Associations Between Variant Repeat Interruptions and Clinical Outcomes in Myotonic Dystrophy Type 1

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Abstract

Objective

To assess the association between variant repeat (VR) interruptions in patients with myotonic dystrophy type 1 (DM1) and clinical symptoms and outcome measures after cognitive behavioral therapy (CBT) intervention.

Methods

Adult patients with DM1 were recruited within the OPTIMISTIC trial (NCT02118779). Disease-related history, current clinical symptoms and comorbidities, functional assessments, and disease- and health-related questionnaires were obtained at baseline and after 5 and 10 months. After genetic analysis, we assessed the association between the presence of VR interruptions and clinical symptoms' long-term outcomes and compared the effects of CBT in patients with and without VR interruptions. Core trial outcome measures analyzed were: 6-minute walking test, DM1-Activ-C, Checklist Individual Strength Fatigue Score, Myotonic Dystrophy Health Index, McGill-Pain questionnaire, and Beck Depression inventory—fast screen. Blood samples for DNA testing were obtained at the baseline visit for determining CTG length and detection of VR interruptions.

Results

VR interruptions were detectable in 21/250 patients (8.4%)—12 were assigned to the standard-of-care group (control group) and 9 to the CBT group. Patients with VR interruptions were significantly older when the first medical problem occurred and had a significantly shorter disease duration at baseline. We found a tendency toward a milder disease severity in patients with VR interruptions, especially in ventilation status, mobility, and cardiac symptoms. Changes in clinical outcome measures after CBT were not associated with the presence of VR interruptions.

Conclusions

The presence of VR interruptions is associated with a later onset of the disease and a milder phenotype. However, based on the OPTIMISTIC trial data, the presence of VR interruptions was not associated with significant changes on outcome measures after CBT intervention.

Glossary

6MWT = 6-minute walking test; **BDI-Fs** = Beck Depression inventory—fast screen; **CBT** = cognitive behavioral therapy; **CIS** = Checklist Individual Strength; **DM1** = myotonic dystrophy type 1; **ePAL** = estimating the progenitor allele length; **MDHI** = Myotonic Dystrophy Health Index; **PROM** = patient-reported outcome measure; **SAP** = statistical analysis plan; **VR** = variant repeat.

Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited, clinically heterogeneous chronic progressive multisystem disorder caused by an expansion of a highly unstable CTG repeat in the *DMPK* gene. Overall, the age at onset and the severity of symptoms are linked to the number of inherited CTG repeats,[1,2] as conventionally analyzed by Southern blot hybridization of restriction-digested genomic blood DNA.[3] Using these methods, the phenotype-genotype correlation is rather weak and only explains the age at onset in approximately 20%–40% of the variation in age at onset.[3–6] However, such measures of the average CTG repeat length fail to take account of the age-dependent somatic expansion observed in the blood DNA,[7–9] and estimating the inherited or progenitor allele length (ePAL, the lower boundary of the expanded allele distribution) using small-pool PCR[8] has been shown to explain much more of the variability in age at onset (~70%).[3] These data also revealed that the degree of somatic instability is an individual determinant of the age at onset in DM1.[3] Furthermore, large cohort studies have shown that the presence of variant repeats (VRs) interrupting the *DMPK* CTG expansions reduces somatic instability and results in a later age at onset and delayed progression.[10–14] VRs have been identified in 3–11% of patients with DM1 across various different ethnicities.[10,14,15] VRs are located either at the 3′ or 5′ ends of *DMPK* expansions, and although CCG, CTC, GGC, and CAG interruptions have been identified, CCG VRs are, by far, the most common.[10–12,15–18] In 2018, Cumming et al.[19] described a later onset of the disease and a milder clinical phenotype in patients with DM1 with VRs in the OPTIMISTIC cohort.

