Neuromuscular Disorders 32 (2022) 893–902

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Masseter muscle volume as a disease marker in adult-onset myotonic dystrophy type 1

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ARTICLE INFO

Article history:
Received 24 February 2022
Revised 21 August 2022
Accepted 20 September 2022

Keywords:
Myotonic dystrophy type 1
MRI
Masseter muscle
Outcome measures

ABSTRACT

The advent of clinical trials in myotonic dystrophy type 1 (DM1) necessitates the identification of reliable outcome measures to quantify different disease manifestations using minimal number of assessments. In this study, clinical correlations of mean masseter volume (mMV) were explored to evaluate its potential as a marker of muscle involvement in adult-onset DM1 patients. We utilised data from a preceding study, pertaining to 39 DM1 patients and 20 age-matched control participants. In this study participants had undergone MRI of the brain, completed various clinical outcome measures and had CTG repeats measured by small-pool PCR. Manual segmentation of masseter muscles was performed by a single rater to estimate mMV. The masseter muscle was atrophied in DM1 patients when compared to controls ($p<0.001$). Significant correlations were found between mMV and estimated progenitor allele length ($p = 0.001$), modal allele length ($p = 0.003$), disease duration ($p = 0.009$) and and the Muscle Impairment Rating Scale ($p = 0.008$). After correction for lean body mass, mMV was also inversely correlated with self-reported myotonia ($p = 0.014$). This study demonstrates that changes in mMV are sensitive in reflecting the underlying disease process. Quantitative MRI methods demonstrate that data concerning both central and peripheral disease could be acquired from MR brain imaging studies in DM1 patients.

1. Introduction

Myotonic dystrophy type 1 (DM1, OMIM 160,900) is an autosomal dominant condition resulting from expansion of a CTG repeat in the 3′-untranslated region of the *DMPK* gene (OMIM 605,377) [1–4]. DM1 is characterized by variable multi-system involvement [5–7], where apart from skeletal muscle involvement, the most commonly affected systems include the central nervous system, endocrine system and smooth and cardiac muscle, among others [7]. There is a broad positive correlation between allele size and disease severity [8], though residual variation is broad, and other factors including presence of variant repeats also affect the expression of the disease phenotype [9,10].

To date, no disease-modifying therapies for DM1 are available for routine clinical use. Nonetheless, recent advancements in the understanding of its molecular mechanisms have allowed for the design of candidate therapeutics [11], which are beginning to reach the stage of clinical trials in humans [12]. In this context, the need to identify objective, quantitative outcomes measures of DM1 symptoms to assess the effects of interventions has become a major research priority.

The challenges of quantitative measurement of skeletal muscle involvement are well-recognised in DM1. The Outcome Measures in Myotonic Dystrophy type 1 Consortium (OMMYD1) [13–15] highlight that available studies focus on muscle strength in the lower limbs, but the results are variable and additional factors affecting muscle involvement are often not well-accounted for (e.g. subtypes of DM1, or disease duration). Their current recommendations are to use QMT (Quantitative Muscle Testing, using a dynamometer) and MMT (Manual Muscle Testing, a grade

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https://doi.org/10.1016/j.nmd.2022.09.005

based on clinical examination) methods, although these clinical outcome measures have inherent limitations including intra- and interrater variability [16,17]. Additionally, MMT might not be sensitive enough to detect smaller changes over time [14] and it is an ordinal scale with no meaningful interpretation of the difference between its scores, and so would very likely require a large sample size to detect an effect of the intervention [15,18].

Recently, magnetic resonance (MR) imaging has been extensively used to assess brain involvement in DM1 [19–25], which may lead to the development of more reliable outcome measures of CNS involvement. However, challenges likewise remain for the identification of CNS outcome measures, in that structure-phenotype relationships are yet to be consistently replicated across studies (reviewed in [26]), and most have had a cross-sectional design (with limited exceptions [27]), and so natural history of changes over time is poorly defined.

