Variant repeats within the DMPK CTG expansion protect function in myotonic dystrophy type 1

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Abstract

Objective
We tested the hypothesis that variant repeat interruptions (RIs) within the DMPK CTG repeat tract lead to milder symptoms compared with pure repeats (PRs) in myotonic dystrophy type 1 (DM1).

Methods
We evaluated motor, neurocognitive, and behavioral outcomes in a group of 6 participants with DM1 with RI compared with a case-matched sample of 12 participants with DM1 with PR and a case-matched sample of 12 unaffected healthy comparison participants (UA).

Results
In every measure, the RI participants were intermediate between UA and PR participants. For muscle strength, the RI group was significantly less impaired than the PR group. For measures of Full Scale IQ, depression, and sleepiness, all 3 groups were significantly different from each other with UA > RI > PR in order of impairment. The RI group was different from unaffected, but not significantly different from PR (UA = RI = PR) in apathy and working memory. Finally, in finger tapping and processing speed, RI did not differ from UA comparisons, but PR had significantly lower scores than the UA comparisons (UA = RI > PR).

Conclusions
Our results support the notion that patients affected by DM1 with RI demonstrate a milder phenotype with the same pattern of deficits as those with PR indicating a similar disease process.
Myotonic dystrophy type 1 (DM1; OMIM 160900) is an autosomal dominant, progressive, multisystem disorder caused by expansion of a CTG repeat in the 3′-untranslated region of DMPK.1–4 DM1 affects many organ systems, including skeletal muscle, heart, gastrointestinal, integumentary, endocrine, and CNS.3,5,6 Although the primary symptoms of DM1 are myotonia and muscle weakness, some of the most disabling symptoms of the disease are those arising from CNS involvement.7,8 These include progressive cognitive and behavioral changes, as well as fatigue and excessive daytime sleepiness, which greatly affect overall quality of life.9–11

In 3%–5% of patients with DM1, the CTG repeat tract is interrupted by naturally occurring variant sequences, such as CCG, CTC, or GGC motifs.12,13 Variant repeats most commonly occur at the 3′-end of the DMPK CTG repeat tract.14,15 These are referred to as variant repeat interruptions (RIs). Increasing evidence from case reports suggests that patients with DM1 who carry RI alleles exhibit a later age at symptom onset, milder muscle symptoms, and atypical patterns of symptoms (smaller proportion of cataracts, cardiac problems, and muscle weakness) compared with those with pure repeats (PRs).12–14,16,17 These effects have now been confirmed in 2 large independent DM1 cohorts.18,19 The attenuation of symptoms is hypothesized to result, at least in part, from a stabilizing effect of RI that reduces expansion-biased instability in somatic cells.13,20,21 In this context, we set out to compare motor, neurocognitive, and behavioral outcome measures of participants with adult-onset DM1 with RIs matched to participants with DM1 with PRs, as well as comparison of both groups to participants unaffected by DM1.

Methods
Recruitment of participants
Participants with adult-onset DM1 were recruited to the University of Iowa “DM1 Brain Study” from across the United States by advertisements through the Myotonic Dystrophy Foundation or word of mouth, as described previously.22 Recruitment was targeted to adult-onset DM1 only, with symptom onset at age 18 years or older. Unaffected participants were primarily recruited from the local community through advertisements. Recruitment for baseline assessments took place between September 2014 and July 2017. Inclusion criteria were as follows: (1) between ages 21 and 65 years; (2) clinical diagnosis of DM1 after age 21 years; (3) committed to completing annual evaluations for 2 years following intake; and (4) commitment of an informant to accompany the participant to study visits. Exclusion criteria included (1) unstable psychiatric illness (including current substance abuse) and (2) history of major head trauma with loss of consciousness for longer than a few minutes and including clinically significant sequelae.

Standard protocol approvals, registrations, and patient consents
All participants gave written informed consent before enrolling in the protocol in accordance with the Declaration of Helsinki. The study was approved by the University of Iowa’s Institutional Review Board.

Data availability
Anonymized data will be shared by request from any qualified investigator.