As part of the OPTIMISTIC trial (NCT02118779[20]), we performed an additional analysis on the association between the presence of VR interruptions and clinical symptoms, sociodemographic characteristics, and outcome measures after cognitive behavioral therapy (CBT) intervention. In the OPTIMISTIC study, we previously demonstrated that most outcome measures significantly improved after CBT.[21] In this trial, CBT was based on a previously constructed DM1 specific model of health status. Major determinants of health status in this model are: chronic fatigue, reduced initiative, and weak social interactions. Patients in the CBT group were assigned to a CBT-trained psychologist. After initial assessment of the main neuropsychological symptoms, an individual overall CBT goal was created by shared decision-making and using specific tools and modules (goal setting, getting started, sleep-wake pattern, activity, helpful beliefs, pain, and optimizing social interactions). Treatment sessions were conducted in person and by telephone on an individual frequency between baseline (V2) and month 10 (V4).[21]

Methods

Protocol and Patient Population

Data analyzed in this work were collected within the OPTIMISTIC trial, a multicenter study on the "Observational Prolonged Trial in Myotonic Dystrophy Type 1 to Improve Quality of Life—Standards, a Target Identification Collaboration." The study purpose of the OPTIMISTIC trial, methods, patient selection and characteristics, and outcome measures were previously described.[20,21] In summary, adult patients with DM1 aged older than or equal to 18 years with severe fatigue, based on a Checklist Individual Strength (CIS)-Fatigue score ≥35 at the screening visit (V1), were enrolled in this 2-arm, multicenter, randomized controlled study to evaluate the effectiveness of CBT on clinical outcome measures. The rationale for this longitudinal study is the model of perpetuating factors for fatigue in patients with DM1.[22] For patients who were not able to provide historical genetic test results verifying a CTG repeat expansion at the screening visit, we performed an additional analysis after informed consent to provide the genetic test result for the presence of a CTG repeat expansion at the baseline visit (V2). In total, 255 patients consented to participate in this study and were enrolled at 4 different centers between April 2014 and May 2015 in the Netherlands, France, Germany, and the United Kingdom. At the baseline visit, the patients were assigned to either the control group or the intervention group 1:1, followed by 5-month (V3), 10-month (V4), and 16-month (V5) visits. CBT intervention was performed in patients assigned to the intervention group between V2 and V4. Disease-related history was obtained by historical medical reports and specific questionnaires filled out at screening or baseline visits by the patient and the investigator. At each visit, functional assessments, including muscular impairment rating scale and the 6-minute walking test (6MWT), were performed. In addition, disease- and health-related questionnaires (patient-reported outcome

Table 1 Demographic Characteristics of Patients With (VR+) and Without Variant Repeats (VR−)

	Missing information	VR+ (n = 21 [8.4%]); 13 women (61.9%)				VR− (n = 229 [91.6%]); 103 women (45%)				
		n	Mean	Median	SD	n	Mean	Median	SD	*p* Value
Age at first med. Problem	25 (10.0%)	18	31.5	29.5	14.8	207	24.8	23.0	13.2	**0.043**
Age at diagnosis	26 (10.4%)	19	33.4	30.0	15.5	205	37.1	36.0	12.9	0.248
Duration of the disease	25 (10.0%)	18	13.6	11.8	8.2	207	20.7	20.4	10.3	**0.035**
Age at baseline visit	0 (0.0%)	21	44.5	43.8	13.0	229	45.9	46.5	11.5	0.609
Inheritance	96 (38.4%)	16	6 (37.5%) maternal			138	47 (34.1%) maternal			0.164

Data were normally distributed; group differences were assessed by a 2-sided *t* test. Bold values indicate significant difference for *p* ≤ 0.05.