With respect to muscle MR imaging in DM1, most studies focus on assessment of lower limb muscles [28,29]. Characteristics of interest include the extent of fat infiltration and loss of muscle volume [30]. Muscle volume, together with muscle shape and structure, are related to its function, including capacity to generate force [31,32]. In fact, a strong correlation was found between MRI biomarkers of tibialis anterior and ankle dorsiflexor strength, confirming muscle imaging measures may meaningfully reflect function [33]. People affected by DM1 frequently exhibit a characteristic, 'myopathic' facial appearance, with ptosis and facial muscle wasting, predominantly affecting the masseter and temporalis muscles [6,7,34]. Masseter muscle involvement may be detectable before overt onset of DM1 symptoms, evident on CT imaging [35], electromyography [36–38] or ultrasound imaging [39]. In contrast, the temporalis muscle is more difficult to quantify (as volume or cross-sectional area) on CT or MR imaging. The clinical impact of masseter wasting includes weak bite and chewing force, which may contribute to functional disability and risk of aspiration [6], which is one of the most common causes of mortality in DM1 patients [40].

To date, no MRI studies have focused solely on masseter muscle involvement in DM1 patients. One study of masticatory muscles in 15 patients showed fat infiltration or reduced muscle volume in 13 participants [41] while another one showed decreased mean maximum masseter area in 10 DM1 patients compared to five controls [42]. No correlations with phenotypic or genetic determinants of disease severity were attempted in these studies.

The objective assessment of clinical outcomes in clinical trials in DM1 is likely to require the participant to undergo a battery of different investigations, which due to the nature of the condition might be poorly tolerated. Given the potential use of brain MRI for assessment of CNS involvement, we hypothesised that the same imaging data could also be used to identify quantitative biomarkers of muscle involvement, therefore maximizing the value of this uncomfortable yet non-invasive investigation. The masseter muscle was chosen because of its common and early involvement, contribution to significant disability, and ease of identification on MR images. Additionally, it is predicted that there will be less inter-patient variability in masseter muscle volumes than in the most thoroughly researched lower limbs muscles, since these muscles must be inevitably used daily for eating, and so will be less affected by individual variations in physical exercise habits.

This study therefore aims to evaluate the volume of the masseter muscle in adult-onset DM1 patients and unaffected controls. It is hypothesized that the volume will be decreased in DM1 patients and will correlate with the genotypic determinants of disease severity (e.g. modal allele length (MAL)), disease duration and functional measures of general muscle involvement as well as other phenotypic features of the disease.

2. Methods

2.1. Patients, Study design & MRI acquisition

The imaging data used in this study was collected as part of the DM1-Neuro study, as previously described [21]. Briefly, 45 DM1 patients (19 males, 26 females) with adult onset DM1 and 20 age-matched unaffected controls (12 males, 8 females) were recruited and those without contraindications (all controls and 17 male and 22 female DM1 participants) underwent MR imaging of the brain. As the study aimed to recruit patients with onset of DM1 symptoms in adulthood, the exclusion criteria included unequivocal presence of DM1-specific symptoms before age 16 years or learning disability diagnosed in childhood. Imaging was carried out on a 3T Siemens Prisma MRI scanner (Software version: VE11B. Erlangen, Germany) with a 20-channel head and neck coil. The original study from which data was used in this work has undergone ethical review (West of Scotland Research Ethics Committee; 15/WS/0189).

DM1 patients additionally completed a clinical evaluation, comprising self-reported symptom questionnaires (Myotonic Dystrophy Health Index (MDHI), DM1-ActivC scale, Fatigue and Daytime Sleepiness Scale, Beck Depression Inventory and McGill Pain Scale) and a battery of neuropsychological evaluations (Stroop test [Golden and Freshwater© Stoelting Co. 2002], Trail Making Tests from the Delis-Kaplan Executive Function System [D-KEFS^{TM}] and the Block Design test from Weschler Abbreviated Scale of Intelligence [WASI-II] and the Edinburgh Cognitive and behavioral ALS Screen [ECAS], Appendix B). Muscle impairment rating scale (MIRS) scores were derived from electronic clinical records, relating to the patient's most closely contemporaneous annual medical review appointment.

Genotyping of the CTG repeat length was completed by small-pool PCR as previously described [43]. The lower boundary of the expanded molecules on gel electrophoresis was used to estimate the inherited, or "progenitor" allele length (ePAL), while the region of greatest band intensity represented the modal allele length (MAL). Samples were also screened for presence of variant repeats sensitive to the AciI restriction enzyme [10].

2.2. MR image segmentation

Manual segmentation of right and left masseter muscles was performed in ITK SNAP (ver. 3.8.0). T1-weighted MRI images were loaded into the software and axial view was chosen as optimal for segmentation of the masseter muscles. Raters, blinded to the DM1 status, delineated contours of each muscle on all slices where it was visible, which allowed for automatic estimation of the volume of the muscle based on the number of same-sized voxels present within the segmented space [44].

