

DMPK gene DNA methylation levels are associated with muscular and respiratory profiles in DM1

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Neurol Genet 2019;5:e338. doi:10.1212/NXG.0000000000000338

Abstract

Objective

To assess the effects of dystrophin myotonia protein kinase (*DMPK*) DNA methylation (DNAm) epivariation on muscular and respiratory profiles in patients with myotonic dystrophy type 1 (DM1).

Methods

Phenotypes were assessed with standardized measures. Pyrosequencing of bisulfite-treated DNA was used to quantify DNAm levels in blood from 90 patients with DM1 (adult form). Modal CTG repeat length was assessed using small-pool PCR. The presence of Acil-sensitive variant repeats was also tested.

Results

DNAm levels upstream of the CTG expansion (exon and intron 11) were correlated with modal CTG repeat length ($r_s = -0.224$, $p = 0.040$; $r_s = -0.317$, $p = 0.003$; and $r_s = -0.241$, $p = 0.027$), whereas correlations were observed with epivariations downstream of the CTG repeats ($r_s = 0.227$; $p = 0.037$). The presence of a variant repeat was associated with higher DNAm levels at multiple CpG sites (up to 10% higher; $p = 0.001$). Stepwise multiple linear regression modeling showed that DNAm contributed significantly and independently to explain phenotypic variability in ankle dorsiflexor (3 CpGs: $p = 0.001$, 0.013, and 0.001), grip ($p = 0.089$), and pinch ($p = 0.028$) strengths and in forced vital capacity (2 CpGs: $p = 0.002$ and 0.021) and maximal inspiratory pressure ($p = 0.012$).

Conclusions

In addition to the CTG repeat length, *DMPK* epivariations independently explain phenotypic variability in DM1 and could thus improve prognostic accuracy for patients.

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Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

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Glossary

DM1 = dystrophy type 1; **DMPK** = dystrophia myotonica protein kinase; **MIRS** = Muscular Impairment Rating Scale.

Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic¹ disorder caused by a CTG repeat expansion in the 3' untranslated region of the dystrophia myotonica protein kinase (*DMPK*) gene.^{2–4} DM1 affects all systems including muscular, cardiac, respiratory, endocrine, and CNS. The hallmark feature of DM1 is the involvement of the muscular system including presence of myotonia, progressive skeletal muscle impairment with a distal to proximal pattern, and respiratory muscle. The phenotypic variability observed in terms of age at onset, clinical presentation, and severity has led to a clinical classification that has evolved over time going from 3⁵ to 5 phenotypes recognized today.⁶ Although patients born with a larger expansion usually present a more severe phenotype than those with a smaller one, the CTG repeat length only partially explains this large phenotypic variability.

DNA methylation (DNAm) regulates gene expression⁷ and alternative splicing⁸ without modifying the nucleic acid sequence of the genes. DNAm occurs mainly on cytosines upstream of a guanine (CpG dinucleotides). The *DMPK* CTG expansion locus harbors a long CpG island, a genomic structure enriched in CpGs prone to DNAm. This led to hypothesize that DNAm contributes to disease development and progression and explains part of the phenotypic variability reported in DM1.^{9–16}

Only a few studies have been conducted, and all have considerable limitations such as small sample size and inclusion of multiple DM1 phenotypes, limiting comparison between studies and the conclusions, which can be drawn. None tested associations with DM1 clinical phenotypes, except for the Muscular Impairment Rating Scale (MIRS) in 1 study.¹² Therefore, we have assessed DNAm levels at the *DMPK* gene locus and tested for association with muscular and respiratory impairments in the largest number of patients with DM1 with the adult phenotype.

Methods

Standard protocol approvals, registrations, and patient consents

The study was approved by the Ethics Review Board of the *Centre de santé et de services sociaux de Chicoutimi*, Canada, and all participants provided written consent.

