



Population frequency of myotonic dystrophy

Bjarne Udd, Tiina Suominen, Linda L. Bachinski, Satu Auvinen, Peter Hackman, Keith A. Baggerly, Corrado Angelini, Leena Peltonen, Ralf Krahe

► To cite this version:

Bjarne Udd, Tiina Suominen, Linda L. Bachinski, Satu Auvinen, Peter Hackman, et al.. Population frequency of myotonic dystrophy. *European Journal of Human Genetics*, 2011, 10.1038/ejhg.2011.23 . hal-00618486

HAL Id: hal-00618486

<https://hal.science/hal-00618486>

Submitted on 2 Sep 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Population frequency of myotonic dystrophy: Higher than expected frequency of myotonic dystrophy type 2 (DM2) mutation in Finland

Running title: Population frequency of myotonic dystrophy

1. Tiina Suominen,¹ ¹Neuromuscular Research Unit, University of Tampere, 33520 Tampere, Finland

2. Linda L. Bachinski,² ²Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

3. Satu Auvinen,³ ³Department of Neurology, Central Hospital of Jyväskylä, 40620 Jyväskylä, Finland

4. Peter Hackman,⁴ ⁴Department of Medical Genetics, Haartman Institute, University of Helsinki and Folkhälsan Institute of Genetics, P.O. Box 63, 00014 Helsinki, Finland

5. Keith A. Baggerly,^{5,6} ⁵Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; ⁶Graduate Program in Human and Molecular Genetics, University of Texas at Houston Graduate School of Biomedical Sciences, Houston, TX, USA

6. Corrado Angelini,⁷ ⁷Department of Neurosciences, University of Padova, 35128 Padova, Italy

6. Leena Peltonen,⁸ ⁸Department of Medical Genetics, University of Helsinki, P.O. Box 63, 00014 Helsinki, Finland

7. Ralf Krahe,^{2,6,9} ²Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA; ⁶Graduate Programs in Human and Molecular Genetics

and ⁹Genes and Development, University of Texas at Houston Graduate School of Biomedical Sciences, Houston, TX, USA

8. Bjarne Udd,^{1,4,10,11*} ¹Neuromuscular Research Unit, University of Tampere, 33520 Tampere, Finland, ⁴Department of Medical Genetics Haartman Institute, University of Helsinki and Folkhälsan Institute of Genetics, P.O. Box 63, 00014 Helsinki, Finland, ¹⁰Department of Neurology, Tampere University Hospital, 33520 Tampere, Finland, ¹¹Department of Neurology, Vaasa Central Hospital, 65100 Vaasa, Finland

*To whom correspondence should be addressed. Bjarne Udd, M.D., Ph.D., Tel: +358 9 19125075; Fax: +358 9 19125073; Email: bjarne.udd@netikka.fi

SUMMARY (Word count: 250)

Myotonic dystrophy is the most common adult-onset muscular dystrophy with an estimated prevalence of 1/8,000. There are two genetically distinct types, DM1 and DM2. DM2 is generally milder with more phenotypic variability than classic DM1. Our previous data on co-segregation of heterozygous recessive *CLCN1* mutations in DM2 patients indicated a higher than expected DM2 prevalence. The aim of this study was to determine the DM2 and DM1 frequency in the general population, and to explore if the DM2 mutation functions as a modifier in other neuromuscular diseases, to account for unexplained phenotypic variability. We genotyped 5,535 Finnish individuals: 4,532 normal blood donors, 606 patients with various non-myotonic NMD, 221 tibial muscular dystrophy patients and their 176 healthy relatives for the DM2 and DM1 mutations. We also genotyped an Italian idiopathic non-myotonic proximal myopathy cohort (n=93) for the DM2 mutation. In 5,496 samples analyzed for DM2, we found three DM2 mutations and two premutations. In 5,511 samples analyzed for DM1, we found two DM1 mutations and two premutations. In the Italian cohort we identified one patient with a DM2 mutation. We conclude that the DM2 mutation frequency is significantly higher in the general population (1/1,830; p -value=0.0326) than previously estimated. The identification of DM2 mutations in NMD patients with clinical phenotypes not previously associated with DM2 is of particular interest and in accord with the high overall prevalence. Based on our results, DM2 appears more frequent than DM1, with most DM2 patients currently undiagnosed with symptoms frequently occurring in the elderly population.

