).

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Abbreviations: CNBP, CCHC-type zinc finger* nucleic acid binding protein; DM, myotonic dystrophy; DMPK, dystrophia myotonica protein kinase gene; EMG, electromyography; EMQN, European Molecular Quality Network; ENMC, European Neuromuscular Centre; PCR, polymerase chain reaction; PROMM, proximal myotonic myopathy; RP-PCR, repeat-primed polymerase chain reaction; SCA1, spinocerebellar ataxia type 1; SCA2, spinocerebellar ataxia type 2; SNP, single nucleotide polymorphism.

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1. Introduction

Myotonic dystrophies type 1 and 2 (DM1, OMIM#605377 and DM2, #602668) are autosomal dominant, multisystem disorders, affecting skeletal and smooth muscle, the heart, the endocrine system and the central nervous system, and are considered the most common muscle dystrophies in adults.

The clinical picture of DM1 has been divided into four overlapping phenotypes: congenital form, childhood onset, adult onset classic and late onset or asymptomatic [1,2]. The most severe, congenital DM1, generally linked to maternal transmission, often develops before birth and presents as hypotonia in infants. Severe generalised weakness and respiratory insufficiency results in high perinatal mortality [3]. Intellectual disability that causes learning and speech disabilities is the main feature of childhood-onset DM1. The core features of adult-onset, classic DM1 comprise distal muscle weakness and wasting, myotonia and posterior subcapsular cataracts. Early frontal baldness in males, facial muscle involvement and typically bilateral mild ptosis are the characteristic features [4]. Myotonia presents as active, percussion or electrical myotonia in almost all patients. The cardiac conduction abnormalities and arrhythmias may cause early mortality and the central nervous system involvement with cognitive impairment is also present. Late-onset oligosymptomatic patients with DM1 are characterised by milder clinical phenotype [5].

The phenotype of DM2 is much more variable and milder than that of DM1. The clinical presentation of DM2 is also known as proximal myotonic myopathy (PROMM) and is characterised by marked weakness and the atrophy of the proximal muscles of the lower limbs, which usually occurs in individuals older than 40 years [6]. Myotonia and cataracts are less frequent than in DM1. Even 50% of DM2 patients may not present with myotonia on clinical examination, and in some, even electrical myotonia is not detected [7].

The muscle pain is present in about 50–80% of DM2 patients and can be the main disabling features of the disease with potential impact on quality of life [8].

Cramps as well as hypertrophy of calves are common, whereas facial muscle weakness and ptosis are rare [6,9]. Nonsustained supra- and ventricular tachycardia are the most common cardiac arrhytmias observed in DM2 patients [10]. Serious cardiac complications were described in a few cases [11]. Anticipation is rare or absent in patients with DM2, unlike families with DM1 [12].

Constellation of the histopathological features like central nucleation, nuclear clumps and type 2 fibres atrophy is considered as a hallmark of DM2 [13,14].

The worldwide prevalence of DM1 is estimated to be between 1:5000 and 1:50,000 in the general population. It ranges from approximately 1:100,000 in some areas of Japan to 1:550 in Northeastern Quebec [15]. DM2 prevalence also seems to differ in various populations. A recently published genetic study in the Finnish population showed that the frequency of the DM2 mutation (1/1830) seems to be much higher than the frequency of type 1 mutations (1/2760) [16]. The DM2 expanded allele is very rare in Japan and only a single case of DM2 has been reported to date in this population [17].

The molecular defect causing DM1 and DM2, involves the expansion of microsatellite repeats in the respective genes. The expansion of CTG repeats in the DM1 mutation is located within the untranslated 3′ region of *DMPK* (dystrophia myotonica protein kinase) gene (19q13.2-q13.3). According to the International Myotonic Dystrophy Consortium (IDMC) in 2000 [18], the range of CTG repeats in a normal alleles is between 5 and 34. Mutable normal alleles (premutation) contain 35–49 CTG repeats and increased meiotic instability may result in the further expansion to the pathogenic range (≥50 CTG repeats). Abnormal CTG repeat lengths are in the range of 50–150 in the mild form, 100–1000 in the classic form and can reach several thousand repeats in infants with the congenital form of DM1 [3]. The pathomechanism of the dynamic mutation leading to multi-organ symptoms acts at the RNA level as an *RNA-toxic gain-of-function mutation* resulting in the abnormal splicing of different genes [19–23].