Participants

In 2002, 200 of the 416 patients with DM1 followed at the Neuromuscular Clinic of the *Centre de santé et des services sociaux de Jonquière* (Québec, Canada) were randomly selected and recruited in a longitudinal study aimed at identifying the determinants of social participation. Selection criteria were

(1) age ≥ 18 years, (2) molecular confirmation of diagnosis (late-onset vs adult phenotypes), and (3) capacity to provide informed consent. The adult phenotype was assigned to participants who had at least 2 of the following characteristics: (1) number of CTG repeats >200 in the standard diagnostic test, (2) Muscular Impairment Rating Score (MIRS) ≥ 3 , and (3) age at symptom onset <40 years.¹⁷ Age at onset was defined as the age at which the participant presented his first symptom. To reduce phenotypic variability that is present between patients with DM1 presenting different phenotypes of the disease, only 90 participants presenting the adult form of the disease were kept for the analysis. Patients with other forms of muscular dystrophies including congenital and childhood DM1 were thus excluded.

Muscle strength measurements

An isometric make test using a MicroFET2 dynamometer (Hoggan Health Industries, West Jordan, UT) was performed twice by a trained physiotherapist to measure the strength of the shoulder abductors, knee extensors, and ankle dorsiflexors. The participant's position was standardized for each muscle group. If there was more than 10% discrepancy between 2 assessments for a muscle group, a third measure was performed, and the mean of the 2 closest values was kept as the final result. The maximal isometric torque was computed by multiplying strength units (N) by length of the corresponding lever arm (m).¹⁸ A Jamar dynamometer (Asimow Engineering Co, Sequim, WA) was used to measure grip strength.¹⁹ Three measures were performed with a standardized protocol, and the mean was used as the final result. A B & L Pinch Gauge (Fabrication Enterprises Inc, New York, NY) was used to measure lateral pinch strength. Again, 3 measures were performed with a standardized protocol, and the mean was used as the final result.¹⁹

Respiratory profile assessment

Forced vital capacity, peak expiratory flow, and maximal inspiratory and expiratory pressure (cm H₂O) were measured by a respiratory therapist at the Jonquière Hospital following a standardized protocol. Percentage of predicted values for forced vital capacity and peak expiratory flow (% predicted) were calculated using the equation as in reference 20. Smoking status was determined using a life habit questionnaire.

DNA extraction and methylation analyses

Blood was collected in EDTA tubes during the same visit muscular and respiratory profiles were assessed. Buffy coats were kept at -80°C until DNA extraction using the Genra Puregene Blood Kit (Qiagen).

DNA methylation analysis takes advantage of the chemical propriety of unmethylated cytosines, which are sensitive to

Table 1 Characteristics of the sample

	Mean ± SD	Range
Age	41.0 ± 7.7	20.0–59.0
Sex: women (n, %)	n = 55 (61%)	NA
Weight (kg)	67.8 ± 16.3	34.9–115.8
Body mass index (BMI) (kg/m ²)	25.21 ± 5.61	14.34–43.05
Smoking (pack-years)	7.3 ± 11.0	0–56.0
Muscular Impairment Rating Scale (MIRS) category (n, %)		
2	n = 7 (7.8%)	NA
3	n = 22 (24.4%)	NA
4	n = 56 (62.2%)	NA
5	n = 5 (5.6%)	NA
Modal CTG repeat length	603.4 ± 267.9	68–1,321
Variant repeats (present n, %)	8 (8.9%)	NA
Age at symptom onset	20.4 ± 7.7	10.0–43.0
Shoulder abductors strength (Nm)	40.8 ± 15.7	14.3–94.2
Knee extensors strength (Nm)	80.9 ± 33.1	18.9–165.3
Ankle dorsiflexors (Nm)	14.2 ± 6.7	2.2–35.6
Grip strength (kg)	8.6 ± 6.4	0.0–28.5
Pinch strength (kg)	5.2 ± 1.5	2.2–9.2
Forced vital capacity (% of predicted value)	82.9 ± 18.7	35.8–130.9
Peak expiratory flow (% of predicted value)	73.1 ± 16.2	33.4–113.8
Maximal inspiratory pressure (H ₂ O cm)	66.4 ± 23.5	26.0–138.0
Maximal expiratory pressure (H ₂ O cm)	58.5 ± 22.4	23.0–123.0