Keywords: myotonic dystrophy, mutation frequency, prevalence, population

INTRODUCTION

Myotonic dystrophy is an autosomal dominant multi-systemic neuromuscular disorder. Two genetically distinct diseases with clinical similarities but also distinct differences have been identified. Myotonic dystrophy type 1 (DM1, Steinert's disease, MIM #160900) is caused by a (CTG)_n expansion mutation in the 3' UTR of *DMPK* (*dystrophia myotonica protein kinase*) in chromosome 19q13.3¹⁻³; myotonic dystrophy type 2 (DM2, MIM #602668) is caused by a (CCTG)_n expansion mutation in the first intron of *ZNF9* (*zinc finger protein 9*) in chromosome 3q21^{4,5}. Similar clinical features of DM1 and DM2 include myotonia and limb muscle weakness, pronounced distal in DM1 and proximal in DM2, and multi-organ involvement including cataracts, insulin resistance, elevated liver enzymes, male hypogonadism and cardiac conduction defects. Muscle atrophy, facial weakness, ptosis and frontal baldness are very prevalent in DM1, while muscle pain and hypertrophy of calf muscles are more characteristic for DM2. Serum CK levels are usually elevated. Overall, clinical manifestations in DM2 appear to be more variable and generally milder than those in classic adult-onset DM1 (for review see references⁶ and⁷).

In DM1 but not in DM2, the length of the expansion mutation generally correlates with the severity of the disease. Normal individuals have 5-37 (CTG)_n repeats at the *DMPK* locus and the number of repeats in DM1 patients varies from mild late-onset cases with ≥ 50 repeats to the most severe congenital form of DM1 containing up to 4,000 (CTG)_n repeats¹. The four main categories of DM1 based on clinical outcome are (1) congenital, (2) childhood-onset, (3) classic adult-onset, and (4) late-onset/oligo-symptomatic. The distinction is not absolute but rather a continuum generally correlating with increasing length of the (CTG)_n expansion, which tends to increase through successive generations causing genetic anticipation.

The DM2 expansion mutation in intron 1 of *ZNF9* consists of several normally polymorphic elements first described as (TG)_n(TCTG)_n(CCTG)_n, but variations with short intervening cryptic repeats (TCTG and/or GCTG) disrupting the (CCTG)_n tract have also been reported ^{4, 5, 8, 9}. It has been proposed that only uninterrupted (CCTG)_n repeats are associated with the DM2 phenotype ^{5, 8, 9}. The range of (CCTG)_n repeats in patients varies between 55 and 11,000, but, unlike DM1, the threshold defining the difference between normal and disease-causing repeats has not yet been firmly established ^{5, 9}. For DM2 correlation between repeat length and disease severity or age-of-onset has not been demonstrated. Phenotypic variability and the large size of most mutant repeats make this determination more complicated.

Before the identification of their respective genetic mutations, the combined prevalence of DM1 and DM2 has generally been estimated at 1 in 8,000 (12.5 per 100,000) based on clinical ascertainment of patients. Since this estimate likely based mostly on more severely affected DM1 patients, DM1 is considered to be the most frequent muscular dystrophy in adults ¹⁰. However, in different populations prevalence estimates vary widely: In an Italian population from the regions around Padova and North-West Tuscany a prevalence of 9.31 per 100,000 inhabitants was reported ¹¹, while a slightly higher prevalence (11.95 per 100,000) was reported in Northern-Ireland ¹², and lower in Belgrade (Serbia) 5.3 per 100,000 ¹³. In Jewish communities an average prevalence of 15.7 per 100,000 was reported in a study including 416 DM1 patients of which 307 were diagnosed genetically and 109 clinically ¹⁴. The same study reported even higher prevalences in two Jewish subgroups: 20.0 per 100,000 in Sephardim/Oriental Jews and 47.3 per 100,000 in Yemenite Jews. Among Jews, Ashkenazi had the lowest prevalence with 5.7 per 100,000. A relatively high prevalence of DM1 in the smaller Istria region in Croatia has been reported (18.1 per 100,000) based on clinical

ascertainment and genetic confirmation¹⁵. The highest known prevalence has been reported in the French Canadian population (1 in 475) due to a founder effect¹⁶. In non-European populations much lower prevalence rates have been observed, for example a prevalence of 0.46 per 100,000 in Taiwan has been estimated based on DM1 patients and families identified by genetic analysis during 1990-2001 (96 subjects belonging to 26 families)^{17,18}.

For DM2 there are currently no established prevalence estimates. DM2 is generally thought to be rarer than DM1, but large scale population studies to confirm this have not been carried out. In Finland 56 new DM2 patients were genetically diagnosed in 2004-2005, and a prevalence estimate of 1/10,000 based on molecular genetic testing has been reported for the Central Finland hospital district¹⁹. However, new clinical ascertainment data suggest at least a two-fold higher prevalence in Central Finland (unpublished data). In Germany 267 mutation-verified DM2 molecular diagnoses were made between 2003 and 2005 compared to 277 DM1 diagnoses within the same period¹⁹. These data support the notion that DM2 is more frequent than previously thought, possibly even as frequent as DM1. Because of a shared ancestral founder haplotype common to all Caucasian DM2 patients⁴, there is no *a priori* reason to expect the frequency of DM2 mutation to be highly variable in different European populations.