measures, PROMs) were obtained, including the primary outcome measure DM1-Activ scale, reflecting as a tool to assess activity and participation in patients with DM1 and health status and cognitive-behavioral measures, as described previously.[20,21] Although the OPTIMISTIC study covered a total of 16 questionnaires, 3 motor function assessments, and 2 cognitive tests, this analysis focused on 6 core outcome measures typically used in the clinical trials and applied in daily clinical practice. Blood samples for DNA testing were obtained at the baseline visit (V2) for determining CTG length by small-pool PCR, i.e., estimated progenitor CTG repeat length (ePAL), modal CTG repeat length, and the presence of VRs (VR+), using digestion with AciI. Patient's samples were shipped to the Newcastle
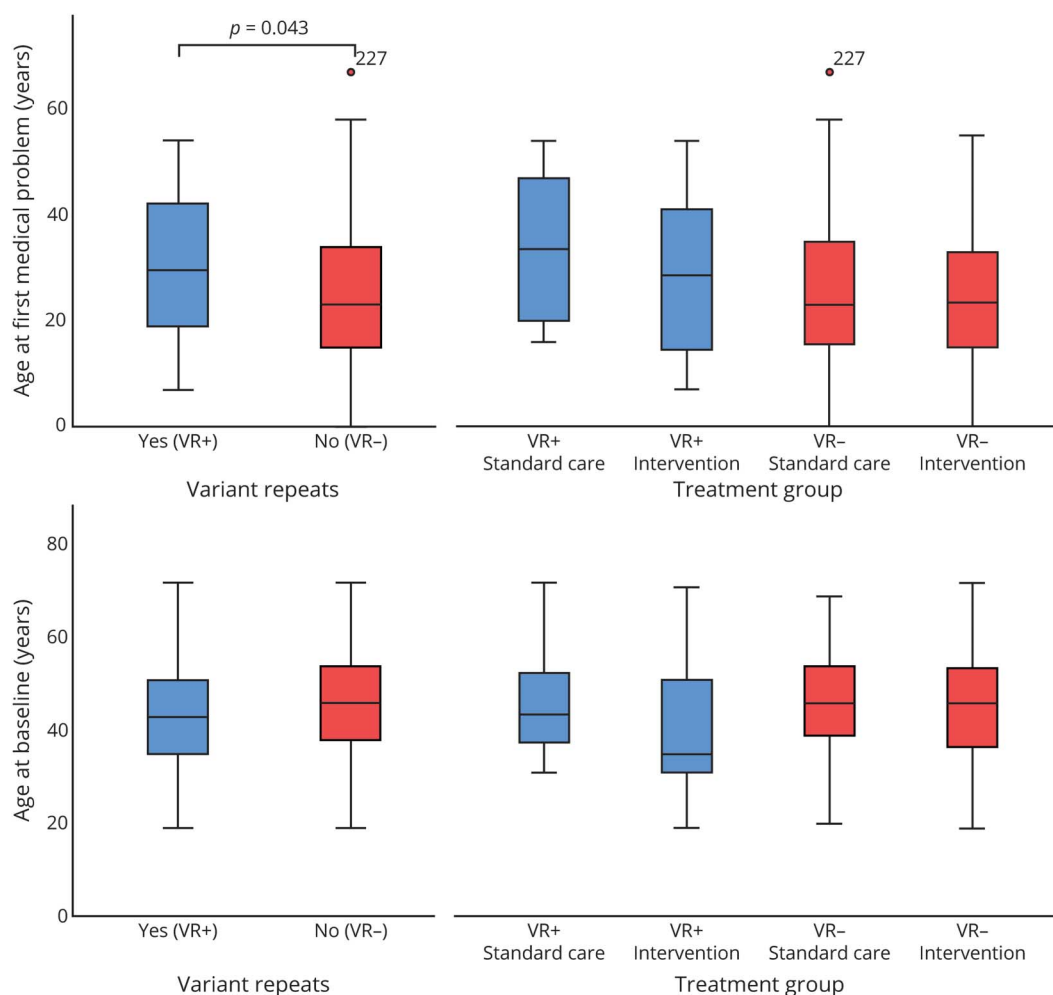
Figure 1 Age at Symptom Onset and at Baseline in Patients With Variant Repeats (VR+) and Without Variant Repeats (VR−)

Table 2 Baseline Clinical Characteristics of the Study Cohort

Baseline characteristics	VR+ (n = 21 [8.4%]), n (%)	VR− (n = 229 [91.6%]), n (%)
BMI, median ±SD	25.4 ± 4.4	26.4 ± 6.8
MIRS		
1	1 (4.8)	7 (3.1)
2	6 (28.6)	38 (16.6)
3	9 (42.9)	82 (35.8)
4	5 (23.8)	95 (41.5)
5	—	7 (3.1)
Myotonia		
Mild	12 (57.1)	138 (60.3)
Severe	5 (23.8)	59 (25.8)
Swallowing problems	14 (66.7)	124 (54.1)
Cataracts	6 (28.6)	75 (32.8)
Fatigue		
Mild	9 (42.9)	87 (38.0)
Severe	12 (57.1)	128 (55.9)
Depression—not severe[a]	4 (19.0)	44 (19.2)
Ventilation		
NIV part-time	1 (4.8)	37 (16.2)
NIV full-time	—	—
TIV	—	1 (0.4)
Mobility (>1 yes possible)		
Walk unaided	20 (95.2)	183 (79.9)
Walk with device	1 (4.8)	46 (20.1)
Wheelchair use part-time	2 (9.5)	35 (15.3)
Cardiac symptoms		
Cardiomyopathy	1 (4.8)	5 (2.2)
Arrhythmia/conduction block	4 (19.0)	71 (31.0)
Not further specified	2 (9.5)	6 (2.6)
Presence of pacemaker	1 (4.8)	34 (14.8)
Presence of cardioverter/defibrillator	—	7 (3.1)
Presence of any pacemaker, not specified	—	5 (2.2)