Abbreviation: NA = not applicable.

sodium bisulfite treatment (NaBis; EpiTect Bisulfite Kit, Qiagen). Treatment of unmethylated cytosines thus generates a C→T (U) transition, whereas methylated cytosines are protected from deamination to uracils in the presence of NaBis, thus remaining cytosines. Combined with NaBis treatment, pyrosequencing is a quantitative real-time sequencing technology that measures the site-specific DNA methylation percentage for each CpG of a given sequence. PCR and sequencing primers were designed using Pyromark Assay Design 2.0 (Qiagen). Primer sequences are provided in table e-1 (links.lww.com/NXG/A157).

The PyroMark PCR Kit (Qiagen) was used for amplification of bisulfite-treated DNA. A Pyromark model q24 (Qiagen) was used to quantify DNA methylation levels.

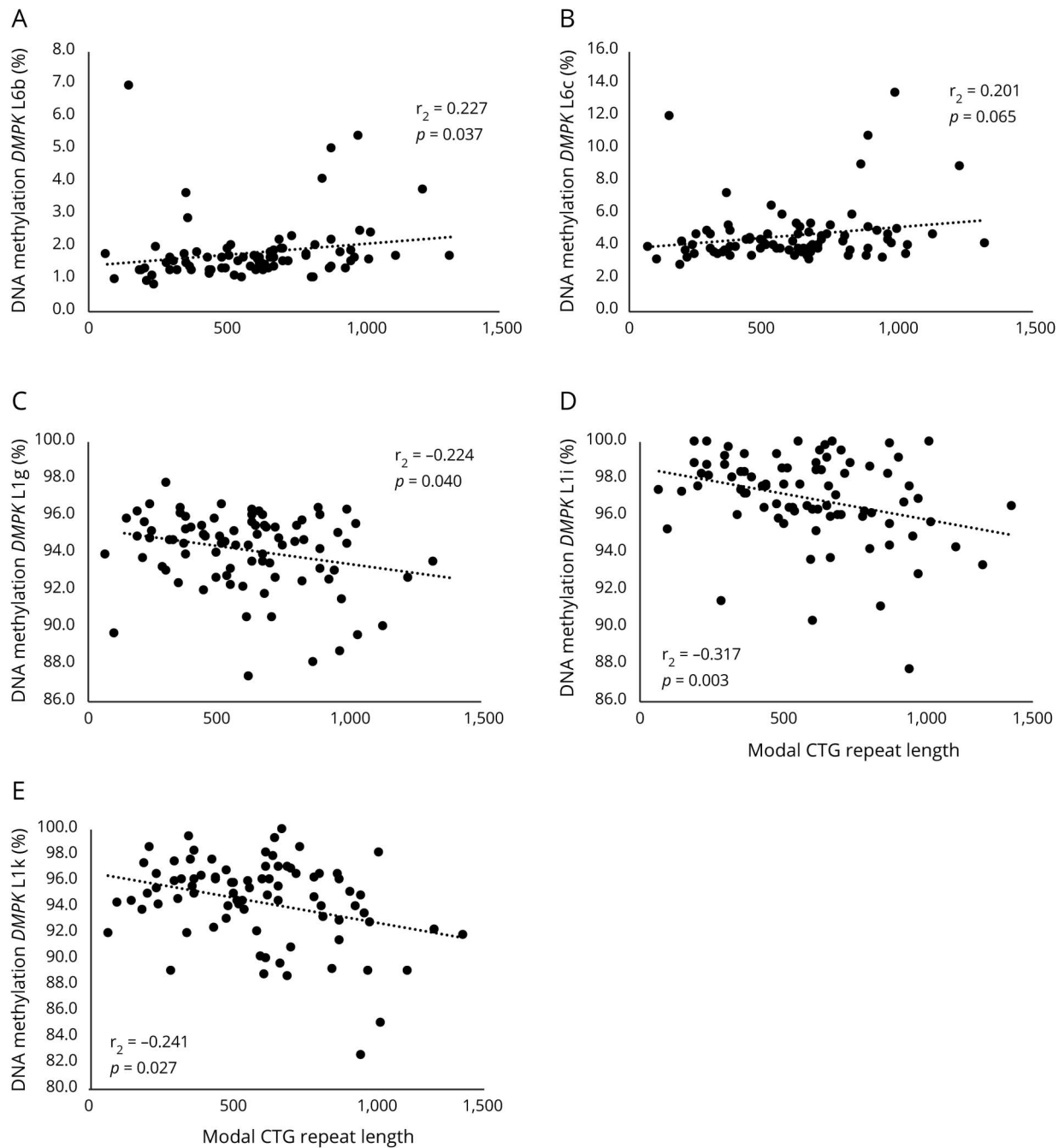
CTG repeat length analysis and variant repeat analysis