To compare the reliability and repeatability of the manual segmentations, three independent raters (AO, CH and MJH) performed segmentations of the left masseter muscle in four subjects (two controls and two DM1 patients).

2.3. Correction for lean body mass

Muscle mass is variable between people with different body build and volume of masseter muscles is likely to vary in accordance with variation in muscle mass [45]. Therefore, lean body mass (LBM) was estimated using the James formula [46]:

$$LBM(females)2 = 1.07 \times weight[kg] - 148 \times \left(\frac{weight[kg]}{height[cm]}\right)^2$$

$$LBM(males) = 1.1 \times weight[kg] - 128 \times \left(\frac{weight[kg]}{height[cm]}\right)^2$$

Subsequently, correction of mMV for eLBM was introduced in part of the analysis:

$$corrected\ masseter\ volume(CMV) = \frac{masseter\ volume(mMV)}{estimated\ lean\ body\ mass(eLBM)}$$

Normal or healthy ranges for LBM are rarely cited in the literature. However, more publications focus on the normal or healthy body fat percentage, which allows to calculate the normal LBM (as total body weight = body fat + lean body mass) [47]. Commonly quoted ranges for normal body fat percentage are around 5–25% for men and 10–35% for women [48,49], although they vary by age and ethnicity [50]. Therefore, a normative range for LBM would be approximately 80–95% of body weight in men and 70–92% in women.

2.4. Somatic instability

Somatic instability (SI) of the CTG repeat expansion was estimated as the difference between ePAL and MAL as previously described [51]:

$$Somatic\ instability(SI) = MAL - ePAL$$

2.5. Statistical analysis

All statistical analysis was performed using R (version 3.6.1) in R studio (version 1.2.5001). Log transformation of ePAL, MAL and SI were used as they better estimate a normal distribution and minimize the effects of extreme values, as described previously [51]. Microsoft Excel was used to produce some of the graphs.

2.5.1. Inter-rater reliability assessment

The reliability of segmentations among the three raters was assessed by comparing the resulting volumes using interclass coefficient (ICC) [52] calculated in R and spatial overlap using the Dice coefficient computed in Convert3D (https://sourceforge.net/p/c3d/git/ci/master/tree/doc/c3d.md).

2.5.2. Comparison of means

Comparison of means was performed using an independent samples *t*-test for variables which were normally distributed (considered as Shapiro-Wilk test $p < 0.05$). For data which was not normally distributed, in either or both groups, a non-parametric two-sample Wilcoxon test was performed (equivalent to Mann-Whitney *U* test).

2.5.3. Corrections for multiple testing

P-values below 0.05 were considered as statistically significant. Given that multiple comparisons of non-independent associations have been performed using linear regression, the Holm-Bonferroni correction of the *p*-values was calculated (https://www.statisticshowto.datasciencecentral.com/holm-bonferroni-method/).

3. Results

3.1. Participants' basic characteristics

Control participants were age- and sex-matched to DM1 patients (Table 1). Control males and females were on average overweight (BMI > 25), consistent with trends in the background population in Scotland [53]. Females with DM1 were also, on average, overweight which is consistent with previous studies [54]. There was no difference between their weight or BMI, and consequently eLBM, when compared to control females (Table 1, $p > 0.05$). Conversely, DM1 males had significantly lower weight and BMI than control males ($p = 0.002$ and 0.005, respectively). Although some of this difference may be due to slightly lower height in DM1 males ($p = 0.046$), their eLBM was still significantly lower than in male controls ($p = 0.002$), which is consistent with generalized muscle atrophy observed in DM1.

Table 1
Basic characteristics of the study participants by sex. ‡ - comparisons using Chi-squared test †- comparisons using two-sample Wilcoxon test, others – independent sample *t*-test. Significant p-values are in bold.