Measurement of CTG repeat length and variant repeat identification
For genotyping of CTG repeats in participants with DM1, we used the same methodology as the one used in previous studies.22,23 For variant repeat identification, small-pool PCR products underwent AciI enzyme digestion (New England Biolabs UK Ltd.; restriction site 5′CCGC-3′) and Southern blotting to indicate the presence of CCG interruptions within the CTG repeat array in the expanded allele as previously described.13

Motor testing
Motor, neurocognitive, and behavioral outcome measures of interest were selected a priori to reduce the number of comparisons. Severity of muscle weakness was measured using the Muscle Impairment Rating Scale (MIRS) during examination by a neuromuscular specialist experienced in DM1, blinded to the participants’ genetic status.24 This scale evaluates muscular impairment severity according to an ordinal 5-point scale as follows: (1) no muscular impairment, (2) minimal signs, (3) distal weakness, (4) mild to moderate proximal weakness, and (5) severe proximal weakness.

Grip strength was measured using a Lafayette Instruments dynamometer. The stirrup of the dynamometer was adjusted to comfortably fit the participant’s hand size, after which they were instructed to squeeze as hard as they possibly could. Strong motivational encouragement was provided by the examiner during each of the 6 trials (3 for the dominant hand and 3 for the nondominant hand) to elicit the participant’s maximal effort. Ultimate scores were the means of 3 trials for each hand.
Neurocognitive and behavioral testing

Neurocognitive and behavioral assessments included the Wechsler Adult Intelligence Scale—Fourth Edition (WAIS-IV), Beck Depression Inventory-II (BDI-II), Apathy Evaluation Scale (AES) self-assessment, and Scales for Outcomes in Parkinson’s Disease (SCOPA). These measures were administered by a trained examiner experienced in DM1 who was blinded to the patient’s clinical condition (CTG expansion length and muscular impairment).

Statistical analysis

All statistical analyses were performed using R (version 3.6.2). Each participant who was determined to have variant repeats was matched by age, sex, and CTG repeat length to 2 participants with pure CTG repeats and 2 unaffected participants. Mixed-effects multivariable linear regression models were used to examine the impact of group, age, sex, and a subject-matching variable on the dependent variables (motor, neurocognitive, and behavioral outcome measures). The coefficient of determination ($R^2$) for the model, semi-partial $R^2$ for each fixed effect (group, age, and sex), and 95% confidence intervals (CIs) were calculated using the Nakagawa & Schielzeth approach.\textsuperscript{25} Effect sizes were considered very weak ($R^2 < 0.1$), weak ($0.1 < R^2 < 0.3$), moderate ($0.3 < R^2 < 0.5$), and strong ($0.5 < R^2$). Post hoc least-squares means tests were used for pairwise comparisons between UA, RI, and PR groups. All outcome measures of interest were selected a priori to minimize multiple testing considerations.

Results

Sample

From a cohort of 57 adults affected by DM1, 6 participants (11%) were identified as positive for variant RIs by Acil enzyme digest. Each RI participant was equivalently matched to 2 PR participants and 2 unaffected healthy comparison participants (UA) by age, sex, and CTG repeat length for a total sample of 125 participants (6 RI, 12 PR, and 12 UA) (table 1). There was an equal proportion of men (66%) and women (33%) within each group ($\chi^2 = 0.0, df = 2, p = 1.0$). Mean age at evaluation was 42.27 years (SD = 11.89) for the UA group, 42.25 years (SD = 12.52) for the RI group, and 40.42 years (SD = 11.03) for the PR group, with no significant group effect ($\chi^2 = 1.96, 16.25$, $df = 1, p = 0.027$) with a higher proportion of PR participants scoring 3 and 4 (mild to moderate proximal weakness) (mean = 2.92; SD = 1.08) than the RI group (mean = 2.00; SD = 0.63). The PR group had 5 participants with a score of 4 (moderate proximal weakness) and no participants with a score of 5 (severe proximal weakness), whereas the RI group had no participants with a score of 4 or 5.

Table 1 Demographics of the study sample

| Sample, n | Control (UA) | Variant (RI) | Pure (PR) |
|-----------|--------------|--------------|-----------|
| Males     | 8            | 4            | 8         |
| Females   | 4            | 2            | 4         |
| Age at evaluation, mean (SD) | 42.27 (11.89) | 42.25 (12.52) | 40.42 (11.03) |
| Age at disease onset, mean (SD) | n/a | 31.75 (5.76) | 24.14 (9.48) |
| CTG       |              |              |           |
| Range     | 12–22        | 157–625      | 125–750   |
| Median    | 13           | 375          | 285       |

Abbreviations: PR = pure repeat; RI = repeat interruption.

