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A DM1 family with interruptions associated with atypical symptoms and late onset but not with a milder phenotype

Short running title: interrupted DM1 patients' atypical and severe signs

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ABSTRACT

Carriage of interruptions in CTG repeats of the myotonic dystrophy protein kinase gene has been associated with a broad spectrum of myotonic dystrophy type 1 (DM1) phenotypes, mostly mild. However, the data available on interrupted DM1 patients and their phenotype are scarce. We studied 49 Spanish DM1 patients, whose clinical phenotype was evaluated in depth. Blood DNA was obtained and analyzed through triplet-primed polymerase chain reaction (PCR), long PCR-Southern blot, small pool PCR, AciI digestion, and sequencing. Five patients of our registry (10%), belonging to the same family, carried CCG interruptions at the 3' end of the CTG expansion. Some of them presented atypical traits such as a very late onset of symptoms (>50 years) and a severe axial and proximal weakness requiring walking assistance. They also showed classic DM1 symptoms including cardiac and respiratory dysfunction, which were severe in some of them. Sizes and interrupted allele patterns were determined, and we found a contraction and an expansion in two intergenerational transmissions. Our study contributes to the observation that DM1 patients carrying interruptions present with atypical clinical features that can make DM1 diagnosis difficult, with a later than expected age of onset and a previously unreported aging-related severe disease manifestation.

INTRODUCTION

Myotonic dystrophy type 1 (DM1, Steinert disease; MIM# 160900) is a multisystemic disorder with an overall estimated prevalence of 1:8000 (Harper PS, 2001), being the most common form of inherited muscular dystrophy in adults. DM1 patients show wide phenotypic heterogeneity, not only in age of onset but also in severity and type of clinical manifestation. DM1 patients can be broadly divided into five subtypes based mainly on their age of onset: congenital (< 1 month), childhood (1 month–10 years), juvenile (10–20 years), adulthood/classic (20–40 years), or late-onset (>40 years) (De Antonio et al., 2016). Classic DM1 symptoms include muscle weakness, myotonia, respiratory failure, cardiac conduction defects, cataracts, and endocrine disturbances. The younger subtypes, congenital and childhood onset, are characterized primarily by cognitive and learning abnormalities (Douniol et al., 2012; Meola & Cardani, 2015).

DM1 is an autosomal dominant disorder caused by a CTG expansion in the 3' untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene. Unaffected individuals carry 5–35 CTG repeats whereas individuals carrying between 35 to 50 repeats are usually asymptomatic. Yet in the latter, *DMPK* alleles have a higher mutation rate and are labelled as 'pre-mutational alleles' (Imbert, Kretz, Johnson, & Mandel, 1993). The length of the CTG expansion varies widely between patients, ranging from 50 to thousands of CTGs and has been associated with age of symptom onset and severity (Groh et al., 2011; Logigian et al., 2004). A CTG repeat size ≤150 CTGs, ≤1000 CTGs and › 1000 CTGs is common to late onset, adulthood/classic and congenital DM1, respectively (Meola & Cardani, 2015). However, a high individual variability exists among DM1 patients of the same subtype and thus caution is needed when using CTG expansion length to

predict disease progression. For instance, congenital cases have been found with CTG repeat lengths clearly below 1000 CTG repeats (Tsilfidis, MacKenzie, Mettler, Barceló, & Korneluk, 1992) and late onset DM1 cases have been reported with over 1000 CTG repeats (Clark, Petty, & Strong, 1998). Another feature of the disease that makes it difficult to infer potential genotype/phenotype correlations is the presence of 'somatic mosaicism'. Indeed, the CTG expansion is highly unstable in both germline and somatic cells, and this instability persists through the lifetime of the patient. Thus, the CTG repeat size of a given patient represents the mean value for different CTG repeat sizes, which in turn can vary depending on the age at which the patient is studied. These potential confounders for sizing CTG repeat makes it difficult to find genotype-phenotype correlations for DM1. In this respect, estimating the inherited allele length has proven to be a more accurate predictor of potential genotype-phenotype correlations in this disease. (Higham, Morales, Cobbold, Haydon, & Monckton, 2012; Morales et al., 2012).

Because the aforementioned fact that CTG expansion instability is also present in germline cells, new alleles with different CTG repeat sizes are constantly generated and children may inherit CTG repeat sizes considerably longer than those found in the transmitting parent. This leads to the so-called 'anticipation' phenomenon, which occurs in DM1 and in other triplet disorders, and is characterized by the fact that the disease may develop earlier in life in each successive generation (Harper, Harley, Reardon, & Shaw, 1992). In DM1, the sex of the transmitting parent plays an important role in anticipation, although both paternal and maternal transmission have been described. The paternal allele seems more unstable and leads more frequently to higher expansions in offspring, especially with CTG expansions below 100 repeats (Brunner, Ashizawa 1992). However, very large expansions

causing congenital DM1 are transmitted almost exclusively by affected mothers (Ashizawa 1994), with few exceptions reported (Di Costanzo et al., 2009; Zeesman, Carson, & Whelan, 2002). On the other hand, the congenital form is frequently observed after transmission from mothers who are carriers of more than 500 CTG repeats. Contractions of the CTG expansion upon transmission have also been reported, with a higher estimated prevalence in paternal transmission compared to maternal transmissions (6.7 vs. 19.5 %) (López de Munain et al., 1996).

In most cases, the CTG expansion in expanded *DMPK* alleles is an uninterrupted sequence. However, in the last decade, pathological variant expansions containing unstable CCG, CTC, GGC and CAG sequence interruptions at the 3´ and 5' ends of the *DMPK* allele have been reported, with a prevalence of 3–5% among DM1 (Botta et al., 2017; Braida et al., 2010; Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017; Santoro et al., 2013; Tomé et al., 2018). In addition, intergenerational transmissions typically lead to smaller CTG expansions when compared to non-interrupted DM1 families, suggesting a stabilizing effect of the expansion on germline transmission (Botta et al., 2017; Pešović et al., 2017; Tomé et al., 2018). These findings might also explain why no congenital cases have been described in maternal transmission of interrupted alleles.