Because of the wide spectrum of milder phenotypic presentations in DM2, the prevalence of the disease is not easy to estimate clinically. Tissue-related differences in repeat instability, modifier genes, or other mechanisms may influence the length of (CCTG)_n repeats needed to elicit symptoms in a given patient and within different organs. In DM2 there is no known correlation between repeat size and disease severity. Our experience clearly indicates a higher prevalence of very late onset and mild manifestations of DM2 than previously reported²⁰.

Because the first descriptions of the clinical phenotype were based on families collected for linkage studies, there may have been an intrinsic ascertainment bias for more severe disease manifestations, resulting in a lack of very mild cases in the phenotypic spectrum. Our objective in this study was to determine the overall frequency of the DM2 and DM1 mutations in the general population.

METHODS

Subjects

To study the frequency of DM2 and DM1 expansion mutations, we analyzed 4,532 Finnish population control DNA samples obtained from anonymous blood donors. The Finnish population is a historically isolated population with very specific features regarding accumulation of certain recessive disorders based on limited numbers of founder populations and bottlenecks. For dominant disorders the outcome is somewhat different and the Finnish population is expected to be a relatively representative Caucasian population in regard to the DM2 mutation, because of the ancestral founder haplotype existing in all Europe. The material was selected because of the availability of suitable and high-quality samples. In order to study the frequency of the DM mutations and to explore whether the presence of the DM2 (or DM1) mutation might function as a modifier in other neuromuscular diseases (NMD), we also studied an additional 1,003 Finnish samples (862 independent chromosomes) consisting of 606 patients with various non-myotonic NMD, 221 tibial muscular dystrophy (TMD) patients, and 176 healthy relatives of the TMD patients from previous linkage studies. We also studied a cohort of 93 Italian patients with undetermined non-myotonic proximal myopathy or asymptomatic hyperCKemia, which were genotyped for

the DM2 mutation only because of their proximal muscle phenotype. The study was approved by the IRB of Tampere University Hospital.

Methods

DNA isolated from peripheral blood leukocytes was genotyped for both the DM2 and DM1 mutations as previously described^{4, 21-23}. Briefly, first DM2 and DM1 repeats were amplified by PCR across the repeat under conditions where only normal-sized alleles are amplified. Samples with a single allele (either two normal alleles of identical size, or only one normal allele due to resistance of expanded mutant alleles to PCR) were further studied using repeat-primed PCR (RP-PCR). The results for both steps were obtained using fluorescent fragment analysis by capillary electrophoresis (ABI 3100 or ABI 3130 Genetic Analyzer) and analyzed using Genotyper or GeneMapper software (Applied Biosystems, Foster City, CA). For the two DM2 premutation alleles from the NMD cohort, single genome equivalent amplification (small-pool PCR) was performed as previously described to determine the stability of the repeat region^{9, 24}, and sequencing of the repeat region to characterize the repeat number and interruptions of the (CCTG)_n repeat in *ZNF9*, as previously described⁹. A single nucleotide polymorphism (SNP, rs1871922) in linkage-disequilibrium (LD) with the DM2 repeat expansion mutation, was also genotyped as previously described^{4, 9} for the samples with large, possibly unstable DM2 alleles in *ZNF9*.

Statistical analyses

To determine statistical significance in the general population cohort, we modeled the presence of a DM mutation (either DM1 or DM2) as a binomial random variable, where in 4,510 tries we observed 4 mutation-positive cases. Using a likelihood function for the detection rate, p , we computed both an equal-tailed 95% interval estimate (excluding the

bottom and top 2.5% of all values) and a maximum likelihood 95% interval estimate (all values in the interval have a likelihood above a threshold value). Computation of these estimates used the fact that the likelihood function is proportional to a $\text{beta}(4+1, 4506+1)$ distribution. We then determined whether $1/8,000$ falls in either interval, and computed the chance that ≥ 4 mutations would be seen in 4,510 trials if this were in fact the true mutation rate.

Statistical significance in the combined general population and NMD cohorts was modeled similarly using a likelihood function that was proportional to a $\text{beta}(5+1, 5495+1)$ distribution, where in 5,500 tries we observed 5 mutation-positive cases. We again determined whether $1/8,000$ falls in either interval, and computed the chance that ≥ 5 mutations would be seen in 5,500 trials if this were in fact the true mutation rate.