Abbreviations: BMI = body mass index; MIRS = muscular impairment rating scale; NIV = noninvasive ventilation; TIV = tracheostomy invasive ventilation; VR = variant repeat.
Data are presented as numbers and % of the subgroup.
[a] Clinically relevant depressive symptoms, not severe; patients with severe depression at baseline (Beck Depression inventory—fast screen score ≥4) were not enrolled.

Research Biobank for Rare and Neuromuscular Diseases, and the genetic analysis was performed at the University of Glasgow. Detailed methods for detecting VRs are described in.[19] Participants with missing data were excluded from relevant analyses.

Standard Protocol Approvals, Registrations, and Patient Consents

The study was approved by the institutional review boards at each of the 4 clinical sites (National Research Ethics Service Committee North East—Sunderland, UK, the Comite de

Figure 2 Muscular Impairment Rating Scale (MIRS) at Baseline and Visit 4 in Patients With Variant Repeats (VR+) and Without Variant Repeats (VR−) in % of the Subgroup
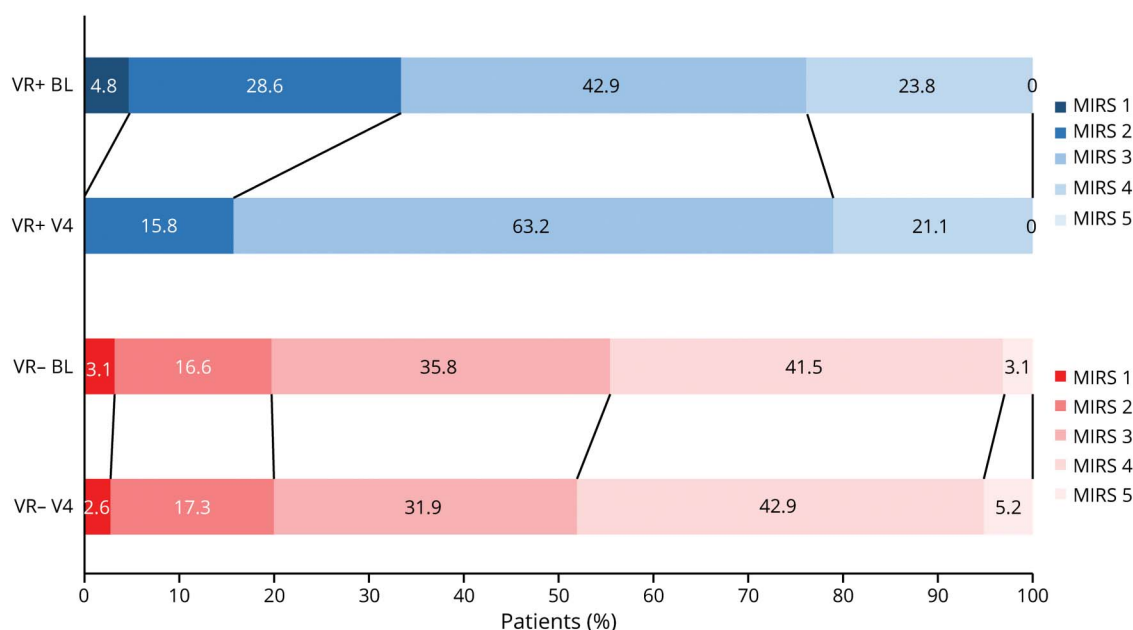
Statistical Analysis

In this additional analysis, we assessed the association between repeat interruptions and clinical symptoms, sociodemographic characteristics, and outcomes of CBT intervention in our OPTIMISTIC cohort. For this, we performed a subgroup analysis on patients with and without VRs (VR+/VR−) and their assignment to the intervention/control group. We ran descriptive and exploratory analysis, where applicable. We tested the data for normal distribution using Shapiro-Wilk tests. We used the 2-sided t test, Pearson χ^2 test, Mann-Whitey U test, or Wilcoxon-rank-sum test, as appropriate. For statistical analysis, we used SPSS Statistics 25 and Microsoft Excel 2016. Significance level (α) was set at 0.05. Because of the small number of patients with VRs and the clear imbalance of patient numbers across the subgroups, we focused on descriptive and group comparison analysis only.