Most of the phenotype consequences of interruptions remain poorly understood and vary considerably between studies, ranging from a complex neurological phenotype to a later age of onset (Annalisa Botta et al., 2017; Braida et al., 2010; Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017; Massimo Santoro et al., 2015). There is an urgent need to determine the phenotypes that associate with the subset of DM1 patients presenting with interruptions. This information is

required for patient management, genetic counselling and future clinical trials. In the literature, only a few families and some isolated cases have been described, and their reported clinical data are scarce. In the present study, we have analyzed a large cohort of Spanish DM1 patients belonging to several families. Our aim was to identify DM1 patients carrying variant repeats and to perform an in-depth analysis of their clinical phenotypes. This might help to gain insight into the modifying effect that these repeat interruptions could have in DM1 diagnosis, clinical manifestation and patient follow-up.

SUBJECTS AND METHODS

Editorial Policies and Ethical Considerations

This study was approved by the ethics committee of the University Hospital *Germans Trias i Pujol* (ref. PI-15-129) and was performed in accordance with the Declaration of Helsinki for Human Research. Written informed consent was obtained from all the participants.

Participants

Forty-nine DM1 patients belonging to 36 different families who were evaluated in our center during the 2015–2018 period participated in this study. Clinical and genetic information was collected and stored in a secure registry. Their clinical phenotype was evaluated by the neurologists of our team. Muscle strength was assessed using the manual Medical Research Council (MRC) scale. The most recent ophthalmological, cardiological and respiratory examinations carried out by the corresponding specialists were reviewed, as well as blood analyses, electrocardiograms, echocardiograms, and functional respiratory and swallowing

tests. Functional status and disability were assessed using the Muscular Impairment Rating Scale (MIRS), the modified Rankin Scale (mRS), and the Rasch-Built Myotonic Dystrophy type 1 activity and participation scale (DM1-Activ).

DNA extraction and bidirectional triplet primed PCR

Total genomic DNA was extracted from peripheral blood samples, as previously described (Miller, Dykes, & Polesky, 1988). To assess the size and the presence of interruptions in the expanded allele, all DM1 blood DNA samples were analyzed by bidirectional triplet primed-PCR (TP-PCR). TP-PCR was performed with primers DM1for-FAM, DM1-CAG-rev, and P3 at the 5'-end of the CTG expansion, or DM1rev-FAM, DM1-CTG-for, and P3 at the 3' end of the CTG expansion, as previously described by Radvansky *et al.* (Radvansky, Ficek, Minarik, Palffy, & Kadasi, 2011). Both TP-PCRs (5' and 3') were performed with 100 ng of genomic DNA, 10x PCR Buffer containing 15 mM of $MgCl_2$, 10 nM of dNTP mixture , 0.5 U of TaKaRa DNA polymerase (TaKaRa), 3% DMSO and 0.2 µM of each primer. PCR amplification conditions were the same for both TP-PCRs: initial denaturation at 94°C for 5 min, followed by 34 cycles at 94°C for 1 minute, 65°C for 1 minute, and 72°C for 2 minutes and a final extension step at 72°C for 7 minutes. Correct amplification was assessed on a 2% agarose gel. PCR products were separated on an ABI PRISM 3130 Genetic Analyzer and data were analyzed with PeakScanner Software v1.0 (Applied Biosystems/MDS Sciex; CA).

AciI digestion and Southern blot

We used a digestion with AciI and Southern blot-long PCR strategy to determine presence of interruptions of the CCG/CGG type. DNA (100 ng) was amplified using the primers MDY1D-F GCTCGAAGGGTCCTTGTAGCCG and DM1-rev

GTGCGTGGAGGATGGAAC. The conditions of the long PCR were as follows: initial denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec and annealing-extension at 65°C for 7 min. Final extension was performed 65°C for 10 min. Fifty microliters of long PCR products were divided in two parts, one digested with AciI and the other not digested. An aliquot (10 µl) of each sample was resolved in an agarose gel and the products were detected by Southern blot hybridization. A DIG-labeled LNA probe (5'-gcAgCagcAgCagCagcAgca-3', with lower and upper-case letters representing an unmodified and an LNA nucleotide, respectively) was used to detect the expansions through chemiluminescence.

Sequencing

To determine the pattern of the interruptions we first amplified the DNA using primers GC1_CC, GC1_CCG, P2-rev and P3, as described elsewhere (Pešović et al., 2017). Products were resolved in a 3% agarose gel and purified using QIAquick gel extraction kit (Werfen, Barcelona, Spain). Purified products were sequenced with BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were analyzed with Chromas version 2.6.2. The *DMPK* gene reference sequence used was NG_009784.1.

Small pool PCR and AciI digestion

To estimate the length of the expanded progenitor allele (ePAL), small-pool PCR (SP-PCR) was carried out using flanking primers DM-C and DM-DR as previously described (Gomes-Pereira, Bidichandani, & Monckton, 2004; Monckton, Wong, Ashizawa, & Caskey, 1995). PCR was performed using Custom PCR Master Mix (Thermo Fisher Scientific, MA, USA) supplemented with 69 mM 2-

mercaptoethanol, and Taq polymerase (Sigma-Aldrich UK, Gillingham) at 1 unit per 10 µL. All reactions were supplemented with 5% DMSO and the annealing temperature was 63.5°C. DNA fragments were resolved by electrophoresis on a 1% agarose gel, and Southern blot hybridized as described (Gomes-Pereira et al., 2004; Monckton et al., 1995). Autoradiographic images were scanned and ePAL estimated from the lower boundary by comparison against the molecular weight ladder, using CLIQS 1D gel analysis software (TotalLab UK, Newcastle upon Tyne). To analyze again the presence of CCG or CGG variant repeats, an additional step was added to the SP-PCR protocol. PCR products were purified using the QIAquick (Qiagen, Venlo, the Netherlands) PCR purification kit and split into two aliquots, one of which was digested with AciI. They were then resolved and blotted as before.