Similarly, to determine statistical significance for the DM2 mutation alone in the general population cohort, we modeled the mutation frequency using a likelihood function that was proportional to a $\text{beta}(3+1, 5497+1)$ distribution, where in 5,500 trials we observed 3 mutation-positive cases. We then determined whether $1/8,000$ falls in either interval, and computed the chance that ≥ 3 mutations would be seen in 5,500 trials if this were in fact the true mutation rate.

RESULTS

General population cohort

The population cohort consisted of 4,532 anonymous blood donor DNA samples. Results for allele sizes were obtained from 4,508 samples for DM2 and 4,520 for DM1 giving very low

failure rates (0.53% for DM2 and 0.26% for DM1). A single allele was seen in 12.6% (572) of samples at the *ZNF9* locus and in 20.7% (938) of samples at the *DMPK* locus. Using RP-PCR on these single-allele samples, two DM2 and two DM1 mutation-positive samples were identified (Table 1). One of the DM1 mutations had a (CTG)₈₀ repeat allele, while for the other the exact repeat number of the expanded allele could not be determined. For the two DM2 mutations the exact (CCTG)_n repeat number could not be determined with the methods used. Both DM2 mutation-positive samples showed homozygosity for the C allele of SNP rs1871922 in LD with the DM2 expansion mutation.

In addition to the normal and mutant alleles, a small number of enlarged alleles that fall in the range between normal and mutant were identified. Such enlarged alleles were found in *ZNF9* in nine samples, of which five showed a pattern consistent with an unstable (CCTG)_n repeat on RP-PCR. These nine samples were genotyped for SNP rs1871922 and the majority (n = 6) were homozygous (C/C) and three samples were heterozygous (A/C).

In *DMPK* one premutation allele of approximately (CTG)₄₅ repeats was identified and in 13 other samples alleles in the range of 35-40 repeats were identified by PCR across the repeat region. In seven additional samples RP-PCR suggested an enlarged allele of unknown repeat size.

NMD patient cohort

Among 988 samples with available molecular diagnostic DM2 data, one DM2 mutation was found (Table 1; Figure 1). Eleven percent (11%) of the individuals studied showed one single allele for the repeat tract in intron 1 of *ZNF9*. Possible large alleles were detected in six individuals by RP-PCR. Sequencing of these DM2 repeat alleles revealed two samples with

uninterrupted short repeat expansions of (CCTG)₂₄ and (CCTG)₂₅ repeats. These expanded alleles were also found to be unstable in small-pool PCR, and the samples were heterozygous for SNP rs1871922 (A/C)⁹. The patient with a DM2 mutation was previously diagnosed with genetically verified tibial muscular dystrophy, but with unusually marked proximal muscle involvement. Clinically the patient had no myotonia. EMG studies were not performed, and the patient is no longer available for follow-up studies. Of the two patients with uninterrupted unstable (CCTG)₂₄₋₂₅ tracts, one was diagnosed with a mitochondrial DNA 3243A>G mutation causing MELAS (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes) syndrome^{25,26}, the other patient had an undetermined asymmetric muscle disorder with pseudohypertrophy of one leg and mild atrophy of the other leg. In light of the lack of any DM-like phenotype, the co-segregating uninterrupted unstable (CCTG)₂₄₋₂₅ tract did not appear to have any discernable effect on the phenotype in these two patients.

Among 991 samples with available molecular diagnostic DM1 data, there were no DM1 mutations (Table 1). One borderline pre-mutation allele of 37 repeats was found in one individual. Nineteen percent (19%) of samples were homozygous for the DM1 repeat in *DMPK* showing one allele size in the locus.

In conclusion, combining our general population cohort and the NMD cohort we found three DM2 mutations in 5,496 individuals and two DM1 mutations in 5,511 individuals. Based on these results the frequency of DM mutations is approximately 1 in 1,830 for DM2 and 1 in 2,760 for DM1. Because all patients with myotonia were excluded from the NMD cohort and DM mutations segregate independently from the underlying cause of disease in these patients, this cohort can be considered an unselected population with respect to both DM mutations.

Cohort of proximal myopathy or asymptomatic hyperCKemia

A cohort consisting of 93 Italian patients with idiopathic proximal myopathy or asymptomatic hyperCKemia was analyzed for the DM2 mutation by PCR across the DM2 repeat and RP-PCR. In this cohort, one DM2 mutation-positive patient was found (Table 1). This patient showed waddling gait, proximal weakness and Gower's sign at age 49, while CK levels were normal. EMG showed findings consistent with a myopathic pattern without myotonic discharges.