	Control	DM1	*p*-value
n	20	39	
Sex = Male (%)	12 (60.0)	17 (43.6)	0.358‡
Age (mean (SD)) [years]	46.05 (13.14)	47.28 (13.06)	0.734
Female	50.88 (15.04)	45.27 (12.95)	0.370
Male	42.83 (11.22)	49.88 (13.11)	0.133
Height (mean (SD)) [cm]	171.90 (9.83)	168.87 (7.69)	0.239
Female	162.00 (6.80)	164.55 (5.85)	0.368
Male	178.50 (4.34)	174.47 (6.05)	**0.046**
Weight (mean (SD)) [kg]	84.91 (18.95)	74.55 (14.95)	**0.041**
Female	71.61 (14.31)	73.96 (16.26)	0.708
Male	93.77 (16.60)	75.32 (13.51)	**0.002†**
BMI (mean (SD)) [m^2/kg]	28.53 (4.82)	26.12 (5.00)	0.081
Female	27.23 (4.92)	27.26 (5.69)	0.991
Male	29.39 (4.77)	24.65 (3.57)	**0.005†**
eLBM (mean (SD))	58.95 (11.55)	52.62 (7.96)	**0.036**
Female	46.91 (5.11)	48.05 (5.43)	0.622†
Male	66.97 (6.18)	58.54 (6.77)	**0.002**

Table 2
Most demographic characteristics of the DM1 patients by sex. ‡ - comparisons using Chi-squared test †- comparisons using Two-sample Wilcoxon test *U* test, others – independent sample *t*-test. Significant p-values are in bold.

DM1 patients			
	Female	Male	*p*-value
N	22	17	
Age at onset of symptoms (mean (SD)) [years]	29.90 (12.93)	32.27 (14.51)	0.619
Disease duration (mean (SD)) [years]	15.73 (9.34)	14.18 (11.24)	0.649
ePAL (mean (SD))	265.09 (113.92)	171.24 (85.22)	**0.006**
MAL (mean (SD))	569.14 (214.46)	329.24 (231.34)	**0.002**
SI (mean (SD))	304.05 (147.04)	158.00 (163.91)	**0.006†**
Presence of variant repeats (+) (%)	1 (4.5)	2 (11.8)	0.816‡

3.2. Demographic characteristics in DM1 patients

Females had significantly greater ePAL and MAL than males ($p = 0.006$ and $p = 0.002$, respectively), while age at symptom onset and disease duration were comparable. Three patients had detectable variant repeats (Table 2).

3.3. Inter-rater reliability of manual segmentation of the masseters

Excellent agreement was found between three independent raters in manually segmenting four masseter muscles with an ICC value of 0.94 (95% CI 0.71–1.00, $p < 0.001$) [55]. Dice coefficient, which is a spatial overlap metric, was also high with a mean of 0.873 (95% CI 0.852–0.894, Appendix C).

3.4. Correlation of right and left masseter volumes

There was overall a strong correlation between the volume of the right and left masseter muscle (Correlation coefficient: 0.965, 95% CI 0.941 – 0.979). However, the relationship was weaker and there was more variability in control participants overall, and both female control participants and female DM1 patients (Table 3, Appendix A, Appendix D) possibly reflecting more bite asymmetry in those groups (example in Fig. 1). The strong correlation in DM1 patients may suggest that atrophy occurs at a similar rate on both sides.

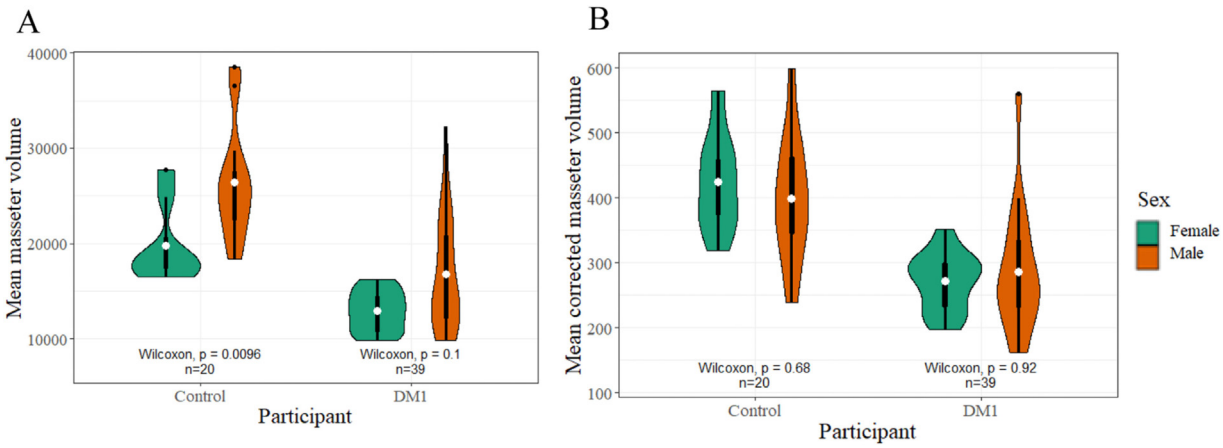

Fig. 1. Bite asymmetry
Example of bite asymmetry in a male control participant.