RESULTS

Clinical phenotypes

Five of 49 DM1 patients (~10%) were found to have interruptions in the 3' end of the CTG expansion. They belonged to the same family (**Figure 1**). Patient P1, P2 and P3 are sisters who paternally inherited the disease, and patient P4 is the son of P2 (**Figure 1**) whereas patient P5 is the daughter of P3. A summary of their clinical characteristics is shown in **Table 1**.

Patient P1 is the oldest of the siblings, and currently the most severely affected of all five patients. The first symptom she reported was weakness at the age of 52. Subsequently, she developed a generalized weakness, which interfered with her ability to cope with daily life activities. We studied her when she was 72-year-old and the most striking feature upon clinical examination was severe axial weakness

with dropped-head. The patient also presented with mild weakness in the upper and lower limbs, with only little myotonia. Another remarkable fact was that she had moderate facial weakness, but almost no ptosis and no temporal atrophy. She also presented with bilateral cataracts, dysphagia for liquids and frontal baldness. She had a heart pacemaker implanted since the age of 71 and used nocturnal noninvasive mechanical ventilation, and had no cognitive impairment.

Patient P2: Symptoms started at the age of 50 with mild fatigue and myotonia. At the moment of inclusion in the study (aged 62), the clinical examination revealed only mild weakness of the neck flexor muscles, with mild handgrip myotonia and minimum ptosis. Complementary explorations showed a first-degree atrioventricular block and low values of maximum inspiratory and expiratory pressure (38% and 22% of normal, respectively), and of both forced vital capacity (81%) and expiratory volume in 1 second (96%). The patient presented with bilateral cataracts and severe baldness. No limb weakness, dysphagia or cognitive impairment was found.

Patient P3: The first sign reported was handgrip myotonia in her fifties. At the moment of examination (age 60) she also had severe axial weakness with mild proximal limb weakness and moderate distal weakness. Like her oldest sister (Patient P1), she had moderate facial weakness with no ptosis or temporal atrophy. She also presented with bilateral cataracts, frontal baldness and dysphagia for liquids. Cardiological studies revealed a first-degree atrioventricular block. No respiratory or cognitive involvement was found.

Patient P4: This male patient (aged 35 years) carrying an interrupted allele was asymptomatic upon clinical examination and had no detectable myotonia or cardiac alterations.

Patient P5: She was diagnosed at age of 25 based on the family history, although clinical manifestation did not start until two years later, starting with handgrip myotonia. At the moment of assessment (age 32) she presented with mild neck flexor and facial weakness and handgrip and percussion myotonia, without limb weakness. She has a first-degree atrioventricular block and cataracts, but no respiratory impairment.

Molecular analysis of interruptions

Interrupted alleles were firstly detected as gaps in the pattern of contiguous peaks detectable by capillary electrophoresis by 3' TP-PCR (**Figure 2**). Patients P2 and P4 showed a similar interruption pattern, while P1, P3 and P5 showed different interruption patterns (**Figure 2**). No alterations where found with 5' TP-PCR (**Supp. Figure S1**)

We performed an AciI digestion of PCR products to test for the presence of CCG or GGC variant repeats in patients P1–5. In all these patients, the results showed a downward shift of the smear in the gel of the digested product compared to the non-digested product (**Figure 3**). This indicated that AciI had cleaved the PCR product and the interruption was likely either a CCG or a GGC triplet. In addition, since the bidirectional TP-PCR performed in the entire cohort was limited to the outer regions of the CTG expansion, we performed an AciI digestion in the entire DM1 cohort to search for possible undetected CCG or GGC interruptions in the

middle region of the CTG expansion. No additional CCG or GGC interruptions were found in our 44 remaining DM1 patients.

Sequencing revealed the presence of several CCG interruptions in the CTG expansion of our five patients carrying interrupted alleles (**Figure 4**). The pattern of CCG interruptions was identical in the mother (patient P2) and son (P4), but different between all the other family members (patients P1, P3 and P5). In P1, we found some isolated CCG repeats scattered across the expansion. Patients P2 and P4 showed a complex CCG pattern, with one pair of CCGs together with other isolated CCG repeats. Patient P3 had a few CCGCTG hexamers, but inside of a more complex pattern including CCG interruptions in other positions. Patient P5 (the daughter of P3) showed a pattern similar to that of her mother with respect to the hexamers, but with some extra CCGs located in different positions, generating three consecutive CCG repeats. During the sequencing process, we purified different bands from the same patient to assess the influence of somatic instability in the interruption pattern (**Figure 4B**). In the different bands analyzed, the same pattern was observed in each patient.

SP-PCR (**Figure 5**) provided information on the repeat size of the ePAL for some of the patients: P1, 319 CTGs; P2, 241 CTGs; P4, 222 CTGs; P5, 547 CTGs. The expansion range due to the instability of the repeat was also determined: P1, 319 to 900 CTGs; P2, 241 to 651 CTGs; P4, 222 to 332 CTGs; and P5 = 547 to 897 CTGs. For patient P3, the expanded allele did not amplify well under these conditions, so it was not possible to determine ePAL or expansion range. This may be due to the specific pattern of variant repeats present. However, in the SP-PCR we could amplify 368 CTGs, which was the only sizing of the expanded allele that we could make. Additionally, the type of interruptions were analyzed through AciI

digestion in SP-PCR experiments, which again showed that the interruptions were of the CCG type (data not shown). By comparing the range of these bands, we could determine a contraction in the repeat size from patient P2 to P4 (*i.e.*, from mother to son) but an expansion from patient P3 to P5 (*i.e.*, from mother to daughter). This expansion was also linked to anticipation, with an early age of onset for P5 when compared to her mother (P3).