Statistical analyses

The previously reported prevalence for DM is 1 in 8,000 ($0.000125 = 1.25\text{E-}4$)¹⁰. Using a likelihood function to model the presence of a DM mutation (either DM1 or DM2) as a binomial random variable in 4,510 anonymous blood donor samples with available genotypes (general population cohort), the observed frequency of four mutation-positive carriers was significantly higher than expected by chance (point estimate (PE): $8.9\text{E-}4$, equal-tailed 95% interval (ET) [$3.6\text{E-}4$, $2.27\text{E-}3$], maximum likelihood 95% interval (ML) [$2.7\text{E-}4$, $2.09\text{E-}3$], $p\text{-value} = 0.0027$). Similarly, the observed frequency of five mutation-positive carriers in the combined general population and NMD cohorts ($n = 5,500$) was significantly higher than expected by chance (PE: $9.1\text{E-}4$, ET: [$4.0\text{E-}4$, $2.12\text{E-}3$], ML: [$3.2\text{E-}4$, $1.98\text{E-}3$], $p\text{-value} = 0.0007$). The frequency of the DM2 mutation by itself was also significantly more frequent (PE: $5.45\text{E-}4$, ET: [$1.98\text{E-}4$, $1.59\text{E-}3$], ML: [$1.30\text{E-}4$, $1.44\text{E-}3$], $p = 0.0326$ for the general population and NMD cohorts combined). These results are presented in Table 2. Starting with the null hypothesis that the true rate of the DM prevalence is $1/8,000$ and testing this by measuring the status of 5,500 individuals with a required a type I error $\alpha \leq 0.05$, observing ≥ 3 mutation-positive DM cases causes us to reject the null hypothesis. If the true rate is $5/5,500$

(or 1/1,100), we have 87.5% power to correctly identify a difference. Thus, our data clearly indicate that DM (DM1 and DM2 combined) and DM2 alone are significantly more frequent than could be expected for the population based on previous estimates.

DISCUSSION

Early estimates of disease frequency for DM relied on clinical ascertainment of patients and have resulted in a calculated overall prevalence of 1/8,000¹⁰. Since the advent of molecular genetic testing for DM1, several prevalence studies have been reported^{11-15, 17}, but so far no large-scale population studies have been performed. Most of the reported studies were based on the number of diagnosed patients related to the corresponding total population in a certain region. In regions with good diagnostic services, this type of ascertainment may be close to 100% for the subcategories congenital and adult-onset DM1. However, the actual DM1 mutation frequency may be higher due to the known existence of a large group of clinically undiagnosed oligosymptomatic mild forms and some undiagnosed childhood-onset forms. For DM2, clinically based ascertainment of patients is even more difficult because of the large phenotypic variability and a large number of individuals with milder symptoms who remain undiagnosed. Since the availability of DM2 molecular diagnostics, our experience indicated that DM2 is far more common than previously estimated^{27, 28}. Milder phenotypes with prominent myalgia may easily be misdiagnosed as fibromyalgia²⁷, and patients with onset of slowly progressive proximal muscle weakness after age 70 may not be referred for neuromuscular investigations. Further evidence for a large proportion of undiagnosed DM2 patients came from our study on diagnosed DM2 patients showing a disproportionally high number of co-segregating heterozygous recessive *CLCN1* mutations²⁸. This study directly suggested that DM2 patients with co-segregating *CLCN1* could be more easily identified and

diagnosed than DM2 patients without the modifier allele, and consequently that the majority of DM2 patients remains undiagnosed even in clinical centers with considerable experience with DM2.

Our present study investigated the prevalence of the DM2 and DM1 mutations in a cohort of 5,535 individuals consisting of anonymous blood donors and patients with various determined and undetermined neuromuscular disorders with exclusion of any myotonic symptoms. The finding of three DM2 mutations in this cohort suggests a mutation frequency of 1 in 1,830, which is >4-fold higher than any previous estimates. In the same cohort we identified two DM1 mutations suggesting a mutation frequency of 1 in 2,760 in the general population. Compared to previous prevalence estimates for DM, this is almost 3-fold higher. These results are significantly different to the previous DM prevalence estimate of 1/8,000 (4/4510, p -value = 0.0027 for the general population cohort, and 5/5500, p -value = 0.0007 when general and NMD cohorts are combined).

The higher than expected DM1 mutation frequency is probably due to a large number of smaller expansion alleles which are known to be asymptomatic or cause very mild symptoms that can be confused with normal aging in elderly individuals²⁹. Clinically this may indicate that a number of elderly patients with cataracts or ptosis may, in fact, have DM1 as the underlying cause. However, if these patients do not develop other symptoms indicative of DM1 during their lifetime there is no need to change clinical practice.