Data Availability

The deidentified participant data are available on reasonable request from the study director Dr. Baziel G.M. van Engelen (baziel.vanengelen@radboudumc.nl). The study protocol and statistical analysis plan (SAP) are available online.[20] The SAP was publicly available before completion of the study.

Results

Variant Repeats

Genetic data on VRs were available from 250 of 255 patients from the OPTIMISTIC cohort (98%). VRs (VR+) were detectable in 21 of 250 patients (8.4%), as described.[19] Divided into subgroups, VRs were detectable in 12 patients in the standard-of-care group (control group) and 9 patients in the intervention group. The frequency of VRs in the whole cohort was 8.4%[19] and was similar in both the intervention (7.03%) and the natural history groups (9.83%; $p = 0.425$).

Demographic and Clinical Baseline Characteristics

Demographic and baseline characteristics are summarized in table 1. Patients with VRs were significantly older (mean 6.7 years) when the first medical problem occurred (table 1, figure 1, $p = 0.043$). Furthermore, patients with VRs had a significantly shorter duration of the disease at baseline ($p = 0.035$). In about 2/3 of patients, inheritance was paternal in both groups. The clinical baseline characteristics are summarized in table 2. We found a tendency toward a milder disease severity in patients with VRs, especially in ventilation status, mobility, and cardiac symptoms (table 2, figure 2). Significantly less patients with VRs had any type of ventilation at baseline, underscoring the milder VR+ phenotype. Further analysis did not reveal any significant difference between the groups (e.g., VR+ treatment group vs VR− standard of care group).

Medical History: First Symptoms

First symptoms were primarily core muscular symptoms myotonia and muscle weakness in 2/3 of the patients in both

Table 3 First Symptoms Recalled by the Patients in Medical History

	VR+ (n = 21 [8.4%]); 13 women (61.9%)	VR− (n = 229 [91.6%]); 103 women (45%)	
	N (%)	N (%)	*p* Value
Muscle symptoms	16 (76.2)	181 (79.0)	0.417
Muscle cramps/stiffness in hands	10 (47.6)	110 (48.0)	
Muscle cramps/stiffness in jaw or tongue	1 (4.8)	—	
Muscle pain	—	10 (4.4)	
Muscle weakness	4 (19.0)	48 (21.0)	
Swallowing problems	—	3 (1.3)	
Dysarthria	1 (4.8)	6 (2.6)	
Ptosis	—	2 (0.9)	
Dyspnoea	—	1 (0.4)	
Neuropsychological	5 (23.8)	24 (10.5)	0.068
Cognitive problems	—	1 (0.4)	
Daytime sleepiness	4 (19.0)	17 (7.4)	
Fatigue	1 (4.8)	6 (2.6)	
Cardiac symptoms	—	4 (1.7)	
Gastrointestinal symptoms	—	1 (0.4)	
Cataracts	—	17 (7.4)	
Other	—		n.a.
Include: voice tone dropped, pain in knees	—	2 (0.9)	

Abbreviations: n.a. = not applicable; VR = variant repeat.
Group differences were calculated by Pearson χ^2 tests.

groups (table 3). There were no significant differences between the 2 groups as to the nature of the first symptoms (i.e., neuromuscular vs neuropsychological).

Association Between VR Interruptions and Intervention Outcomes

We analyzed the association between VR interruptions and core outcome measures after CBT, which included the motor function assessment 6MWT and the PROMs DM1-Activ-C, the CIS, subscore fatigue, the Myotonic Dystrophy Health Index (MDHI), the McGill-Pain questionnaire, and the Beck Depression Inventory—fast screen (BDI-Fs). In patients with VRs (VR+), a significant change between baseline (V2) and after 10 months (V4) was only found in the CIS-Fatigue score. In the VR− group, significant changes between baseline and V4 were found for the 6MWT only in the intervention group and in the CIS-Fatigue score and MDHI score in both groups (table 4, figure 3). Effect size *r* for significant differences between V2 and V4 was high (>0.5) for CIS-fatigue in the VR+ CBT group (*r* = 0.728), moderate (0.3–0.5) for the CIS fatigue in the VR− standard care and intervention group, and low (<0.3) for MDHI in the VR− standard care group and intervention group. Except

for MDHI in the VR− standard-of-care group, the results of significant changes remained valid after Bonferroni correction for multiple testing and setting the significance level to <0.008. There was no significant difference in any outcome assessment between patients with and without VRs.