Discussion

The effect of variant repeat patterns on DM1 clinical phenotype is still unclear. On one hand, this genetic alteration has been shown to be associated (albeit in one family only) with a complex co-segregated neurological phenotype, including an intermediate Charcot-Marie-Tooth neuropathy, early hearing loss and encephalopathic attacks (Braida et al., 2010). On the other hand, variant repeats have been associated with a milder or atypical phenotype, including a later age of onset (Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017), a DM2-like muscle phenotype (Pešović et al., 2017), as well as with an absence of muscular dystrophy (Musova et al., 2009) or central nervous system symptoms (Santoro, Masciullo, Silvestri, Novelli, & Botta, 2017). These reports have led to a tendency to believe that patients with interrupted alleles have some atypical symptoms, but overall a milder phenotype than their age-matched DM1 non-interrupted peers with a similar repeat length. In this respect, we had the unique possibility to study a family containing interrupted cases of whom three were aged above 60 years. In this regard, although our data were obtained in a small number of patients within the same family, our results support the occurrence of atypical DM1 features and late age of onset, but not of a milder phenotype in patients carrying interruptions.

Despite the fact that several of the classical symptoms of DM1 could be found in the three sisters — such as myotonia, cataracts and cardiopathy — some peculiarities need to be highlighted. An atypical trait was the distribution pattern of muscle weakness in two of the sisters. Indeed, besides the distal limb weakness commonly found in DM1 patients, these two sisters presented with proximal limb weakness and severe axial involvement. One of them also had a dropped-head, which resembled a limb-girdle muscle dystrophy and severely affected her ability to perform activities of daily living. Another atypical trait of these patients is that they did not have the typical myopathic face expected in DM1 patients, despite the presence of moderate facial weakness. Although the interrupted cases showed several classic DM1 symptoms, the presentation of atypical symptoms could interfere with (and thus delay) the diagnosis.

Based on the algorithm published by Morales et al (Fernando Morales et al., 2012), the ePAL of patients P1, P2 and the expanded allele size of P3 should be theoretically associated with an age of onset around 30s whereas in our patients symptoms did not actually start until they were in their 50s. In this regard, it should be first noted that it is very difficult to assess the age of onset in DM1 patients. The definition of age of onset refers to the age at which an individual starts to develop one or more clinical features or symptoms of a disease. In actual clinical practice, this depends on the capacity of the patient to report such symptoms or to remember the time when they started, and also on the ability of the physician to recognize them. Thus, the reported age of onset can be quite variable, depending on which symptoms are searched for by the physician and on the patient's own reports. Our patients P1–3 (the three sisters) reported their first symptoms in their 50s (being myotonia, and difficulty to walk, the first abnormalities that made them suspect

they had a major medical condition). Patient P4 was a 35-year-old and was still asymptomatic. Based on his ePAL length (222 CTGs), he should show a classic DM1 phenotype (Morales et al., 2012), but no signs could be detected upon neurological examination. This late onset of symptoms has been previously reported in interrupted DM1 families and seems to be a fingerprint for most of the cases (Botta et al., 2017; Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017).

In the family we studied, anticipation was observed in one of the two intergenerational transmissions that we assessed, since in the other intergenerational transmission one of the patients (P4) was still asymptomatic. In patient P5 (whose first symptom was myotonia, at the age of 27), we found a bigger size of the expansion and an earlier age of onset than her progenitor. Although this anticipation in interrupted families has been previously reported (Pešović et al., 2017), after reviewing all the published families (**Table 2**), we assessed anticipation in every single reported family. In the rest of intergenerational transmissions reported, and in the case of our patients P2 and P4, anticipation could not be assessed since patients in the next generation are still asymptomatic. The explanation for these findings is not apparent, since anticipation is not expected in these families; indeed, interruptions are thought to be related to a stabilization or even contraction of the pathological expansion (Braida et al., 2010; Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017; Tomé et al., 2018). However, anticipation was found in our studied intergenerational transmission, with this finding being also reported in other interrupted DM1 patients based on reported age of onset (**Table 2**). In our family, no congenital, childhood or juvenile cases of DM1 were observed. Among the interrupted families

reported in the literature (**Table 2**), at least three juvenile DM1 cases (age < 18 years) have been described (Braida et al., 2010; Pešović et al., 2017), but no congenital or childhood case. Thus, absence of infantile DM1 seems also to be a distinctive trait for interrupted expansions.

The prevalence of interrupted alleles among our patients was ~10%, and 3% among the studied DM1 families. This is in overall agreement with previous studies in which the prevalence in families ranged from 3 to 5% (Botta et al., 2017; Braida et al., 2010; Musova et al., 2009; Pešović et al., 2017). The type of interruption present in our cohort was CCG, which is currently the most frequently reported variant repeat. However, the difficulties we experienced in characterizing the pattern of interruptions in our family members must be emphasized, with such difficulties mainly due to a technical limitation of TP-PCR and sequencing, which have a limited ability to detect interruptions deeper inside the expansion. In addition, characterization is affected by the PCR slippage and by somatic mosaicism (implying more noise in readouts and thus a higher difficulty to identify the interrupted pattern). Our sequences showed in some cases double peaks of C and T at the same position, and we decided to consider only those interruptions where the C peaks were above T in the electropherograms, which might have resulted in loss of CCG interruptions in our patients' sequences. We sequenced several amplified bands coming from the same TP-PCR to determine whether somatic mosaicism was also affecting the pattern of CCG interruptions, but the same patterns were found in all the sequences. Despite the aforementioned limitations, we determined the interrupted pattern in all the studied family members. We observed a substantial change in the interruption pattern in every transmission, and the number and position of the CCGs changed in every