Motor performance

Detailed statistics for each outcome measure from the mixed-effects multivariable linear regression model with post hoc least-squares means tests are shown in table 2 and table 3. The figure summarizes group differences for all outcome measures, which show that the RI group is always intermediate between the UA and PR groups.

As shown in figure A, there was a significant difference between the RI and PR groups in MIRS scores ($t(25) = -2.2, 95\%\ CI [-1.95, -0.003]$) with an overall moderate effect size of the model ($R^2 = 0.308, 95\%\ CI [0.090, 0.668]$) and a significant group effect ($\chi^2 = 4.87, df = 1, p = 0.027$) with a higher proportion of PR participants scoring 3 and 4 (mild to moderate proximal weakness) (mean = 2.92; SD = 1.08) than the RI group (mean = 2.00; SD = 0.63). The PR group had 5 participants with a score of 4 (moderate proximal weakness) and no participants with a score of 5 (severe proximal weakness), whereas the RI group had no participants with a score of 4 or 5.

Finger tapping test results for the dominant hand are shown in figure, B. The mean scores were 43.98 (SD = 8.98) for the UA group; 36.83 (SD = 5.54) for the RI group; and 29.16 (SD = 11.78) for the PR group. There was an overall moderate effect size ($R^2 = 0.459, 95\%\ CI [0.264, 0.694]$) and significant group effect in the model ($\chi^2 = 16.84, df = 2, p < 0.001$), with no significant difference between the UA and RI groups ($t(25) = 1.61, 95\%\ CI [-1.96, 16.25]$), a significant difference between the UA and PR groups ($t(25) = 4.10, 95\%\ CI [7.41, 22.34]$), and

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\chi^2 < 0.001. \]

As expected for CTG length, there were significant differences between the UA group and the RI ($t(17.9) = -5.10, 95\%\ CI [-545.39, -227.07]$) and PR ($t(18.3) = -5.01, 95\%\ CI [-468.87, -192.23]$) groups and no significant difference between the RI and PR groups ($t(17.5) = 0.82, 95\%\ CI [-86.25, 197.62]$) (table 1).
no significant difference between the RI and PR groups ($t(25) = 1.74$, 95% CI $[-1.40, 16.86]$).

As shown in figure, C, for grip strength (dominant hand), the mean was 41.08 (SD = 11.12) for the UA group, 29.19 (SD = 15.7) for the RI group, and 19.25 (SD = 14.42) for the PR group. The group effect was significant in the model ($\chi^2 = 22.01, df = 2, p < 0.001$) with a strong overall effect size ($R^2 = 0.505$, 95% CI $[0.312, 0.722]$), no significant difference between the UA and RI groups ($t(22) = 2.03, 95\%$ CI $[-0.25, 24.02]$), a significant difference between the UA and PR groups ($t(22) = 4.69, 95\%$ CI $[12.56, 32.45]$), and no significant difference between the RI and PR groups ($t(22) = 1.81, 95\%$ CI $[-1.54, 22.79]$).

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**Neurocognitive functioning**

As shown in figure, D through F shows group differences on the WAIS-IV Full Scale IQ, Working Memory Index (WMI), and Processing Speed Index (PSI), respectively. The mean Full Scale IQ was 120.08 (SD = 13.52) for the UA group; 107.17 (SD = 8.77) for the RI group; and 94.67 (SD = 7.67) for the PR group. There was a significant group effect in the model for Full Scale IQ ($\chi^2 = 33.39, df = 2, p < 0.001$), with a strong overall effect size ($R^2 = 0.537$, 95% CI $[0.346, 0.741]$), a significant difference between the UA and RI groups ($t(25) = 2.37, 95\%$ CI $[1.72, 24.11]$), a significant difference between the UA and PR groups ($t(25) = 5.78, 95\%$ CI $[16.55, 34.90]$), and a significant difference between the RI and PR groups ($t(25) = 2.35, 95\%$ CI $[1.59, 24.02]$).