Discussion

This is the first analysis on the association between VR interruptions and core clinical symptoms, sociodemographic characteristics, and treatment effects of CBT in a large cohort of patients with myotonic dystrophy type 1. This additional analysis was performed on data collected in the OPTIMIS-TIC trial.[20,21] Of 255 patients enrolled, data on VR interruptions were available from 250 patients. The frequency of VRs in the whole cohort was 8.4%[19] and was similar in both the intervention and the natural history groups, which is within the rate of VR interruptions previously described in different cohorts.[10,11,14] Overall, our additional analysis revealed that the presence of VRs resulted in a later onset of the disease and a tendency to a milder severity of the disease at baseline assessments, which is consistent with previous studies

Table 4 Outcome Measures in the 4 Groups

	S*	Standard care group (n = 122)								p Value[b] VR+/VR− group
		VR− (n = 110)				VR+ (n = 12)				
		Mean V2	Mean V4	Mean Δ, %	p Value[a] change V2/V4	Mean V2	Mean V4	Mean Δ, %	p Value[a] change V2/V4	
6MWT, m	H	398.1	400.4	1	0.982	465.6	438.0	−6	**0.041**	0.221
DM1-Activ-c	H	61.9	60.4	−2	0.306	73.8	67.3	−10	0.221	0.291
CIS-fatigue	L	45.2	40.4	−12	**<0.001; r = 0.307**	42.8	43.0	0	0.833	0.058
MDHI	L	36.8	33.0	−12	0.010	30.8	31.0	1	0.575	0.886
McGill-pain	L	30.9	28.7	−8	0.828	29.8	35.3	16	0.514	0.597
BDI-Fs	L	4.1	3.6	−14	0.167	3.8	3.7	−3	0.916	0.680

	S*	Intervention group (n = 128)								p Value[b] VR+/VR− group
		VR− (n = 119)				VR+ (n = 9)				
		Mean V2	Mean V4	Mean Δ, %	p Value[a] change V2/V4	Mean V2	Mean V4	Mean Δ, %	p Value[a] change V2/V4	
6MWT, m	H	386.1	417.6	8	**<0.001; r = 0.119**	426.1	454.8	6	0.515	0.744
DM1-Activ-c	H	61.1	63.8	4	0.241	62.2	65.1	4	0.373	0.535
CIS-fatigue	L	45.0	36.9	−22	**<0.001; r = 0.417**	44.0	29.3	−50	**0.008; r = 0.728**	0.057
MDHI	L	37.9	32.1	−18	**<0.001; r = 0.153**	36.8	28.4	−30	0.086	0.567
McGill-pain	L	30.9	26.9	−15	0.628	26.1	22.6	−15	0.397	0.382
BDI-Fs	L	4.2	4.1	−2	0.311	6.0	4.0	−50	0.127	0.187

Abbreviations: 6MWT = 6-minute walking test; BDI-Fs = Beck Depression inventory—fast screen score; CIS-fatigue = checklist individual strength score, subscore fatigue; McGill-pain = McGill-Pain Inventory fast screen score; MDHI = Myotonic Dystrophy Health Index score; S* = score direction: H = higher values indicate improvement; L = lower values indicate improvement; VR = variant repeat.
All data were not normally distributed.
[a] Wilcoxon test was used for the comparison of outcomes at 2 time points.
[b] Mann-Whitney test was used for the comparison of change (V2 to V4) in outcome measures between the VR+ and VR− groups. Bold values: significant difference for $p \leq 0.008$.
Effect size r is provided for p values ≤0.008.