The DM2-associated gene *CNBP* (3q13.3-q24) codes for a CCHC-type zinc finger, nucleic acid binding protein and contains a complex repeat motif – $(TG)_n(TCTG)_n(CCTG)_n$ – in the intron 1; these three repeat tracts are present in both normal and expanded alleles. Moreover, the CCTG tract in normal alleles contains tetranucleotide interruptions (TCTG or GCTG). DM2 results from the expansion of the pure $(CCTG)_n$ repeats. The size of the complex in normal alleles ranges from 104 to 176 bp and is usually reported in base-pair lengths because of its polymorphic TG and TCTG repeat tracts [24,25]. The characteristic feature of the DM2 mutation is pure $(CCTG)_n$ expansion with 75–11,000 repeats and loss of interruptions. The pathogenic mechanism of CCTG expansion causes RNA gain-of-function toxicity leading to the formation of ribonuclear foci and splicing disturbances. The clinical picture of DM2 may also result from aberrant *CNBP* expression, decreased both on the mRNA and the protein levels, which may be a consequence of multiple factors, for example, the inappropriate pre-mRNA processing of the *CNPB* mutant allele [26,27].

The aim of this study was to investigate the genetic status of a large group of patients with a clinical diagnosis of DM and to assess the relative proportions of DM1 and DM2 in Polish patients.

2. Material and methods

The study group consisted of 742 patients with an initial diagnosis of myotonic dystrophy type 1 or type 2. Additionally, 133 individuals and prenatal cases were tested as asymptomatic members of the families, leading to a total number of 875 individuals genetically evaluated for the presence of DM1 and DM2.

The patients referred for DM1 genetic testing met the main inclusion criteria established by Harper [28] which included distal muscle weakness and atrophy, myotonia, facial muscle involvement and cataracts. The clinical criteria for DM2 genetic testing were consistent with the recommendation presented by Udd at al. in 2006 at an ENMC meeting [6] and were extended during the study according to modifications published in 2011 [29].

16 patients were genetically tested because of the result of muscle biopsy characteristic for DM2: central nucleation, nuclear clumps and type 2 muscle fibres atrophy were observed.

The control group comprised 181 unrelated subjects of Polish ethnicity aged 18–98 years, with neither neurologic nor muscular complaints. Typically, they were recruited from healthy spouses of patients (other than DM patients) visiting the Genetic Counselling Unit and presented no apparent disorders.

The informed consent for the genetic testing was obtained from all tested individuals. The study protocol was approved by the appropriate Bioethics Committee.

DNA samples were extracted from the peripheral blood using the standard phenol/chlorophorm method described elsewhere [30,31] or automated isolation with Magna Pure Compact Nucleic Acid Isolation Kit I – Large Volume (Roche Diagnostics, IN, US) according to the manufacturer recommendations. Testing for CTG/CCTG repeats were performed via PCR reactions with primers according to Brook et al. [32] and Liquori et al. [24]; the reverse primer was fluorescently labelled. The exclusion of the disease was made if two normal alleles were present.

For DNA samples in which only one allele was observed (homozygous individuals) a repeat primed PCR (RP-PCR) reaction was performed with a reverse primer, a primer specific to the CTG repeats and a P3R tail-specific primer [33]. The RP-PCR testing for the DM1 locus was performed in two directions as recommended by EMQN to avoid false negative tests results. For the DM2 molecular analysis, the RP-PCR reaction [34] was performed with a reverse labelled primer, a primer specific to CCTG repeats and a P3R tail-specific primer: RP-DM2 P4CCTG 5′-TAC GCA TCC CAG TTT GAG ACG CCT GCC TGC CTG-3′.

The RP-PCR method detects large expansions and is a qualitative technique only because it does not reveal the exact number of repeats both for DM1 and DM2.

All PCR products were separated on 4% polyacrylamide gels with the automatic sequencer ABI PRISM 377 (Applied Biosystems, CA, US). The length of products was established by comparison with the internal size standard (GeneScan 500 TAMRA).

The analysis for the single nucleotide polymorphism (SNP) rs1871922 was performed with primers described by Bachinski et al. [35], but conditions were modified by the authors. The 172 bp fragment obtained in the PCR reaction was digested with the Hae III restriction enzyme. Within the amplicon, one restriction site is present that yields two products (65 and 107 bp). The presence of another restriction site depends on the presence or absence of a C nucleotide. If the allele with the C nucleotide is present, the restriction digestion yields 3 products (65, 37 and 70 bp), whereas the A allele yields two products. Products labelled fluorescently were separated in 4% polyacrylamide gel in the ABI PRISM 377 sequencer.