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### Table 2 Outcome comparisons between groups

| Variable                  | Group 1     | Group 2     | Estimate | Estimate 95% CI [LL, UL] |
|---------------------------|-------------|-------------|----------|-------------------------|
| MIRS                      | Variant     | Pure        | -0.97    | [-1.95, -0.003]         |
| Tapping                   | Controls    | Variant     | 7.15     | [-1.96, 16.25]          |
|                           | Controls    | Pure        | 14.88    | [7.41, 22.34]           |
|                           | Variant     | Pure        | 7.73     | [-1.40, 16.86]          |
| Grip strength             | Controls    | Variant     | 11.89    | [-0.25, 24.03]          |
|                           | Controls    | Pure        | 22.51    | [12.57, 32.46]          |
|                           | Variant     | Pure        | 10.62    | [-1.54, 22.79]          |
| WAIS Full Scale IQ        | Controls    | Variant     | 12.92    | [1.73, 24.11]           |
|                           | Controls    | Pure        | 25.73    | [16.56, 34.90]          |
|                           | Variant     | Pure        | 12.81    | [1.59, 24.03]           |
| WAIS working memory       | Controls    | Variant     | 18.34    | [4.84, 31.84]           |
|                           | Controls    | Pure        | 25.88    | [14.82, 36.95]          |
|                           | Variant     | Pure        | 7.54     | [-5.99, 21.08]          |
| WAIS processing speed     | Controls    | Variant     | 8.51     | [-5.19, 22.20]          |
|                           | Controls    | Pure        | 26.84    | [15.62, 38.06]          |
|                           | Variant     | Pure        | 18.33    | [4.61, 32.06]           |
| Beck Depression Inventory | Controls    | Variant     | -5.25    | [-10.33, -0.18]         |
|                           | Controls    | Pure        | -10.50   | [-14.66, -6.34]         |
|                           | Variant     | Pure        | -5.25    | [-10.34, -0.16]         |
| Apathy Evaluation Scale   | Controls    | Variant     | -8.51    | [-16.30, -0.71]         |
|                           | Controls    | Pure        | -11.65   | [-18.04, -5.26]         |
|                           | Variant     | Pure        | -3.14    | [-10.96, 4.68]          |
| SCOPA daytime             | Controls    | Variant     | -2.75    | [-4.93, -0.57]          |
|                           | Controls    | Pure        | -5.56    | [-7.35, -3.77]          |
|                           | Variant     | Pure        | -2.81    | [-4.99, -0.62]          |

Abbreviations: CI = confidence interval; MIRS = Muscle Impairment Rating Scale; SCOPA = Scales for Outcomes in Parkinson’s Disease; WAIS = Wechsler Adult Intelligence Scale.

*All regression coefficients (estimate) and 95% CIs were calculated in the linear mixed-effects regression model with least-squares means post hoc tests, using group, age, sex, and a matching variable as predictors. LL and UL represent the lower limit and upper limit of the regression coefficient, respectively.*
For WMI, the mean score was 120.17 (SD = 11.57) for the control group, 101.83 (SD = 11.55) for the RI group, and 94.75 (SD = 14.59) for the PR group, with a significant group effect in the model ($\chi^2 = 24.06$, $df = 2$, $p < 0.001$) and moderate overall effect size ($R^2 = 0.458$, 95% CI [0.262, 0.694]). There was a significant difference between the UA and RI groups ($t_{(25)} = 2.79$, 95% CI [4.84, 31.84]), a significant difference between the UA and PR groups ($t_{(25)} = 4.82$, 95% CI [14.82, 36.95]), and no significant difference between the RI and PR groups ($t_{(25)} = 1.14$, 95% CI [−5.99, 21.08]).

PSI exhibited a similar pattern as WMI, with the UA group having a mean score of 114.5 (SD = 16.71), the RI group having a mean score of 106.00 (SD = 12.88), and the PR group having a mean score of 88.08 (SD = 8.15). There was a significant group effect in the model ($\chi^2 = 24.90$, $df = 2$, $p < 0.001$) and moderate