Small-pool PCR and Southern blot hybridization were used to measure CTG repeat length. PCR amplification of the *DMPK* CTG repeat was performed using the flanking primers DM-C and DM-DR, as previously described in reference 21. The PCR buffer was Custom PCR Master Mix-No Taq (Thermo Scientific #SM 0005) supplemented with 69 mM 2-mercaptoethanol. Taq polymerase (Sigma-Aldrich, United Kingdom) was used at 1 unit per 10 μL. Where required, reactions were supplemented with 10% dimethyl sulfoxide (Sigma-Aldrich), and the annealing temperature reduced from 68°C to 63.5°C. PCR products were digested with Acil (New England Biolabs UK Ltd) in accordance with the manufacturer's instructions. PCR-amplified DM1 alleles were run on 1% agarose gels in 0.5X tris-borate-EDTA, blotted, and hybridized as described in reference 21. α-32P-dCTP (Perkin Elmer UK) and the Invitrogen Random Primers DNA Labeling System (Thermo Fisher Scientific UK) were used to label the molecular weight marker (Invitrogen 1 kb+ ladder, Thermo Fisher Scientific UK) and DNA probes. Flanking primers DM-C and DM-DR and a patient with DM1 with 56 CTG repeats were used to amplify the CTG probe. After autoradiography, blot images were scanned, and then, estimated progenitor allele length and baseline modal allele lengths were estimated from the lower boundary²¹ and the densest part of the expanded alleles,²² respectively. Allele length was estimated by comparison to the 1 kb+ ladder, using CLIQS 1D gel analysis software (TotalLabs UK). Somatic instability was computed as modal allele length minus progenitor allele length.

Statistical analyses

Normality of distribution was tested using the Kolmogorov-Smirnov test and then visually confirmed on histograms. The following variables were rank transformed to respect the normality condition of the regression analysis: age, modal and progenitor CTG repeat length, DNA methylation levels, smoking, weight, shoulder abductors, knee extensors, ankle dorsiflexors, grip and pinch strengths, percentage of predicted forced vital capacity, percentage of predicted peak expiratory flow, maximal expiratory and inspiratory pressure, age at symptom onset, and instability. Spearman correlation coefficients were used to assess the association between DNAm and CTG repeat length. Mann-Whitney *U* tests were then applied on residual scores to assess the differences in DNAm between patients with and without repeat variants. For Spearman and Mann-Whitney *U* analysis, linear regression and resultant unstandardized residuals were used to adjust DNAm levels for sex and age of the participants. Stepwise multiple linear regression analyses were applied to identify variables independently contributing to explain muscular- and respiratory-related phenotypes. Confounding factors were added to each model based on their association with the corresponding phenotype. The 4 CpGs correlated with CTG repeat length without correction for age and sex were selected to be included into the stepwise regression model. We used relaxed 0.1 and 0.15 cutoff *p* values, respectively, for entry and

Figure 1 Spearman correlation between *DMPK* DNA methylation and modal CTG repeat length in patients with myotonic dystrophy type 1 (DM1)



Each panel (A to E) represents a different CpG. Results corrected for age and sex.

removal of variables within the regression. Significance levels were set to $p < 0.05$. Bonferroni correction was applied to adjust for multiple testing. Statistical analyses were performed on IBM SPSS statistics version 24 (IBM) software.