generation, except one intergenerational transmission. Thus, interruption patterns can be conserved or vary upon transmission. Both situations have been previously described in interrupted families (Musova et al., 2009; Pešović et al., 2017; Tomé et al., 2018). Our CCG interruptions were found in blocks of two or three (in hexamers of CCGCTG that were repeated two or three times), and also as isolated cases. Due to technical limitations, we cannot be certain that other interruptions are not present deeper in the CTG expansion. TP-PCR and sequencing allowed us to study the flanking regions of the CTG expansion, but the middle part remained undetected. We detected contraction of the expansion between patients P2 and P4, but expansion between patients P3 and P5. Previous studies suggest that CTG expansion containing variant repeat patterns display more frequently stable, or even contracted, *DMPK* alleles instead of further expanded *DMPK* alleles (Cumming et al., 2018; Musova et al., 2009; Pešović et al., 2017; Tomé et al., 2018). However, some studies have also found expansion of the interrupted alleles from one generation to the other (Braida et al., 2010; Cumming et al., 2018; Pešović et al., 2017). Perhaps these expansions are less frequent than in pure CTG expansions transmission, but they do occur. Therefore, caution is needed with genetic counseling with regard to prospective parents with DM1.

Our study contributes to the observation that DM1 patients carrying interruptions may have atypical symptoms that can make the diagnosis of DM1 difficult, with a later age of onset and a previously unreported aging-related severe disease manifestation. Indeed, some of our older patients needed mechanical ventilation and a pacemaker, and besides their cardiorespiratory problems, they had muscle weakness with subsequent impairment in daily life activities and walking ability. Despite the small sample size of our study sample, our results challenge the notion

that interrupted patients who remain asymptomatic until their late 30s or 40s are not at risk for having a severe phenotype later in life. Indeed, our patients developed a classical DM1 phenotype after their 50s. These patients require clinical follow-up and genetic counselling similar to non-interrupted DM1 patients. In the family we studied, we found some characteristics that add to the current body of knowledge regarding interrupted families: a later age of onset, variation of CCG repeat pattern between intergenerational transmission, anticipation due to the earlier age of onset of symptoms in next generation and no cases of congenital or childhood onset of DM1. In addition, we have found other previously undescribed characteristics, such as a predominant axial weakness. However, the small number of interrupted patients present in the DM1 population makes it hard to perform genotype-phenotype correlations and there is still much uncertainty. Studies with larger DM1 cohorts, preferably with DM1 families, are needed to unravel the phenotypic consequences of variant repeat patterns and to study their effect on intergenerational transmissions of the *DMPK* expanded allele.

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Conflicts of Interest

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Molecule RNA and Lion Therapeutics and he also had a research contract with AMO Pharma. The remaining co-authors declare no conflicts of interest.

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Figures

Figure 1. Pedigree of the interrupted patients in our cohort. Patient P1 = P1; Patient P2 = P2; Patient P3 = P3; Patient P4 = P4; Patient P5 = P5. Years = yr; Number of repeats in CTGs = CTGs. The father of P1, P2 and P3 died (sudden cardiac death).

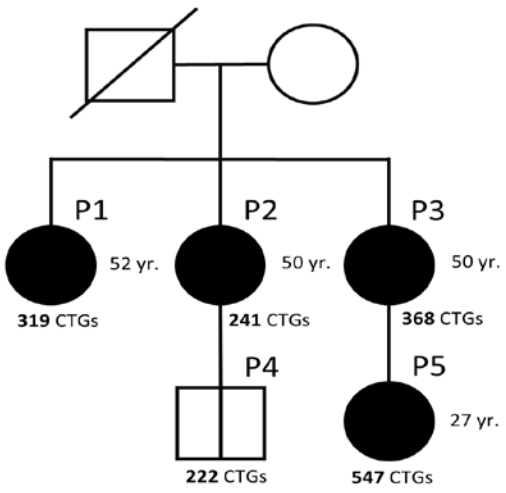

Figure 2. Peak scan results of TP-PCR of the 3' end, obtained with DNA extracted from blood. Interruptions indicated by black box. P1 = Patient P1; P2= Patient P2; P3= Patient P3; P4 = Patient P4; P5 = Patient P5.

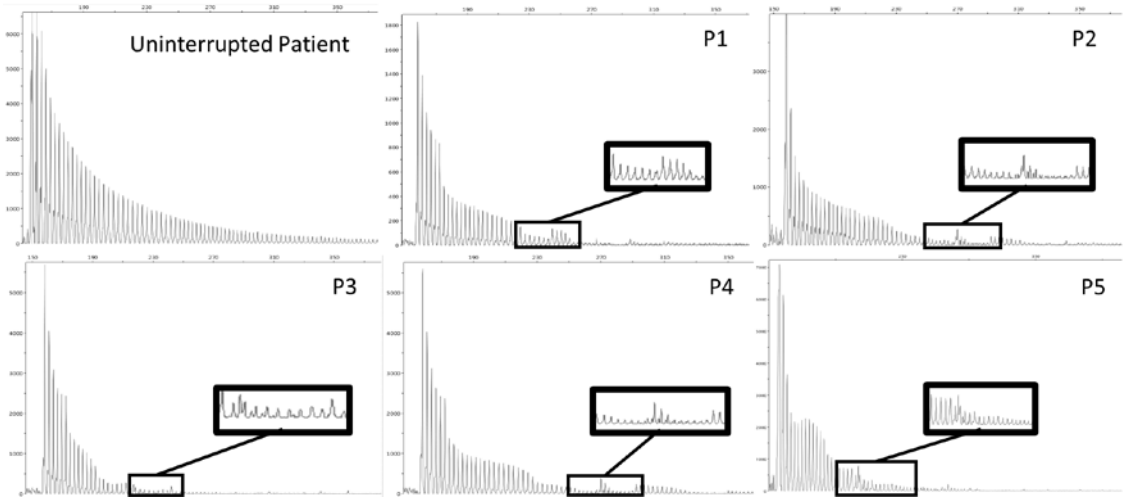

Figure 3. Southern blot of long PCR products from patients carrying variant repeats. For each patient, we show two conditions: digestion with (+) and without (-) the enzyme AciI (recognizing the pattern CCGC). P1= Patient P1; P2 = Patient P2; P3 = Patient P3; P4 = Patient P4; P5 = Patient P5; M = molecular weight marker; WT = wild type; bp = base pairs.