Table 3
Pearson correlation coefficient for the relationship between right and left masseter volumes in study participants. Significant *p*-values are in bold.

	n	Correlation coefficient (95% CI)	*p*-value
All	59	0.965 (0.941 – 0.979)	**< 2.2e-16**
DM1 patients	39	0.970 (0.942 – 0.984)	**< 2.2e-16**
Females	22	0.857 (0.681 – 0.939)	**3.61e-07**
Males	17	0.982 (0.949 – 0.994)	**3.14e-12**
Controls	20	0.919 (0.803 – 0.967)	**1.05e-08**
Females	8	0.834 (0.314 – 0.969)	**0.01005**
Males	12	0.930 (0.762 – 0.980)	**1.22e-05**

3.5. Mean & corrected masseter volumes

To minimize potential effect of bite asymmetry, the mean of left and right masseter volumes was used for further analysis. Consistent with generalized muscle atrophy observed with the condition, the mean masseter volume (mMV) was lower in DM1 patients than in the control participants (Table 4, $p < 0.001$) and the trend was maintained after correction for eLBM (Table 4, $p < 0.001$, Fig. 2A, Appendix D). Despite the larger absolute difference between DM1 patients and controls for males (Table 4), the larger variability in males resulted in the effect size of this difference being larger for females (Cohen's D, Table 4).

Regarding sex differences, it is well-established that females tend to have lower muscle mass, including masseter muscle volumes, than males (Appendix E.1) and this was confirmed in our study for control participants (Table 4, Fig. 2A). This effect was, however, not present for DM1 patients with the means for males and females being not significantly different from each other ($p = 0.100$), which, again, could suggest that masseter volume might decrease towards a similar minimum in both sexes. Another observation that supports this hypothesis is the fact that females had longer allele lengths than males (Table 2), i.e. despite their more severe disease, the mean volume was still not significantly different from males.

After correction for eLBM, which aimed to correct for different whole body muscle masses, differences in corrected mMV (cmMV) means between sexes in control participants were diminished (Fig. 2A,B), suggesting that the difference in mMV between the sexes is highly influenced by body build.

All three patients with variant repeats had lower mMV and cmMV than the average for the DM1 patients in the study and markedly lower volumes than closely age- and sex-matched DM1 patients without variant repeats and controls (Table 5), however none had any proximal muscle weakness (all MIRS≤3) or significant limitations in physical activity (DM1Activ score ≥88 centile).

3.6. Correlation of mMV in DM1 with their genetic and clinical measures

3.6.1. Univariate models

Univariate linear regression was performed to explore the relationships between mMV and genetic determinants of disease severity (ePAL, MAL) detailed clinical measures of disease severity and burden, neuropsychological tests and measures of brain involvement (detailed in Appendix B). Significant negative correlations were found between disease duration, log(MAL), log(ePAL), MIRS and mMV for the whole DM1 cohort (all $p < 0.05$, Table 6). Additionally, increasing mMV correlated with increasing ICV ($p = 0.002$, Table 5). Surprisingly, there was no relationship between mMV and age in either cohort (Table 5, Appendix F.2). However, most participants were of a similar age (IQR 37 – 57 years old).

Table 4
Mean masseter volumes and corrected masseter volumes in female and male DM1 patients and controls. †- comparisons using Two-sample Wilcoxon test *U* test, others – independent sample *t*-test. Significant *p*-values are in bold.

	Control	DM1	*p*-value	Effect size (Cohen's D)	% Difference between mean MVs
Masseter volume (mean (SD)) [mm^3]					
Mean	23,795.96 (6231.26)	14,614.94 (4699.50)	**< 0.001†**	1.66	38.6
female	19,826.86 (4169.75)	12,949.49 (2089.77)	**< 0.001†**	2.09	34.7
male	26,442.02 (6075.18)	16,770.22 (6162.08)	**< 0.001†**	1.58	36.6
Corrected masseter volume (mean (SD)) [mm^3/kg]					
Mean	408.67 (89.27)	277.49 (70.76)	**< 0.001†**	1.63	32.1
female	423.89 (78.12)	271.36 (43.58)	**< 0.001**	2.41	36.0
male	398.53 (97.98)	285.42 (96.33)	**< 0.001**	1.16	28.4