With DM2 the situation is different, however. Because all DM2 mutation carriers are expected to develop disease symptoms, and patients currently remain undiagnosed, their symptoms are currently incompletely understood. This may cause significant differences in

DM2 ascertainment in different populations, because the prevailing practice for directing a patient for genetic testing of DM2 mutation may vary. In the most typical families selected for linkage studies, the DM2 mutation was 100% penetrant. However, it is not settled if this is true in all situations or if the phenotype can extend beyond the currently known variation. Since the DM2 mutation may also cause cardiac conduction defects and sudden cardiac death as early as middle age³⁰, the timely identification of DM2 mutations in carriers is of major clinical importance. Mutation carriers may present to a wide range of clinical specialities: neurology, cardiology, internal medicine, ophthalmology, rheumatology, endocrinology, etc. The mutation frequency determined in this study suggests a need for increased consideration of DM2 as a possible cause of symptoms. At least those patients undergoing neurological evaluation should be assessed by molecular diagnostic testing with a rather low phenotypic threshold⁸.

Because the DM2 mutation has a single European founder haplotype⁴, the frequency of DM2 in the Finnish population is suggestive for a high mutation frequency also in other Caucasian populations. However, specific historical population bottlenecks and genetic drift may cause somewhat skewed frequencies in different sub-populations. Samples from healthy blood donors are expected to be relatively representative of the population at-large from which they are drawn.

Uninterrupted unstable DM2 repeat tracts of (CCTG)₂₄ and (CCTG)₂₅ repeats were found in two individuals from the NMD cohort. One of these patients had a diagnosis of molecularly verified MELAS syndrome, while the other had an undetermined asymmetric muscle hypertrophy in one leg and atrophy in the other. However, deleterious consequences of these small expansion alleles on the phenotype in these individuals are unlikely. In the general

population cohort we found nine samples with possibly unstable enlarged alleles, suggesting a DM2 premutation. These nine samples in addition to the two small expansion samples found in the NMD cohort were all either heterozygous or homozygous for the C allele at SNP rs1871922, which is in LD with the DM2 haplotype ⁴. The occurrence of such uninterrupted unstable repeat tracts is compatible with the recent identification of DM2 premutation alleles ⁹.

The single DM2 mutation in the NMD cohort was found in a patient with a genetically confirmed diagnosis of tibial muscular dystrophy. Besides the conventional weakness of ankle dorsiflexion this patient had an unusually marked proximal muscle involvement at the age of 55 years. The DM2 mutation mainly affects proximal muscles, suggesting that the marked proximal muscle involvement in this particular DM2-positive TMD patient could be associated with the co-segregating DM2 mutation, although its impact cannot be definitively determined. Most TMD patients have a highly selective involvement of the anterior compartment muscles of the lower legs and marked proximal lower limb weakness occurring only after age 70-75. In a study of 209 TMD patients, 9% had very unusual phenotypes, including onset in proximal muscles, despite an identical *TTN* founder mutation in all patients ³¹. The reason for these phenotypic variations remains unknown and was not associated with a co-segregating DM2 mutation. In the Italian cohort with 93 patients of undetermined non-myotonic proximal myopathy or asymptomatic hyperCKemia, one patient proved to have a molecular diagnosis of DM2. The fact that myotonia is frequently absent in DM2 patients, even on EMG examination, is not a new observation ^{7, 19}. However, the identification of DM2 in a patient without otherwise suggestive features of the disease is of interest and suggests that the DM2 diagnosis may easily be missed in a neuromuscular diagnostic setting because of incomplete or uncharacteristic phenotypic expression.

Two DM1 mutations were identified in the general population cohort. A (CTG)₈₀ allele was identified for one individual, while for the other size could not be determined. Two premutation alleles were found in both the population cohort [(CTG)₄₅] and the NMD cohort [(CTG)₃₇].

Taken together our data indicate that mutations for DM (DM1 and DM2) are much more prevalent than previously estimated, and that DM2 may even be the most common inherited muscle disease in European populations. The results also indicate that the vast majority of DM2 patients currently remain undiagnosed. At least two conclusions can be drawn: first, DM2 patients with symptoms for myalgia, muscle weakness in advanced age, insulin resistance or cardiac conduction abnormalities are currently not correctly identified, and secondly, a more comprehensive study of the natural history to characterize the entire spectrum of disease presentations is urgently needed for DM2.