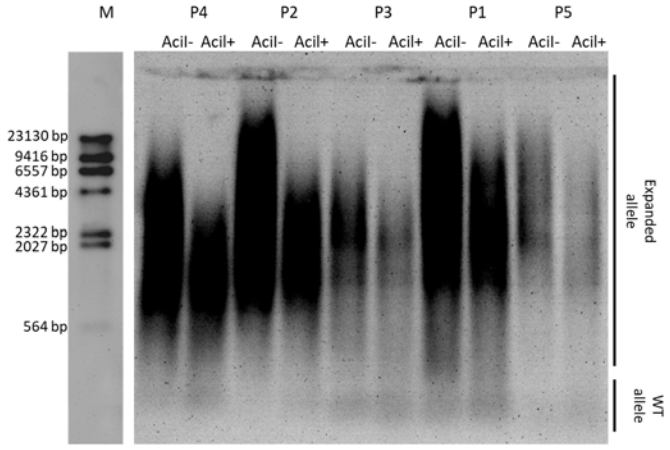

Figure 4. Sequencing the interrupted alleles. A) Schematic structure of *DMPK* expanded alleles of the interrupted DM1 family in our study. CTG repeats are shown in white, CCG repeats in black. Indicated size ranges were estimated by Southern Blot analysis. B) Cutting and purifying strategy for several bands of PCR product (indicated by arrows), from each interrupted patient, which are affected by somatic instability. C) Sequences showing the CCG interruptions are marked by black rectangles. P1= Patient P1; P2 = Patient P2; P3 = Patient P3; P4 = Patient P4; P5 = Patient P5; M = molecular weight marker; Bl = PCR reaction with no DNA. The *DMPK* gene reference sequence used was NG_009784.1.

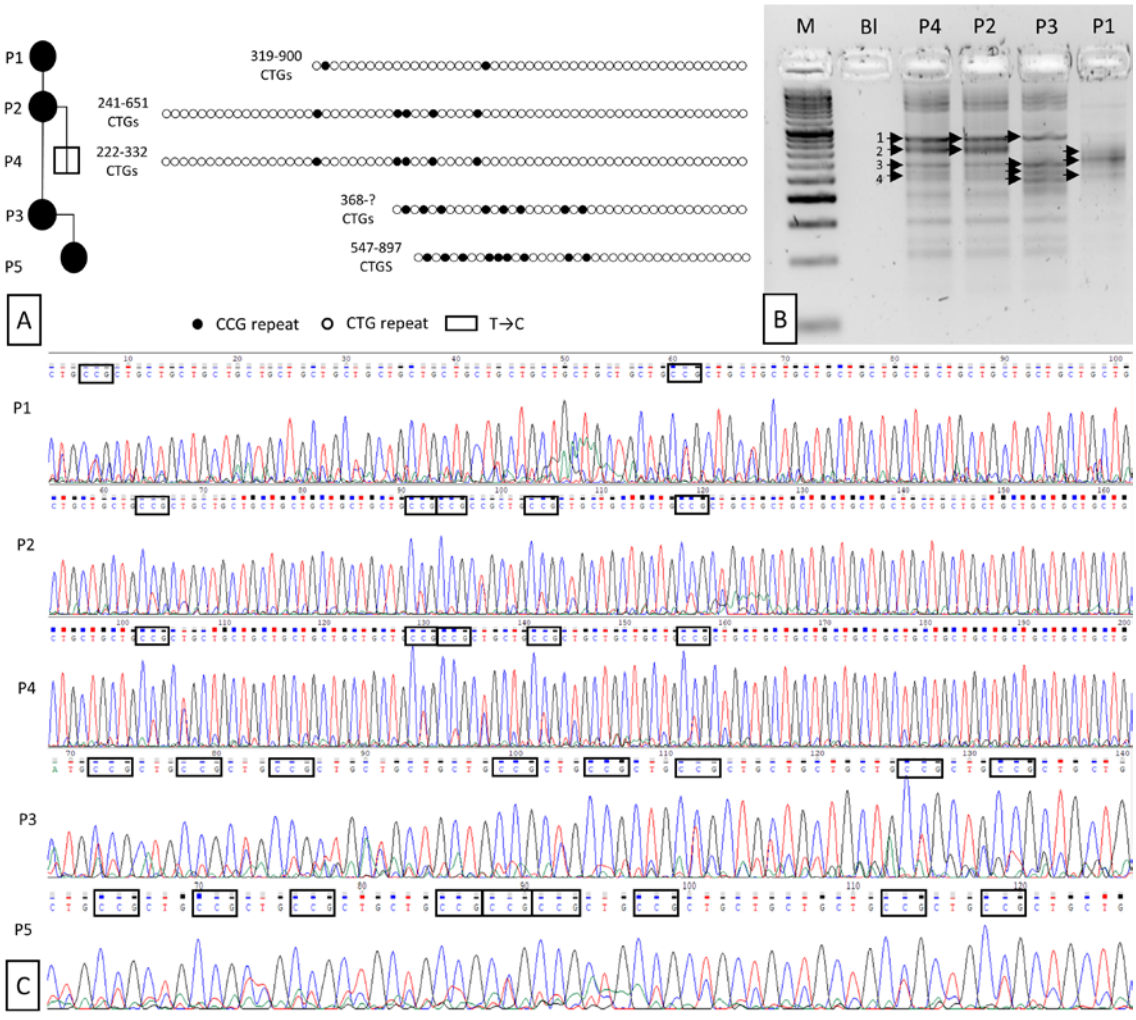

Figure 5 Small pool PCR from patients carrying variant repeats. For each patient several lines show the normal and the expanded alleles. P1= Patient P1; P2 = Patient P2; P3 = Patient P3; P4 = Patient P4; P5 = Patient P5; CTGs = number of repeats; WT allele = wild type allele.