Data availability

The data sets generated and/or analyzed during the current study are not publicly available for ethical reasons, but are available from the corresponding author on reasonable request.

Results

Description of study participants and overall DName profile within the *DMPK* locus

The participants are, on average, middle aged and normal weight, with women being slightly overrepresented. Participants were aged 41.0 years (± 7.7) and 603.4 repeats (± 267.9) on average (table 1). Eight of the 90 participants were carriers of a CTG repeat variant (8.9%).

Table 2 Multiple linear regression analysis of muscle impairments of patients with DM1

	Shoulder	Knee	Ankle	Grip	Pinch
Age	NR	$\beta = -0.223$ $p = 0.022$	NR	NR	$\beta = -0.173$ $p = 0.071$
Sex	$\beta = 0.703$ $p < 0.001$	$\beta = 0.482$ $p < 0.001$	NR	NR	$\beta = 0.339$ $p = 0.001$
Modal CTG repeat length	$\beta = -0.146$ $p = 0.066$	NR	$\beta = -0.442$ $p < 0.001$	$\beta = -0.476$ $p < 0.001$	$\beta = -0.387$ $p < 0.001$
DNA methylation L6b	NR	NR	NR	NR	NR
DNA methylation L6c	NR	NR	$\beta = 0.340$ $p = 0.001$	$\beta = 0.173$ $p = 0.089$	$\beta = 0.219$ $p = 0.028$
DNA methylation L1i	NR	NR	$\beta = -0.363$ $p = 0.013$	NR	NR
DNA methylation L1k	NR	NR	$\beta = 0.467$ $p = 0.001$	NR	NR
Variant repeat (presence/absence)	NR	$\beta = -0.225$ $p = 0.021$	NR	NR	NR
R ² of the model (<i>p</i> value)	0.529 (<0.001)	0.336 (<0.001)	0.323 (<0.001)	0.222 (<0.001)	0.305 (<0.001)
<i>p</i> Value, Bonferroni corrected	<0.001	<0.001	0.001	0.002	<0.001

Abbreviations: ankle = ankle dorsiflexor strength; DM1 = myotonic dystrophy type 1; grip = grip strength; knee = knee extensors strength; NR = variables not retained in the model in the final model; pinch = pinch strength; shoulder = shoulder abductors strength.

DNAm levels were quantified at 8 different loci (L1 to L8) within the CpG island covering 35 and 17 CpG sites upstream and downstream of the CTG expansion, respectively (figure e-1, links.lww.com/NXG/A154). Some of these CpGs/loci have previously been studied in patients with DM1 or in mouse models.^{9–16} Upstream of the CTG expansion, DNAm profiles are multidimensional with those proximal to the CTG expansion (L3, 4, and 5 in intron 14 and exon 15) being unmethylated (<5%) and those more distally located showing increasing levels of DNAm from ~50% methylation, at L2 (intron 13) to close to fully methylated CpGs at site L1 (exon 11). Downstream of the CTG expansion, the CpG sites tested showed low (L6; <10%) to very low (L7 and L8; unmethylated to <5%) levels of DNAm.

Associations between DNAm and CTG repeats

Although the coefficients are similar, we observed that DNAm levels were correlated with the CTG repeat length (figure 1) in opposite directions depending on whether they were upstream or downstream of the CTG expansion. Briefly, DNAm levels in exon and intron 11 were correlated with modal CTG repeat length after correction for age and sex (L1g [$r_s = -0.224$; $p = 0.040$], L1i [$r_s = -0.317$; $p = 0.003$] and L1k [$r_s = -0.241$; $p = 0.027$]), whereas those downstream showed a correlation (L6b [$r_s = 0.227$; $p = 0.037$] and L6c [$r_s = 0.201$; $p = 0.065$]). The results remain significant with slightly stronger correlation coefficients after excluding patients with variant repeat.