The sequencing of DM2 alleles was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and capillary electrophoresis in POP 7 polymer.

A custom NGS (Next Generation Sequencing) ''Myotonia'' panel including 9 genes: *CACNA1S*, *CLCN1*, *HINT1*, *HSPG2*, *KCNJ18*, *KCNJ2*, *KCNJ5*, *MBNL1* and *SCN4A* was applied to screen for causative variants in the patient with CCTG repeats instability in the DM2 locus. The Agilent SureSelectQXT Target Enrichment kit was used to generate the libraries and sequencing performed on MiSeq Illumina platform.

The *CLCN1* gene variants identified in the patient were evidenced by Sanger sequencing also in her affected father and healthy mother.

Statistical analysis was performed using Statistica v. 6.1.

3. Results

3.1. *Molecular analysis of DMPK gene in healthy controls and patients affected with DM*

The analysis of the CTG allelic distribution in the *DMPK* gene in a Polish control group of 181 individuals revealed a range of 5–31 CTG repeats with the most frequent allele with 5 CTGs (36%) and a heterozygosity of 80% (Fig. 1). The distribution of DM1 alleles was in accordance with the Hardy–Weinberg equilibrium ($p = 0.049$).

Molecular tests in the studied group of 875 individuals provided positive results for either DM1 or DM2 in 588 cases: DM1 – 400 subjects (including predictives) from 196 pedigrees and DM2 – 188 subjects (including 41 predictives) from 122 pedigrees.

In 8 of 16 patients tested for DM2 on the basis of characteristic pattern of muscle biopsy the test was positive.

3.2. *Low expanded alleles of DMPK gene (DM1)*

Among 400 DM1 subjects, we found 17 from 16 families (mean age at examination was 54.7 years) with small range of expanded alleles (50–97 CTG) (Table 1). 10 of these were asymptomatic persons (with 50, 55, 74, 75, 75, 84, 88, 88, 90 and 97 CTG repeats) and 7 individuals showed mild form of the disease and classic DM1 phenotype (41.2%). The transmission was paternal in 11and maternal in 6 cases. All the 17 mentioned subjects produced offspring with full DM1 mutation

3.3. *Two rare familial cases of coexistence of premutation and full range mutation in DMPK locus*

In 2 out of 197 pedigrees, we came across the coexistence of a DM1 premutation and a full mutation. In the first case a patient aged 23 affected with congenital form of the DM1 had two alleles: one with full mutation inherited from the affected mother and the second one with premutation of 40 CTG repeats inherited from the healthy father. In the second case (a patient aged 16 affected with congenital form of DM1), a full mutation was transmitted by the father and premutation of 40

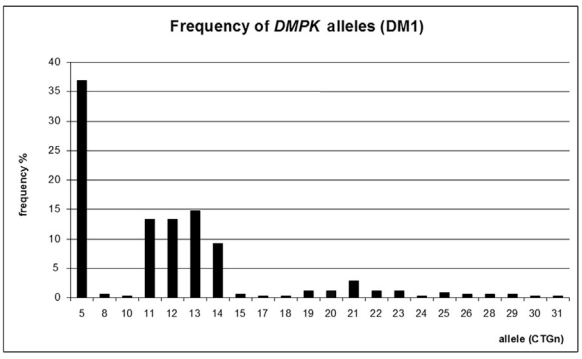

Fig. 1 – The frequency of *DMPK* alleles in a Polish control group composed of 181 healthy individuals ($n = 362$ chromosomes).

Table 1 – Clinical characteristics of patients with low expanded alleles in *DMPK* gene.

Patients no.	Date of birth	Age at examination	CTG number	Clinical symptoms
1.	1946	58	50	Asymptomatic
2.	1939	73	54	Symptomatic (symptoms unknown)
3.	1948	59	55	Asymptomatic
4.	1958	46	58	Arrhythmia
5.	1944	60	63	EMG – slight abnormalities
6.	1954	51	65	Cataract at 46 years
7.	1965	46	74	Asymptomatic
8.	1961	47	75	Asymptomatic
9.	1965	43	75	Asymptomatic
10.	1958	53	80	Cataracts
11.	1950	58	80	Myocardial infarction
12.	1967	46	84	Asymptomatic
13.	1951	60	85	Myotonia, distal weakness of upper limbs
14.	1961	45	88	Asymptomatic
15.	1937	66	88	asymptomatic
16.	1946	63	90	Asymptomatic
17.	1954	57	97	Asymptomatic

CTG from the mother. This rare coincidence requires special attention in genetic counselling. In both mentioned families the alleles of 40 CTG reminded stable at transmission.