Acknowledgements

Tamer Ahmed is gratefully acknowledged for his technical assistance with DM2 and DM1 mutation analysis. Merja Soininen and Satu Luhtasela are acknowledged for their assistance with sample preparations and logistics. TS was funded by Finnish Cultural Foundation, Pirkanmaa Regional fund and Pirkanmaa Hospital District research fund. CA was supported by the Telethon Genetic Biobank project GTB07001, the Eurobiobank project n° QLRI-CT-2002-02769 and Cariparo Grant. RK was supported by grants from NIH (AR48171), Muscular Dystrophy Association, USA and the Kleberg Foundation. BU was supported by grants from the Vaasa Central Hospital district and Pirkanmaa Hospital district medical research funds, and the Liv och Hälsa medical research foundation.

Conflict of interest statement:

We declare that we have no conflicts of interest.

References

- 1 Brook JD, McCurrach ME, Harley HG, *et al.* Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell* 1992;**69**:385.
- 2 Fu YH, Pizzuti A, Fenwick RG,Jr, *et al.* An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science* 1992;**255**:1256-1258.
- 3 Mahadevan M, Tsilfidis C, Sabourin L, *et al.* Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science* 1992;**255**:1253-1255.
- 4 Bachinski LL, Udd B, Meola G, *et al.* Confirmation of the type 2 myotonic dystrophy (CCTG)_n expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. *Am J Hum Genet* 2003;**73**:835-848.
- 5 Liquori CL, Ricker K, Moseley ML, *et al.* Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. *Science* 2001;**293**:864-867.
- 6 Machuca-Tzili L, Brook D, Hilton-Jones D. Clinical and molecular aspects of the myotonic dystrophies: a review. *Muscle Nerve* 2005;**32**:1-18.
- 7 Krahe R, Bachinski LL, Udd B. Myotonic dystrophy type 2: Clinical and genetic aspects. In: Ashizawa T, Wells D, editors. Genetic instabilities and neurological diseases. Second Edition ed. : Academic Press; 2006. pp. 131-132-150.
- 8 Liquori CL, Ikeda Y, Weatherspoon M, *et al.* Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. *Am J Hum Genet* 2003;**73**:849-862.

- 9 Bachinski LL, Czernuszewicz T, Ramagli LS, *et al.* Premutation allele pool in myotonic dystrophy type 2. *Neurology* 2009;**72**:490-497.
- 10 Harper PS. Myotonic dystrophy 3rd ed. London, UK: W.B. Saunders; 2001.
- 11 Siciliano G, Manca M, Gennarelli M, *et al.* Epidemiology of myotonic dystrophy in Italy: re-appraisal after genetic diagnosis. *Clin Genet* 2001;**59**:344-349.
- 12 Magee A, Nevin NC. The epidemiology of myotonic dystrophy in Northern Ireland. *Community Genet* 1999;**2**:179-183.
- 13 Mladenovic J, Pekmezovic T, Todorovic S, *et al.* Epidemiology of myotonic dystrophy type 1 (Steinert disease) in Belgrade (Serbia). *Clin Neurol Neurosurg* 2006;**108**:757-760.
- 14 Segel R, Silverstein S, Lerer I, *et al.* Prevalence of myotonic dystrophy in Israeli Jewish communities: inter-community variation and founder premutations. *Am J Med Genet A* 2003;**119**:273-278.
- 15 Medica I, Markovic D, Peterlin B. Genetic epidemiology of myotonic dystrophy in Istria, Croatia. *Acta Neurol Scand* 1997;**95**:164-166.
- 16 Bouchard G, Roy R, Declos M, Mathieu J, Kouladjian K. Origin and diffusion of the myotonic dystrophy gene in the Saguenay region (Quebec). *Can J Neurol Sci* 1989;**16**:119-122.
- 17 Hsiao KM, Chen SS, Li SY, *et al.* Epidemiological and genetic studies of myotonic dystrophy type 1 in Taiwan. *Neuroepidemiology* 2003;**22**:283-289.
- 18 Ashizawa T, Epstein HF. Ethnic distribution of myotonic dystrophy gene. *Lancet* 1991;**338**:642-643.

- 19 Udd B, Meola G, Krahe R, *et al.* 140th ENMC International Workshop: Myotonic Dystrophy DM2/PROMM and other myotonic dystrophies with guidelines on management. *Neuromuscul Disord* 2006;**16**:403-413.
- 20 Day JW, Ricker K, Jacobsen JF, *et al.* Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. *Neurology* 2003;**60**:657-664.
- 21 Sallinen R, Vihola A, Bachinski LL, *et al.* New methods for molecular diagnosis and demonstration of the (CCTG)_n mutation in myotonic dystrophy type 2 (DM2). *Neuromuscul Disord* 2004;**14**:274-283.
- 22 Sermon K, Seneca S, De Rycke M, *et al.* PGD in the lab for triplet repeat diseases - myotonic dystrophy, Huntington's disease and Fragile-X syndrome. *Mol Cell Endocrinol* 2001;**183 Suppl 1**:S77-85.
- 23 Warner JP, Barron LH, Goudie D, *et al.* A general method for the detection of large CAG repeat expansions by fluorescent PCR. *J Med Genet* 1996;**33**:1022-1026.
- 24 Coolbaugh-Murphy MI, Xu J, Ramagli LS, Brown BW, Siciliano MJ. Microsatellite instability (MSI) increases with age in normal somatic cells. *Mech Ageing Dev* 2005;**126**:1051-1059.
- 25 Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. *Ann Neurol* 1984;**16**:481-488.
- 26 Montagna P, Gallassi R, Medori R, *et al.* MELAS syndrome: characteristic migrainous and epileptic features and maternal transmission. *Neurology* 1988;**38**:751-754.