We then assessed the links between DNAm and presence or absence of a variant repeat within the CTG expansion. We found that the presence of a variant repeat was associated with

higher DNAm levels only with CpGs just downstream of the repeat. On average, DNAm levels at any of the 5 CpGs tested were 3–4 times higher (L6; figure e-2, links.lww.com/NXG/A155). For example, we observed that the mean DNAm level at L6c for patients with a variant repeat was 15.5%, whereas it was 4.7% for patients without Acil-sensitive sequence ($p < 0.001$), even after correction for modal CTG and progenitor allele length. Although a nonparametric statistical test was applied, these associations seem primarily driven by 3 participants. We have carefully reviewed their familial, metabolic, and genetic characteristics, and the 2 patients with the highest levels of DNAm are siblings. No associations were found between the presence of a variant repeat and DNAm levels upstream of the CTG expansion.

Stepwise multiple linear regression analyses

We then assessed whether DNAm levels measured at the *DMPK* gene locus were associated with muscular strength and respiratory profile. Using multiple linear regression models and stepwise procedures, we found that DNAm contributed to explain variability in muscular and respiratory profiles independently of the CTG repeat length. These results are shown in detail in table 2 for tested muscular strength phenotypes and table 3 for respiratory measures. Very briefly, DNAm levels at one or more loci contributed significantly to every DM1-phenotype tested, except for knee and shoulder strengths and peak expiratory flow. Of interest, robust and persistent associations were observed between DNAm levels at L6c and all 3 muscular-related phenotypes tested, whereas our results suggest a similar pattern of associations between DNAm levels measured at L6b and all respiratory-related phenotypes.

Table 3 Multiple linear regression analysis of respiratory impairments of patients with DM1

	FVC	PEF	MIP	MEP
Age	NA	NA	$\beta = -0.332$ $p = 0.008$	NR
Sex	NA	NA	NR	$\beta = 0.417$ $p < 0.001$
Modal CTG repeat length	$\beta = -0.283$ $p = 0.012$	NR	NR	NR
DNA methylation L6b	$\beta = 0.368$ $p = 0.002$	$\beta = -0.236$ $p = 0.039$	$\beta = 0.266$ $p = 0.083$	$\beta = -0.211$ $p = 0.067$
DNA methylation L6c	NR	NR	$\beta = -0.399$ $p = 0.012$	NR
DNA methylation L1i	NR	NR	NR	NR
DNA methylation L1k	$\beta = 0.249$ $p = 0.021$	NR	NR	NR
Variant repeat (presence/absence)	NR	NR	NR	NR
Smoking (pack-years)	NR	$\beta = -0.228$ $p = 0.046$	NR	NR
Weight	$\beta = -0.266$ $p = 0.013$	NR	NR	NR
R ² of the model (p value)	0.283 (<0.001)	0.107 (0.018)	0.167 (0.012)	0.219 (0.001)
p Value, Bonferroni corrected	<0.001	0.072	0.048	0.004

Abbreviations: DM1 = myotonic dystrophy type 1; FVC = percentage of predicted forced vital capacity; MEP = maximal expiratory pressure; MIP = maximal inspiratory pressure; NA = variables not considered in the model based on their lack of association with the phenotype; NR = variables not retained in the final model; PEF = percentage of predicted peak expiratory flow.

DNAme levels were also tested for associations with somatic instability of the CTG repeat and age at symptom onset. We found that DNAme levels were associated with variability in somatic instability (differences between progenitor and average repeat length) of the CTG repeat, but not with age at symptom onset (table 4). All models except that for peak expiratory flow remain significant after Bonferroni correction. In addition to DNAme levels at other loci, age, sex, and smoking status contributed to explain one or more of the phenotypes tested.