and our first analysis of the impact of VR interruptions on disease severity in patients with DM1.[3,11,12,14,19] However, the presence of VRs was not associated with significant changes in clinical outcome measures after CBT intervention. Although the limited power because of the small number of patients in the VR+ group has to be considered, our results may have a major influence on the interpretation of outcome measures in future clinical trials. Despite the later onset of symptoms in these patients, we did not identify significant differences in baseline characteristics, socio-demographic characteristics, or the occurrence of first symptoms. However, we could observe some tendencies toward a milder disease severity in patients with VRs, based on historic and current medical data on cardiac symptoms, mobility, and the use of assistive devices and ventilation status. Evaluating clinical symptoms and assessments at baseline relative to ePAL and age, patients with VRs tended to have, on average, a milder affected phenotype in several different domains.[19] Subgroup analysis has to be interpreted with care because of the low number of patients

with VRs. Where assessable, outcome measures showed an improvement in the 6MWT for patients with VRs in the standard-of-care group with low effect size, but not in the intervention group. A significant change between baseline and 10-month visit after CBT was found for the CIS-fatigue in the intervention group both for VR+ and VR− patients but without a significant difference between the 2 subgroups. For CIS-fatigue, effect size was high in the VR+ intervention group ($r = 0.728$) and moderate in the VR− intervention and in the standard-of-care group, respectively ($r = 0.417$ and $r = 0.307$). For MDHI, a significant change was only detectable in patients without VRs both in the standard care and intervention groups with a low effect size.

One of the limitations of this analysis is the imbalance in patient numbers between subgroups, especially the low number of patients in the VR+ CBT group, so that group comparison analyses should be interpreted with care to avoid a statistical type II error. Because of the OPTIMISTIC protocol, a

Figure 3 Core Outcome Measures for the 4 Subgroups for the 6MWT (A), DM1-Activ-c Score (B), Checklist Individual Strength, Subscore Fatigue (CIS-Fatigue) Score (C), and the Myotonic Dystrophy Health Index (MDHI) (D)
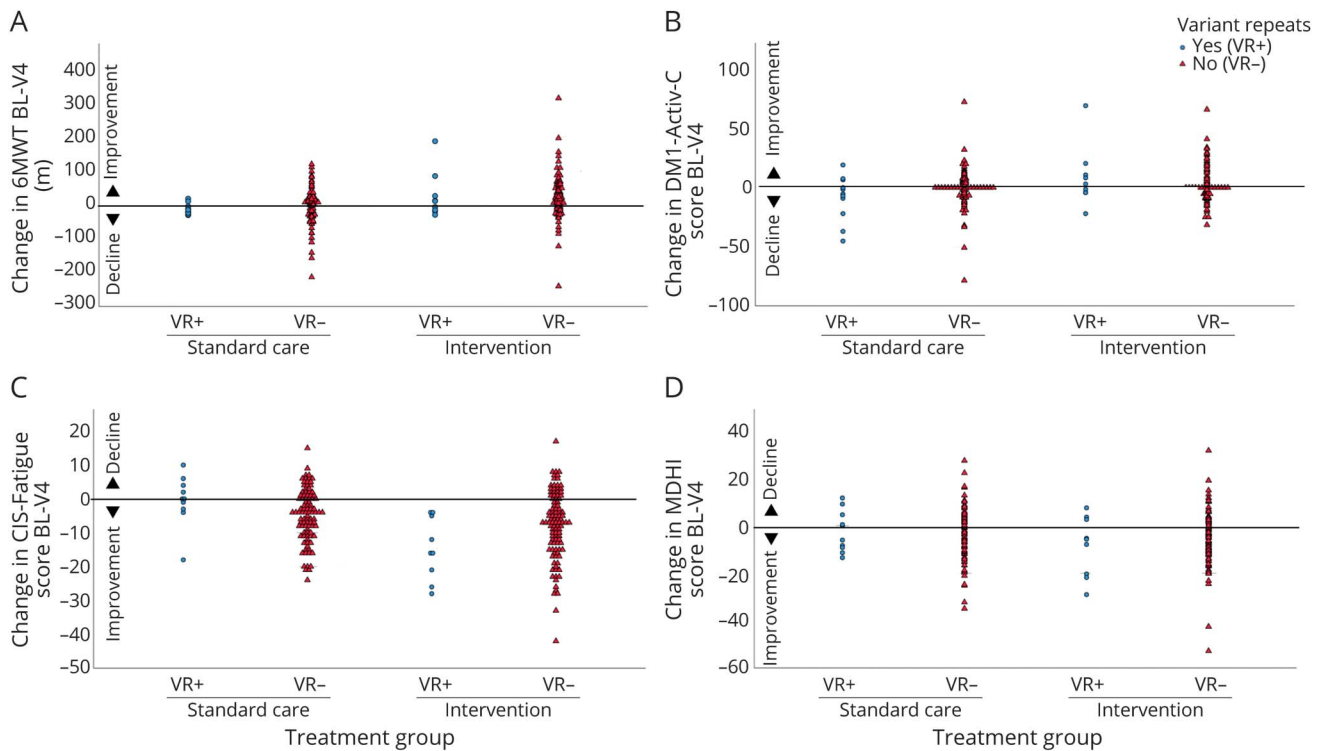
Higher values in 6MWT and DM1-Activ-c, and lower values in CIS-fatigue and MDHI indicate improvement. 6MWT = 6-minute walking test; DM1 = myotonic dystrophy type 1.