3.4. *Molecular investigation of CNBP gene in healthy controls and patients affected with DM2*

For the complex repeat motif $(TG)_n(TCTG)_n(CCTG)_n$ in the *CNBP* gene, the control range established in our study was 110–158 bp. One exception was an individual, aged 92 years, with one allele of 228 bp, apparently unaffected female. An allele of 140 bp was found to be the most common (22%) and the *CNBP* gene heterozygosity was found in 89% (Fig. 2 and Table 2). The distribution of DM2 alleles was in accordance with the Hardy–Weinberg equilibrium with $p = 0.999$.

The presence of expanded alleles in the *CNBP* gene was found in 188 of the subjects from 122 families. Moreover, 41 predictive tests were performed with 22 positive results.

3.5. *Atypical alleles size in myotonic dystrophy type 2 and SNP analysis*

Interestingly, among the patients examined towards DM2 we identified a family with CCTG repeats instability – a 196 bp fragment in the affected girl and a 188 bp fragment in her father, i.e., they exhibited instability with an 8 bp expansion (2 tetranucleotide repeats) during the father-to-daughter transmission.

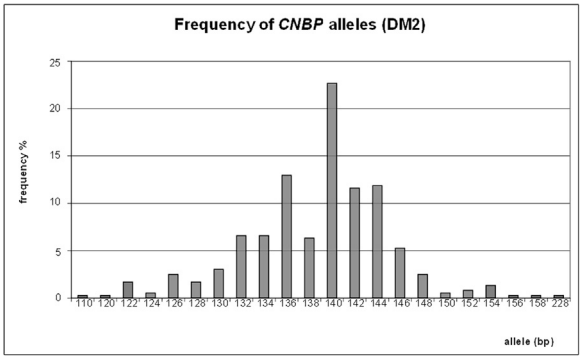

Fig. 2 – The frequency of *CNBP* alleles in a Polish control group composed of 181 healthy individuals ($n = 362$ chromosomes).

These alleles were clearly above the normal range of 104–176 bp according to Liquori et al, but below the range of 75–11,000 CCTG repeats that was previously observed and considered to be pathogenic in patients with DM2 [24].

A patient with 188 bp allele was a 33 year male complaining of cramps and muscle stiffness and myalgia after exercises from about 20 years of age. His neurological examination was normal except of percussion myotonia. EMG revealed some myotonic discharges and neurogenic changes in vastus lateral muscle. The CK level was mildly elevated and dyslipidemia was observed. A 24-h ECG revealed supraventricular arrhythmias. His 7-years-old daughter with myotonia. complained of stiffness of the lower limbs and difficulties in initiating walking from about 3 years of age and her EMG showed myotonic discharges. Her phenotype met the criteria of nondystrofic myotonia. As the father presented a mild myotonia with multisystemic disorders, the test for DM1 and DM2 were performed in both of them.

The sequencing analysis in both patients revealed the following structure:

Father NA (normal allele): $[TG]_{19}[TCTG]_{11}[CCTG]_5GCTGCCTGTCTG[CCTG]_5$ (134 bp).
Father AA (abnormal allele): $[TG]_{16}[TCTG]_9[CCTG]_{30}$ (188 bp).
Daughter NA: $[TG]_{21}[TCTG]_9[CCTG]_5GCTGCCTGTCTG[CCTG]_7$ (138 bp).
Daughter AA: $[TG]_{16}[TCTG]_9[CCTG]_{32}$ (196 bp).

Considering the relatively high frequency of DM2 in Poland and the presence of atypical alleles of *CNBP* gene in patients and in one control subject (228 bp), the analysis of SNP rs1871922 was carried out. The 26 patients with DM2 with a full mutation and as well as 2 above mentioned cases, possessed at least one C allele known to be linked to the European founder mutation [35]. The genotypes frequency: A/C – 50% and C/C – 50% were observed in the patient group. In 36 individuals with

Table 2 – Frequencies of particular alleles of *CNBP* gene with the length of combined motif $(TG)_n(TCTG/CCTG)_n$ in Polish and other control populations.