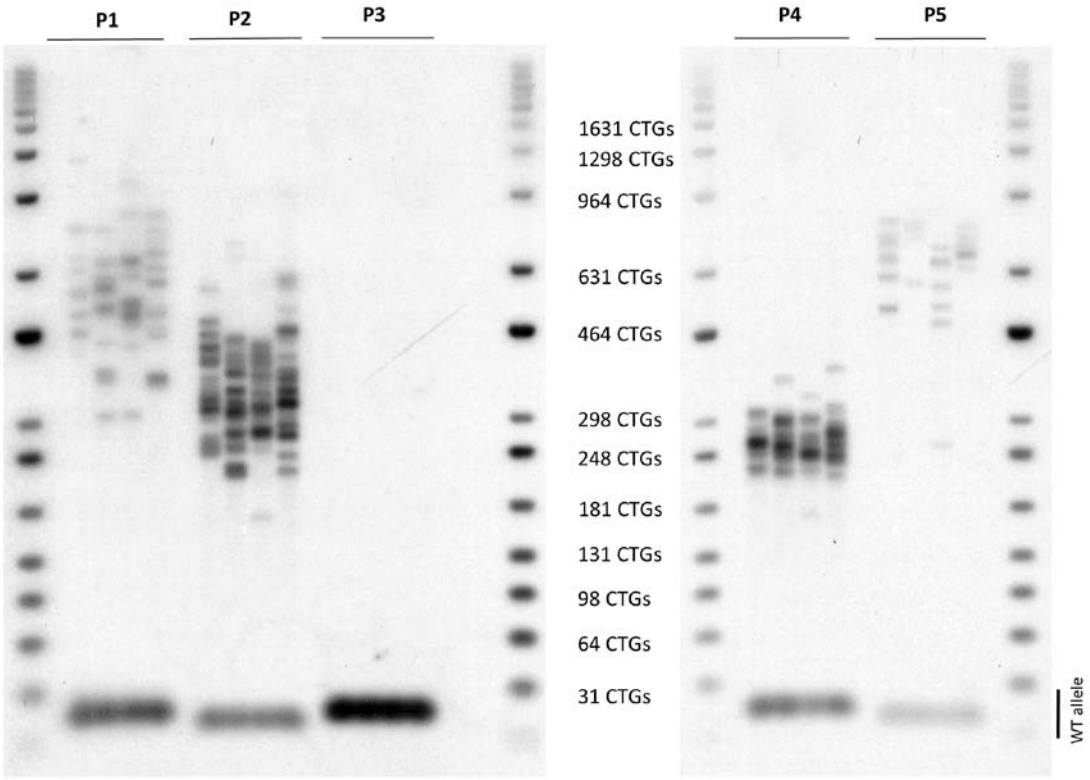

Table 1. Clinical characteristics of the interrupted cases

	P1	**P2**	**P3**	**P4**	**P5**
Sex	Female	Female	Female	Male	Female
Age of onset (years)	52	50	50	Asymptomatic	27
Age of	72	62	60	35	32

assessment (years)					
Cardiopathy	Pacemaker	1st degree AV-block	1st degree AV-block	None	1st degree AV-block
Respiratory disturbance	Yes, nocturnal NMV	Alteration MIP & MEP	None	None	None
Dysphagia	Liquids	No	Liquids	None	None
Cognitive impairment	None	None	None	None	None
Cataracts	Yes	Yes	Yes	None	Yes
Metabolic disturbance	None	None	Hypothyroidism	None	None
Myotonia	Yes	Yes	Yes	None	Yes
Polyneuropathy	None	None	None	None	None

CK level	Normal	Normal	213 U/L	Normal	ND
Limb Weakness					
Facial ptosis	Yes	Mild	Yes	None	Mild
Flexor/extensor neck	1 (Dropped head)	4	2	5	5
Axial weakness	Severe	None	Severe	None	None
Upper Limb proximal (MRC)	4	5	4	5	5
Upper Limb distal (MRC)	4	5	3	5	5
Lower Limb proximal (MRC)	4	5	4	5	5
Lower Limb distal (MRC)	4	5	3	5	5

6 MWT (meters)	250	436	240	658	800
MIRS	4	2	4	1	2
mRS	3	1	3	0	2
DM1-Activ	23	37	23	40	39

CK, creatine kinase; ND, not determined; MRC, Medical Research Council; 6 MWT, six-minute walking test; MIRS, Muscular Impairment Rating Scale; mRS, modified Rankin Scale; DM1-Activ, Rasch-built myotonic dystrophy type 1 activity and participation scale; AV, atrioventricular; NMV, non-invasive mechanical ventilation; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure.