selection bias was predetermined because only patients with severe fatigue were enrolled (based on a CIS-fatigue score ≥35). Furthermore, patients aged younger than 18 years and patients with severe depression at baseline (BDI-Fs score ≥4) were not included in the study.[20]

In summary, based on data collected from this study cohort, the presence of VRs has no detectable effect on outcomes after CBT treatment. However, according to our data, the presence of VRs suggests a later onset of the disease and is associated with a milder phenotype. To date, it remains unclear whether the presence of VR interruptions in the DMPK gene is associated with improvement of clinical symptoms after investigational therapy. Further studies will investigate the effects of VR interruptions, in which case the percentage of VR+ and VR− patients should be balanced between the intervention and control arms of the trial.

Publication History

Appendix 1 Authors

Name	Location	Contribution
Stephan Wenninger, MD	Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany	Patient acquisition and examination, acquisition of data, statistical analysis and interpretation of data, first manuscript draft, critical revision of the manuscript for intellectual content, and final manuscript draft
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 acquisition of genetic data, analysis and interpretation of data, and critical revision of the manuscript for intellectual content
Kristina Gutschmidt, MD	Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany	Acquisition of clinical data and critical revision of the manuscript for intellectual content

Appendix 1 *(continued)*

Name	Location	Contribution
Kees Okkersen, MD	Radboud University Medical Center, Donders Institute of Brain, Imaging and Cognition, Nijmegen, The Netherlands	Major role in recruitment of participants and acquisition of biological samples and clinical data and critical revision of the manuscript for intellectual content
Aura Cecilia Jimenez-Moreno, PhD	University of Newcastle, United Kingdom	Major role in recruitment of participants and acquisition of biological samples and clinical data and critical revision of the manuscript for intellectual content
Ferroudja Daidj, MSc	Neuromuscular Reference Centre, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, France	Major role in recruitment of participants and acquisition of biological samples and clinical data and critical revision of the manuscript for intellectual content
Hanns Lochmüller, MD	Children's Hospital of Eastern Ontario Research Institute; Division of Neurology, Department of Medicine, The Ottawa Hospital; and Brain and Mind Research Institute, University of Ottawa, Ontario, Canada	Coordination of DNA sample collection, clinical study design, and critical revision of the manuscript for intellectual content
Fiona Hogarth, PhD	Tayside Clinical Trials Unit, University of Dundee, United Kingdom	Major role in acquisition of clinical data, discussion of results, and critical revision of the manuscript for intellectual content
Hans Knoop, MD	Medical Psychology of the Amsterdam University Medical Centers, University of Amsterdam, The Netherlands	Supervision of recruitment, data and sample acquisition and clinical study design, and critical revision of the manuscript for intellectual content
Guillaume Bassez, MD	Neuromuscular Reference Centre, Pitié-Salpêtrière Hospital, Assistance Publique-Hôpitaux de Paris, France	Supervision of recruitment, data and sample acquisition and clinical study design, and critical revision of 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	Genetic study concept and design, study supervision, data analysis, and critical revision of the manuscript for intellectual content
Baziel G.M. van Engelen, MD	Department of Neurology, Donders Institute for Brain Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands	Supervision of recruitment, data and sample acquisition, and the overall study, clinical study design, and critical revision of the manuscript for intellectual content
Benedikt Schoser, MD	Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany	Supervision of recruitment, data and sample acquisition and clinical study design, and critical revision of the manuscript for intellectual content

Appendix 2. Coinvestigators

Coinvestigators are listed at links.lww.com/NXG/A398.

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