- 27 Auvinen S, Suominen T, Hannonen P, Bachinski LL, Krahe R, Udd B. Myotonic dystrophy type 2 found in two of sixty-three persons diagnosed as having fibromyalgia. *Arthritis Rheum* 2008;**58**:3627-3631.
- 28 Suominen T, Schoser B, Raheem O, *et al.* High frequency of co-segregating CLCN1 mutations among myotonic dystrophy type 2 patients from Finland and Germany. *J Neurol* 2008;**255**:1731-1736.
- 29 Arsenault ME, Prevost C, Lescault A, Laberge C, Puymirat J, Mathieu J. Clinical characteristics of myotonic dystrophy type 1 patients with small CTG expansions. *Neurology* 2006;**66**:1248-1250.
- 30 Schoser BG, Ricker K, Schneider-Gold C, *et al.* Sudden cardiac death in myotonic dystrophy type 2. *Neurology* 2004;**63**:2402-2404.
- 31 Udd B, Vihola A, Sarparanta J, Richard I, Hackman P. Titinopathies and extension of the M-line mutation phenotype beyond distal myopathy and LGMD2J. *Neurology* 2005;**64**:636-642.

Figure Legend

Figure 1. Representative results for DM2 mutation genotyping by PCR and RP-PCR.

PCR across the (CCTG)_{DM2} in intron 1 of *ZNF9* showed either a single amplifiable allele (A) or two amplifiable alleles (B), which excludes DM2 mutation. A patient with DM2 mutation from the population cohort showed a peak pattern in *ZNF9* RP-PCR characteristic for the DM2 (CCTG)_n repeat expansion mutation (C and D). In the zoomed-in figure (D) adjacent stutter peaks can be seen with base pair difference of 4 bp. Enlarged unstable (but not actually expanded) DM2 alleles have a distinct pattern in RP-PCR (E and F). In the zoomed-in figure (F), the stutter peaks can be seen, but the pattern ends rapidly. Figures (G) and (H) show a typical example of a DM2 negative sample in RP-PCR. Stutter peaks characteristic to DM2 mutation are not seen in the zoomed-in figure (H).

Table 1. Summary of samples analyzed for DM2 and DM1 mutation. General population and NMD cohorts were analyzed for both DM2 and DM1 mutations; the proximal myopathy or asymptomatic hyperCKemia cohort was analyzed for the DM2 mutation only.

Samples analyzed*	DM2 (CCTG)_n		DM1 (CTG)_n	
	n	DM2 positive n	n	DM1 positive n
General population cohort (FIN)	4,508	2	4,520	2
NMD cohort (FIN)	988	1	991	0
Proximal myopathy or asymptomatic hyperCKemia cohort (I)	93	1	na	na
Total	5,589	4	5,511	2

*FIN, Finland; I, Italy. na, not applicable.

Table 2. Results of the statistical analysis. The presence of DM1 and DM2 mutations together or DM2 mutation alone is modeled as a binomial random variable to determine statistical significance compared to previously reported prevalence for DM, which is 1 in 8,000 ($0.000125 = 1.25\text{E-}4$).

Statistical analysis	PE	ET	ML	p-value
Presence of DM mutations in general population cohort (4 in 4,510)	8.9E-4	[3.6E-4, 2.27E-3]	[2.7E-4, 2.09E-3]	0.0027
Presence of DM mutations in combined general population and NMD cohorts (5 in 5,500)	9.1E-4	[4.0E-4, 2.12E-3]	[3.2E-4, 1.98E-3]	0.0007
Presence of DM2 mutation in combined general population and NMD cohorts (3 in 5,500)	5.45E-4	[1.98E-4, 1.59E-3]	[1.30E-4, 1.44E-3]	0.0326

DM, DM1 and DM2 combined; PE, point estimate; ET, equal-tailed 95% interval; ML, maximum likelihood 95% interval