Control group, origin (no. of chromosomes)	*CNBP* gene				Reference
	The length of the $(TG)_n(TCTG/CCTG)_n$ tract (range)	Most frequent allele	Heterozygosity (%)	Alleles > 160 bp (%)	
European Caucasians (973)	92–190 bp	132 bp	n.d.	1.8%	[38]
African Americans (180)	95–212 bp	126 and 132 bp	n.d.	8.3%	[38]
European Caucasians (1360)	104–176 bp	140 bp	89	0.22%	[25]
Polish (362)	110–228 bp	140 bp	89	0.27%	This study

n.d. – not determined.

normal CCTG repeats number the following genotypes were detected: A/A – 19.4%, A/C – 69.5%, C/C – 11.1%.

NGS approach revealed two known pathogenic variants in the *CLCN1* gene in the affected female c.[1701C>A]; p.(Asn567Lys) known as dominantly inherited and c.[2680C>T]; p.(Arg894Ter) recognised as recessive one. In the affected father c.[1701C>A]; p.(Asn567Lys) variant was found and the mother is a carrier of the c.[2680C>T]; p.(Arg894Ter) variant.

By analysing medical files and pedigrees with confirmed DM1 or DM2 patterns, we were able to determine that there were 89 familial cases of DM1 and 48 familial cases of DM2 as well as 107 and 74 sporadic cases of DM1 and DM2, respectively. It is notable that family history was not always available before the molecular confirmation of DM1 or DM2.

4. Discussion

The frequencies of the particular alleles of the *DMPK* gene obtained in the control group are similar to frequencies reported in other populations. The range of 5–31 CTG in the *DMPK* gene is comparable to the range observed in the European descent populations: (5–27 CTG; 5–30; 5–35) [32,36,37]. The 5 CTG allele in *DMPK* gene was the most common in all the studies.

Liquori et al. were the first to report the structure of the tract containing polymorphic CCTG repeat in *CNBP* gene and its range of 104–176 bp [24] in a group of 680 controls. Other published analyses of two control groups, European Caucasian and African American revealed the length ranged from 92–190 and 95–212 bp, respectively. Alleles greater than or equal to 160 bp were more common in African Americans (8.3%) than in European Caucasians (1.8% or 0.22%) [24,38]. Our results, obtained from 181 controls, revealed a range of 110–228 bp; the 228 bp allele is clearly above the ranges published to date (Table 2). However, if we eliminate this single, particularly large, allele (228 bp), the range of (TG)n(TCTG/CCTG)n in Polish population is quite low: 110 – 158 bp (Fig. 2). The presence of large alleles (above 160 bp – 0.27%) in the Polish population seems to be less common than in other control groups reported in the literature, however, the prevalence of DM2, surprisingly seems to be relatively high. It seems that beside the microsatellites number, distribution pattern and location of interruptions within the microsatellite tracts of long normal alleles, are the major genetic risk factors contributing to the loss of stability and the occurrence of diseases caused by dynamic mutations, as was shown for SCA1 and SCA2 [39]. Therefore, studies of larger control groups are required to evaluate the frequency and structure of large alleles in order to evaluate them as a potential reservoir for DM2 mutations.

The high proportions of heterozygosity in DM1 and DM2 in Polish healthy individuals (80% and 89%, respectively) is similar to those reported by Liquori et al. [24] but higher than in other studies – 75% (DM1) [33] or 81% (DM2) [38].

Considering that the normal *CNBP* alleles size ranges between 104 and 176 bp and the known pathogenic range is between 75 and 11,000 repeats, there is a ''gap'' range of 177–372 bp that corresponds to approximately 27–74 CCTG repeats [40]. In the study of Polish control group, except the longest allele of 228 bp found in the unaffected 92-year-old woman, no allele longer than 158 bp has been identified. The same 228 bp allele was observed together with an expansion of 3400 CCTG repeats in a 59-year-old Japanese woman who suffered from progressing weakness of four limbs, diabetes mellitus and cataracts [41].

Interestingly, among our study patients, we have detected two *CNBP* alleles of atypical size belonging to the ''gap'' range (188 and 196 bp) in an affected father and daughter.