Discussion

Although epigenetics (DNAme) has been a mechanism of interest in DM1 since 1993, only 7 studies have been published so far. Of them, 5 focused on the DM1 congenital phenotype.^{9,11,13–15} Two other studies reported DNAme analyses related to *DMPK* and *SIX5* gene expression,^{10,16} and only one tested association between a clinical phenotype, namely the MIRS.¹² We are the first to study and demonstrate that DNAme at the *DMPK* gene locus contributes to variability of both muscular strengths and respiratory profiles. Our results also support that DNAme contributes to DM1 phenotypic variability independently of the CTG repeat length. Our study has been conducted exclusively in 90 patients with

the adult form of the disease, which we believe reduces the confounding effects of extreme phenotypic and age at sampling variability that characterizes broader DM1 cohorts and allows us to reveal new insights into the role of DNAme in DM1.

Figure e-3 (links.lww.com/NXG/A156) summarizes the results published so far. Our results for sites L1 and L2 are in line with some studies^{15,16} showing constitutive DNAme in intron 11, but not with 1 study,¹³ although we report differences in DNAme levels between individuals at these CpG sites. Our results are also in agreement with those previously published^{13,15,16} showing that the L3 region is unmethylated, although they found some DNAme when patients with DM1 had more than 300 CTG repeats.^{13,15,16} For the L4 and L5 regions, hypomethylation of the region has been observed in other studies,^{9,12–15} although 1 study¹² found DNAme at various degrees in patients with pure CTG repeats, as did another.¹¹ For L6, the region has been found to present some variability in our study and in mouse,^{10,11} in patients with variant repeats,¹² and patients with DM1.⁹ This region has been associated with hypomethylation in patients with DM1^{11,13,15} and patients with DM1 with a pure CTG tract.¹² The L7 and L8 sites have been found to be hypomethylated both by our group and in a previous study.¹³ The studies published so far are of small to very small sample size. Also, inconsistencies between the studies might be explained by the different DNAme quantification

Table 4 Multiple linear regression analysis of phenotypic variability of age at onset of patients with DM1 and somatic instability of their repeats

	Age at onset		Somatic instability	
	Modal CTG	Progenitor CTG	Modal CTG	Progenitor CTG
Age	NA	NA	NR	NR
Sex	NR	NR	$\beta = -0.079$ $p = 0.054$	$\beta = -0.137$ $p = 0.078$
Modal CTG repeat length	$\beta = -0.380$ $p = 0.001$	NA	$\beta = 0.978$ $p < 0.001$	NA
Progenitor allele length	NA	$\beta = -0.339$ $p = 0.003$	NA	$\beta = 0.811$ $p = <0.001$
DNA methylation L6b	NR	NR	$\beta = -0.202$ $p = <0.001$	$\beta = -0.254$ $p = 0.005$
DNA methylation L6c	NR	NR	NR	NR
DNA methylation L1i	NR	NR	NR	NR
DNA methylation L1k	NR	NR	NR	NR
Variant repeat (presence/absence)	NR	NR	NR	NR
R ² of the model (p Value)	0.144 (0.001)	0.115 (0.003)	0.870 (<0.001)	0.533 (<0.001)
p Value, Bonferroni corrected	0.002	0.006	<0.001	<0.001

Abbreviations: age at onset = age at symptom onset; DM1 = myotonic dystrophy type 1; NA = variables not considered in the model based on their lack of association with the phenotype; NR = variables not retained in the final model; somatic instability = somatic instability of the repeat.

methods, which are in some cases far from ideal. For example, methylation-sensitive restriction enzymes have been routinely used, but do not allow detection of small changes in DNAm unlike pyrosequencing. These studies have also analyzed a broader range of DM1 phenotypes than in our study. The other DM1 phenotypes should thus be also investigated.

Of interest, we also report that the CTG repeat length was correlated with DNAm, but in opposite directions depending on which side of the CTG expansion DNAm levels were assessed. We report a correlation with DNAm measured in intron 11. Some groups reported similar associations for DNAm measured in closer vicinity of the CTG expansion,^{9,10,12,15,16} although discrepancies exist.^{11,14} Overall, ours and previous results support the hypothesis that DNAm and modal CTG repeat length are correlated with each other. However, we cannot yet conclude which of the 2 influences the other.