| Table 3 Mixed-effects multivariate model results$^a$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variable        | Predictor       | $\chi^2$ | df  | $p$ Value | Model $R^2$ 95% CI [LL, UL] | Semi-partial $R^2$ | Semi-partial $R^2$ 95% CI [LL, UL] |
| MIRS            | Group           | 4.87     | 1   | 0.027     | 0.308 [0.090, 0.668] | 0.206$^b$ | [0.004, 0.559]$^b$ |
|                 | Age             | 1.75     | 1   | 0.185     |                          | 0.108   | [0.000, 0.460]      |
|                 | Sex             | 0.14     | 1   | 0.703     |                          | 0.010   | [0.000, 0.293]      |
| Tapping         | Group           | 16.84    | 2   | 0.000     | 0.459 [0.264, 0.694] | 0.367/0.083$^c$ | [0.131, 0.611]/(0.000, 0.336)$^c$ |
|                 | Age             | 0.02     | 1   | 0.863     |                          | 0.001   | [0.000, 0.166]      |
|                 | Sex             | 5.44     | 1   | 0.019     |                          | 0.158   | [0.006, 0.428]      |
| Grip strength   | Group           | 22.01    | 2   | 0.000     | 0.505 [0.312, 0.722] | 0.422/0.085$^c$ | [0.184, 0.651]/(0.002, 0.385)$^c$ |
|                 | Age             | 2.34     | 1   | 0.126     |                          | 0.085   | [0.001, 0.340]      |
|                 | Sex             | 1.85     | 1   | 0.173     |                          | 0.069   | [0.000, 0.316]      |
| WAIS Full Scale IQ | Group     | 33.39    | 2   | 0.000     | 0.537 [0.346, 0.741] | 0.535/0.163$^c$ | [0.313, 0.727]/(0.007, 0.433)$^c$ |
|                 | Age             | 0.67     | 1   | 0.411     |                          | 0.023   | [0.000, 0.231]      |
|                 | Sex             | 0.02     | 1   | 0.881     |                          | 0.001   | [0.000, 0.165]      |
| WAIS working memory | Group          | 24.06    | 2   | 0.000     | 0.458 [0.262, 0.694] | 0.667/0.482$^c$ | [0.208, 0.667]/(0.024, 0.482)$^c$ |
|                 | Age             | 1.02     | 1   | 0.310     |                          | 0.034   | [0.000, 0.256]      |
|                 | Sex             | 0.09     | 1   | 0.753     |                          | 0.003   | [0.000, 0.174]      |
| WAIS processing speed | Group       | 24.90    | 2   | 0.000     | 0.468 [0.272, 0.700] | 0.456/0.053$^c$ | [0.220, 0.674]/(0.000, 0.292)$^c$ |
|                 | Age             | 0.819    | 1   | 0.365     |                          | 0.033   | [0.000, 0.254]      |
|                 | Sex             | 0.994    | 1   | 0.319     |                          | 0.027   | [0.000, 0.242]      |
| Beck Depression Inventory | Group | 27.04    | 2   | 0.000     | 0.523 [0.331, 0.733] | 0.483/0.135$^c$ | [0.250, 0.693]/(0.003, 0.403)$^c$ |
|                 | Age             | 5.93     | 1   | 0.014     |                          | 0.170   | [0.009, 0.440]      |
|                 | Sex             | 3.83     | 1   | 0.051     |                          | 0.117   | [0.002, 0.381]      |
| Apathy Evaluation Scale | Group | 14.71    | 2   | 0.000     | 0.400 [0.208, 0.658] | 0.327/0.148$^c$ | [0.096, 0.580]/(0.004, 0.417)$^c$ |
|                 | Age             | 5.93     | 1   | 0.014     |                          | 0.170   | [0.009, 0.440]      |
|                 | Sex             | 1.28     | 1   | 0.257     |                          | 0.042   | [0.000, 0.272]      |
| SCOPA daytime   | Group           | 41.13    | 2   | 0.000     | 0.598 [0.419, 0.777] | 0.587/0.189$^c$ | [0.379, 0.760]/(0.014, 0.460)$^c$ |
|                 | Age             | 3.76     | 1   | 0.052     |                          | 0.115   | [0.001, 0.379]      |
|                 | Sex             | 0.92     | 1   | 0.336     |                          | 0.031   | [0.000, 0.249]      |

Abbreviations: CI = confidence interval; DM1 = myotonic dystrophy type 1; MIRS = Muscle Impairment Rating Scale; SCOPA = Scales for Outcomes in Parkinson’s Disease; WAIS = Wechsler Adult Intelligence Scale.

$a$ Chi-square, degrees of freedom ($df$), and $p$ values were calculated in the mixed-effects multivariate regression model with type III (Wald) $\chi^2$ tests, using group, age, sex, and a matching variable as predictors. Coefficient of determination ($R^2$) for the model, semi-partial coefficient of determination (semi-partial $R^2$) for fixed effects (group, age, and sex), and 95% CIs were calculated using the Nakagawa & Schielzeth approach on the linear mixed-effects model. LL and UL represent the lower limit and upper limit, respectively.

$^b$ Variant vs DM1 only.

$^c$ Control vs variant/control vs DM1.
overall effect size ($R^2 = 0.468, 95\% \text{ CI} [0.272, 0.700]$), with no significant difference between the UA and RI groups ($t_{(25)} = 1.27, 95\% \text{ CI} [-5.19, 22.20]$), a significant difference between the UA and PR groups ($t_{(25)} = 4.93, 95\% \text{ CI} [15.62, 38.06]$), and a significant difference between the RI and PR groups ($t_{(25)} = 2.75, 95\% \text{ CI} [4.61, 32.06]$).