Table 2: Analysis of the literature reported interrupted families

Article	**Cases**	**Patient Code**	**Age S**	**Age O**	**Relationship**	**Anticipation**	**Atypical findings in examination**	**CTG Repeats Number**	**Type of Interruption**
Musova et al 2009	3 families								
	Family A	A-1 (Fetus)	0		Fetus of A2			230	CTC & CCG
		A-2	31	-	Daughter of	NPS		300	CTC & CCG

					A4				
		A-3	23	-	Daughter of A4	NPS		400-500	CTC & CCG
		A-4	54	40s	Brother of A5			600-800	CTC & CCG
		A-5	53	40s	Sister of A4		Electromyography confirmed myotonia but no dystrophy	450-650	CTC & CCG
		A-6	29	-	Daughter of A5	NPS		600-750	CTC & CCG
		A-7	31	-	Son of A5	NPS		270	CTC & CCG
	Family B	B-1	50	40s	Father of B2		No muscle weakness or muscle atrophy	450	CCG
		B-2	25	-	Son of B2	NPS		400	CCG
	Family E	E-1	20	1 year *	Son of E2		Upper limbs showed no muscle atrophy, weakness or myotonic reaction	43	CCG
		E-2	56	-	Father of E1	A		43	CCG
Braida et al 2010	1 family								
	Family 1	III-9	55	25	Cousin of III-17 and III-16		Charcot-Marie-Tooth disease,	229[P]	CCG & GGC

						acute encephalopathy and early hearing loss		
III-16	57	44	Sister of III-17			Charcot-Marie-Tooth disease, acute encephalopathy and early hearing loss	170[P]	CCG & GGC
IV-19	37	20	Son of III-16	A		Charcot-Marie-Tooth disease, acute encephalopathy and early hearing loss	213[P]	CCG & GGC
IV-20	34	24	Son of III-16	A		Charcot-Marie-Tooth disease	213[P]	CCG & GGC
III-17	61	35	Sister of III-16			Charcot-Marie-Tooth disease, acute encephalopathy and early hearing loss	179[P]	CCG & GGC
IV-21	30	17 #	Son of III-17	A		Charcot-Marie-Tooth disease	220[P]	CCG & GGC
IV-22	28	25	Daughter of III-17	A		Charcot-Marie-Tooth	225[P]	CCG & GGC

							disease		
Botta et al 2016	3 families								
	Family A	A1	66	58	Father of A2		Absence of myotonia and cataracts	1000-1400	CCG
		A2	39	31	Daughter of A1	A	Absence of muscle weakness	475-640	CCG
		A3	0	N/A	Fetus of A2			500	CCG
	Family B	B1	55	51	Mother of B2			740-930	CCG
		B2	28	-	Dauther of B1	NPS		450-550	CCG
	Family C	C1	58	58	Mother of C2		Absence of muscle weakness and cataracts	140	CCG
		C2	40	37	Daughter of C1	A	Absence of muscle weakness and cataracts	121	CCG
		C3	0	N/A	Fetus of C2			113	CCG
Pesovic et al 2017	3 families								
	Family DF1	DF1-1	57	39	Mother of DF1-2 and DF1-3			5200-1250	CCG
		DF1-2	37	30	Son of DF1-1	A	Absence of:	370-730	CCG

							percussion myotonia, ptosis, cataracts and muscle wasting. Presence of calf hypertrophy, suggesting DM2		
		DF 1-3	30	15 #	Son of DF1-1	A	Calf hypertrophy	450-970	CCG
	Family DF 2	DF 2-1	45	40	Father of DF2-2		Similar involvement of both proximal and distal muscles and winging scapulae in the right side.	320-600	CCG
		DF 2-2	14	12 #	Daughter of DF-1	A	Mild ptosis and mild percussion myotonia	200-240	CCG
	Family DF 5	DF 5-2	27	22	Sister of DF5-3		Normal strength of the sternocleidomastoid muscle and very mild myotonia	250-350	CTC
		DF 5-3	22	21	Sister of DF5-2			300-620	Non-Interrupted
Cumming et al	3 families								

						Absence of muscle weakness, myotonia and cataracts	381[e] PAL	CCG
Family 1	14	25, 5	-	Daughter of 165	NPS			
	57	20, 5	5	Son of 165	A		597[e] PAL	Non-Interrupted
	165	59	28	Brother of 83			383[e] PAL	Non-Interrupted
	83	46	38	Brother of 165			105[e] PAL	Non-Interrupted
						Absence of muscle weakness, mild masseter myotonia and peripheral membrane irritability on EMG.	293[e] PAL	CCG
Family 2	182	35, 5	-	Brother of 184	NPS			
	184	28	20	Brother of 182			288[e] PAL	Non-Interrupted
	206	70	60	Father of 182 and 184			90[eP] AL	Non-Interrupted
	242	65	ND	Sister of 206			80[eP] AL	Non-Interrupted
						No clinical apparent weakness or myotonia and no cataracts	303[e] PAL	CCG
Family 3	15	39	-	Daughter of 234	NPS			

		54	40	35	Brother of 15		146[e] PAL	Non-Interrupted
		234	ND	ND	Father of 15 and 54		496[e] PAL	Non-Interrupted
Our study	1 family							
	Family 1	Patient 1	72	52	Sister of Patient 2 and 3	Severe axial weakness with dropped-head. Mild weakness in upper and lower limb muscles, with only little myotonia. Moderate facial weakness, almost no ptosis and no temporal atrophy	319[e] PAL	CCG
		Patient 2	62	50	Sister of Patient 1 and 3	Mild weakness of the neck flexor muscles, no limb weakness and minimum ptosis	241[e] PAL	CCG
		Patient 3	60	50	Sister of Patient 1 and 2	Severe axial weakness, mild proximal limb	368	CCG

					weakness and moderate distal weakness. Moderate facial weakness with no ptosis or temporary atrophy		
Patient 4	35	-	Son of Patient 3	NPS		222[e] PAL	CCG
Patient 5	32	27	Daughter of Patient 2	A	Mild neck flexor and facial weakness, but no limb weakness.	547[e] PAL	CCG

Age S= age of Sampling; Age O= age at Onset; N/A= not applicable; - = asymptomatic; ND= no data; NPS = not possible to establish yet; A = anticipation; *authors clarify in the paper that he had isolated symptoms, cannot be considered childhood DM1. [e]PAL estimated progenitor allele.