Of interest, we also found that presence of a variant repeat within the CTG expansion was associated with higher DNAm levels downstream of the CTG repeat (L6). Similar results have been reported showing an association driven by participants who had more “CG” sequences in their repeat.¹² The “CG” content has not been assessed in our study, precluding us from drawing a clear conclusion. However, we found that 2 of the 3 patients with higher DNAm levels were siblings. Variant repeats were first observed in a small number of families,^{24,25} suggesting that they might be inherited. As such, the pattern of each variant repeat is also probably inherited and similar within families.

Next, we tested whether DNAm was also associated with key impairments in DM1. DNAm at 2 different CpG sites, both within the same L6 region downstream of the CTG expansion, contributed independently of the CTG repeat length to variability of muscular- (L6c; ankle dorsiflexors, handgrip, and pinch strength) and respiratory-related phenotypes (L6b; forced vital capacity, maximal inspiratory pressure, and maximal expiratory pressure). This suggests that DNAm at those CpG sites might affect distinctive muscle groups by changing affinity for different transcription factors. However, no specific transcription factor consensus sequences covering each of the CpG sites were found in the ENCODE database. Also, we did not find that DNAm contributes to strength variability of proximal muscle groups (shoulder abductors and knee extensors), which could be explained by several reasons including measurement errors, muscle impairment level, and other factors.

At least 3 hypotheses could explain how DNAm might drive clinical variability in DM1. First, DNAm might block CTCFs binding to one or both of the CTCF consensus sequences located on each side of the CTG expansion, leading to CTCF insulator disruption ability between *DMPK* and *SIX5*.²⁶ Second, DNAm might reduce the mRNA levels of *DMPK* and *SIX5* and thus reducing the number of *DMPK* and *SIX5* protein.^{10,16} Third, DNAm might alter CTG repeat instability^{27,28} through disruption of CTCF binding,²⁸ although another study reported that it was DNA demethylation that was promoting repeat instability²⁷ with another one showing

no link between DNAm and CTG repeat instability.¹⁴ Of interest, we found that increased DNAm was associated with lower somatic instability. Over time, this might be beneficial for the patients, as longer repeats usually means a worse prognosis.

Strengths and limitations

Our study is the largest in terms of number of patients with DM1 with the same phenotype (adult form) and the second largest overall. Our participants were extensively phenotyped and genotyped, including measures of CTG repeat length and occurrence of CTG variant repeats. DNAm was measured in blood only (this could also be seen as a limitation) and using pyrosequencing, the gold standard method to assess CpG-specific DNAm levels, thus reducing potential technical variability. One limitation is that we only covered a small part of the DM1 locus (<3%), leaving many other CpG sites to be tested. Another limitation is that we did not have access to non-DM1 participants, and thus, comparisons with what could be considered a normal DNAm profile were not possible. However, the *DMPK* region has previously been found to be hypomethylated near the CTG repeat and hypermethylated in the region of exons 11 and 12 in controls and the general population in various studies.^{9,11,12,29} No new data on the methylation status of the *DMPK* region are available on the epigenome roadmap than the data already published.²⁹

DNAm at the *DMPK* gene locus contributes to specific clinical phenotypes (muscular strengths and respiratory profiles) in DM1. These associations are independent of the CTG repeat length. Our results thus provide evidence that measuring DNAm might help to predict progression of the disease and to establish a more reliable prognosis for those patients. We nevertheless agree that further studies, including longitudinal studies and in other DM1 forms, are needed before strong conclusions can be drawn and clinically applied.

Acknowledgment

Pascal-Denis Grenier performed the quantitative muscle testing during a single visit. Céline Bélanger revised the language of the manuscript.

Study funding

This project has been supported by the Marigold Foundation, the Canadian Institutes of Health Research (CIHR) (#JNM-108412), the Fondation du grand défi Pierre Lavoie, and Muscular Dystrophy UK.

Disclosure

Disclosures available: Neurology.org/NG.

Publication history

Received by *Neurology: Genetics* January 31, 2019. Accepted in final form April 4, 2019.

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Neurol Genet 2019;5;

DOI 10.1212/NXG.0000000000000338

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