Sequencing of their *CNBP* alleles revealed a loss of interrupting repeats in both large alleles and uninterrupted tracts of CCTG in the father and the daughter of 30 and 32 CCTG, respectively, which also confirms meiotic instability during paternal transmission of 188 bp allele. Studying the length and structure of normal chromosomes, Bachinski et al. distinguished the class of uninterrupted alleles into two subtypes: premutation range (166–186 bp corresponding to 24–32 CCTG) and small full mutation (276–300 bp corresponding to 55–61 CCTG repeats) [38].

After completing the two described projects a new research project on non-dystrophic myotonias allowed us to perform NGS screening of genes related to non-dystrophic myotonias in this family. The more severely affected female is the compound heterozygote, carrying the dominant variant c.[1701C>A]; p.(Asn567Lys) as the result of the transmission from the relatively mildly affected father and recessive variant c.[2680C>T]; p.(Arg894Ter) from the asymptomatic mother-carrier.

Other causes of nondystrophic myotonias were excluded. Obviously the daughter's phenotype is mainly attributed to the *CLCN1* gene mutations and it is difficult to predict a role of unstable allele of *CNBP* gene as a phenotype modifier in this case. A relation between the phenotype of the father, who carries the dominant mutation in the *CLCN1* gene and an unstable allele of *CNBP* is not evident. A further observation of the patients is necessary to draw final conclusions.

Cases of co-segregation of mutations in the *CLCN1* gene interpreted as genetic modifying factors of the DM2 phenotype were reported in Finland, Germany and Norway [42–44].

Moreover, SNP rs1871922 analysis showed the genotype A/C or C/C in all 24 of those with a full DM2 mutation as well as in the 2 above-mentioned cases, which is in accordance with the data reported by Bachinski et al. [35] Allele C seems to be common, but usually segregates with the mutation and shows a different frequency in controls.

In our large group of patients, with both DM1 and DM2, 318 pedigrees were identified. The relative frequencies of DM1 and DM2 in the investigated material of unrelated cases were 61.6% and 38.4%. The Polish control group was not numerous enough to estimate the population frequency of DM1 and DM2 mutations. Other studies were undertaken for other different populations and revealed conflicting results. The prevalence of DM1 in the European population was estimated at 1/25,000, whereas the prevalence in the French-Canadian population in the Quebec province was much higher – 1/514 [45]. The lower prevalence of DM1 in the Japanese, Albanian, Egyptian and Italian population is thought to be associated with the low frequency of the ''at risk'' alleles, or more than 19 CTG repeats [37,46,47]. The frequency of myotonic dystrophy in the Finnish population seems to be much higher than previously reported estimates, which were 1/8000 for DM1 and DM2 together, and a mutation rate of DM1 and DM2 at 1/2760 and 1/1830, respectively [16]. The proportion of DM2 cases identified in our study is higher than expected according to the length of normal alleles in healthy controls. Moreover, because of a milder clinical phenotype, DM2 may be less frequently recognised or perhaps even misdiagnosed [12]. Clinicians should also consider the possibility that patients with DM2 may often present as isolated cases. In our study, the mean number of affected subjects per pedigree was 1.3 for DM2 and 1.6 for DM1.

Our study, and the results of the study by Suominen et al. [16], suggest that DM2 seems to be more prevalent than previously expected and a detailed investigation is essential to explore the whole spectrum of the disease's clinical presentation.

The presence of low expanded DM1 alleles detected in 17 individuals suggests that some patients may remain asymptomatic or present with very subtle clinical symptoms that may not be recognised. Patients with a low number of CTG expansions were previously described as being relatively frequent in the Canadian population; most of them had 50–99 CTG repeats and showed no clinical symptoms except for cataracts [5]. Out of the 17 individuals described here with 50–97 CTGs, 6 had mild symptoms or conditions not necessarily related to the detected mutation (Table 2). The clinical status of these individuals may depend on the age at examination, i.e., the clinical expression can be detected mainly in elderly carriers.

The diagnostic protocol for molecular testing to identify DM1 and DM2 in a group of 875 individuals (patients, predictives and prenatal) included a combination of regular PCR and RP-PCR that tested for normal and expanded alleles, as well as premutation or the expansion of 50–100 CTG repeats [30]. This approach represents a valuable tool for mutation and premutation detection in DM1 and DM2 patients and asymptomatic carriers.

Conflict of interest

None declared.

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