Behavioral outcomes

Figure, G shows scores for the BDI across groups. The mean score was 1.92 (SD = 1.83) for the UA group, 7.17 (SD = 7.05) for the RI group, and 12.00 (SD = 6.63) for the PR group, with a significant difference between the RI and PR groups ($t_{(25)} = 2.75, 95\% \text{ CI} [4.61, 32.06]$).

Results for the AES are shown in figure, H. The mean score for the UA group was 22.33 (SD = 3.73), 31.83 (SD = 6.31) for the RI group, and 34.33 (SD = 11.37) for the PR group, with a significant group effect ($\chi^2 = 14.71, df = 2, p < 0.001$) and moderate overall effect size ($R^2 = 0.400, 95\% \text{ CI} [0.208, 0.658]$). Age also had a significant effect ($\chi^2 = 5.93, df = 1, p = 0.014$) with weak effect size ($R^2 = 0.170, 95\% \text{ CI} [0.009, 0.440]$), and sex had a significant effect on the model ($\chi^2 = 3.83, df = 1, p = 0.051$) with weak effect size ($R^2 = 0.117, 95\% \text{ CI} [0.002, 0.381]$). There were significant differences between the UA and RI groups ($t_{(25)} = -2.13, 95\% \text{ CI} [-10.33, -0.18]$), the UA and PR groups ($t_{(25)} = -5.20, 95\% \text{ CI} [-14.66, -6.34]$), and the RI and PR groups ($t_{(25)} = -2.12, 95\% \text{ CI} [-10.34, -0.16]$).
A limitation of this present study is small sample size, with only 6 participants with variant repeats, 12 participants with PRs, and 12 unaffected participants. This decreased the statistical power with which we could possibly detect significant differences in additional motor, neurocognitive, and behavioral domains. Another limitation is the overall mild nature of symptoms of the study cohort. A lower age at disease onset could have possibly revealed more evident changes between groups. In addition, although the mean age at disease onset for the variant repeat group was greater than 7 years than the PR group (31.75 years vs 24.1 years), it was not statistically significant. However, in larger cohorts, AciI sites are statistically significantly associated with later onset. A follow-up neuroimaging study in a larger sample could possibly help increased risk for weight loss and aspiration. Consistent with recent findings from the OPTIMISTIC and Saguenay cohorts, our data confirm a significant difference in muscle power between PR and RI participants, detectable clinically by MIRS assessment,
under study conditions in which the evaluating clinician is blinded to the participants' genetic status.
elucidate the specific brain changes behind this pattern of deficits. Nonetheless, motor, cognitive, and behavioral measures were significantly better in the presence of variant repeats. Our group has demonstrated that cognitive deficits in DM1 are associated with altered brain structure.41 We would expect that the RI group will exhibit a milder neuroanatomic phenotype than their PR counterparts.

Our study supports the hypothesis that variant RIs within the CTG repeat tract of the DMPK gene have a protective effect in multiple systems in DM1, including the CNS. Further exploration of the mechanisms underlying this effect is required to improve prognostic information available to affected patients and may reveal potential targets for novel therapy.

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Disclosure
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Appendix

| Name              | Location                                      | Contribution                                      |
|-------------------|-----------------------------------------------|--------------------------------------------------|
| Mark Hamilton, PhD| West of Scotland Clinical Genetics Service, Queen Elizabeth University Hospital, Glasgow, United Kingdom; Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom | Designed and conceptualized the study and revised the manuscript for intellectual content |
| Timothy R. Koscik, PhD | University of Iowa Hospitals and Clinics | Designed and conceptualized the study; interpreted the data; and revised the manuscript for intellectual content |
| Laurie Gutmann, MD | University of Iowa Hospitals and Clinics | Major role in the acquisition of data; interpreted the data; and revised the manuscript for intellectual content |
| Sarah A. Cumming, PhD | Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom | Major role in the acquisition of data and revised the manuscript for intellectual content |
| Darren G. Monckton, PhD | Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom | Major role in the acquisition of data and revised the manuscript for intellectual content |
| Peggy C. Nopoulos, MD | University of Iowa Hospitals and Clinics | Interpreted the data and revised the manuscript for intellectual content |

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