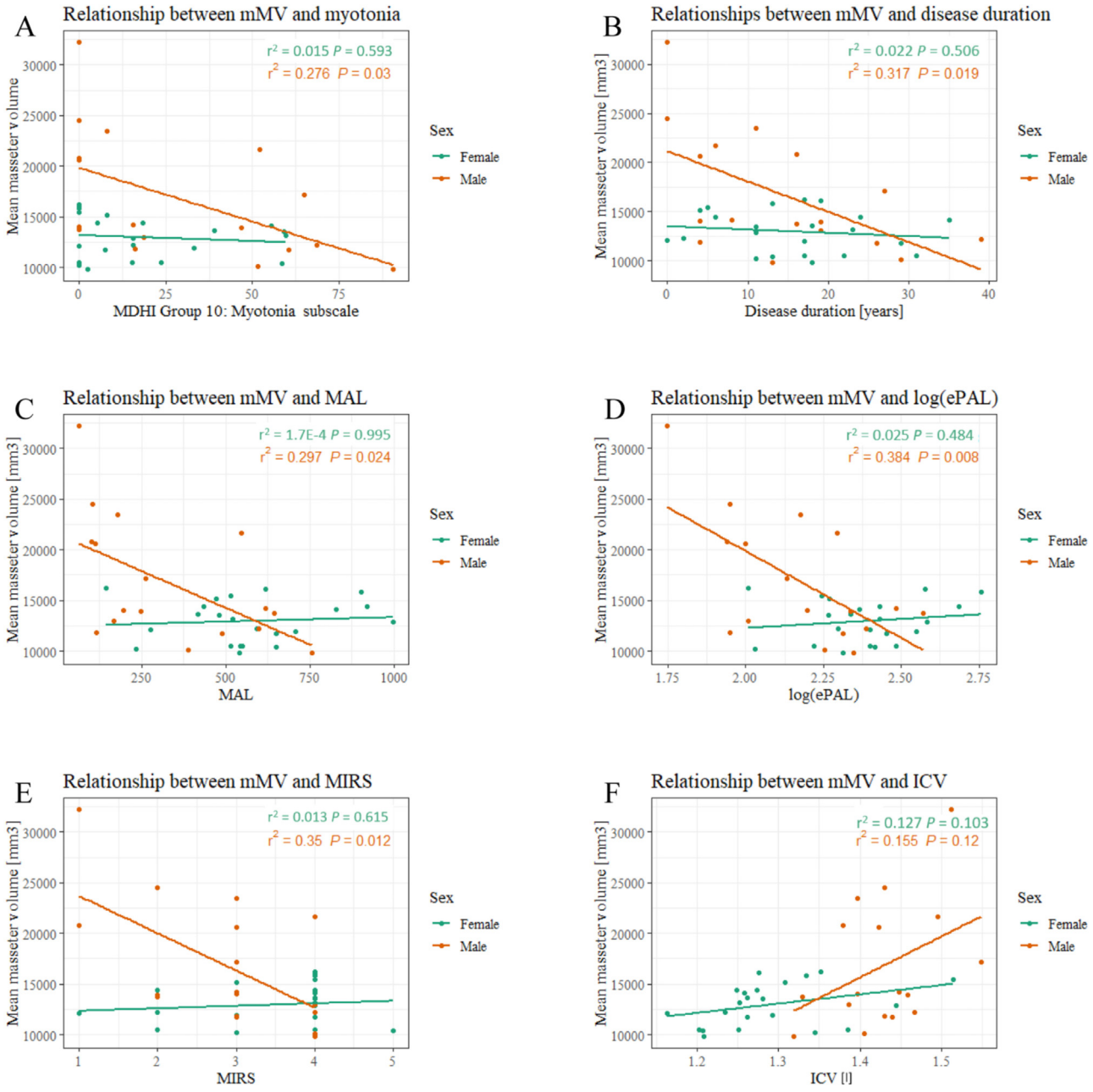

Fig. 2. Mean and corrected mean master volumes
mMV and cmMV in DM1 patients and control participants presented on violin plots by sex. Thin black bar indicates the range of values, the thick black bar indicates the interquartile range and the white dot the mean. (A) mMV (B) cmMV.

Table 5
Demographic characteristics and mean masseter volumes (mMV) of DM1 patients with variant repeats and age- and sex- matched DM1 patients without variant repeats and controls.

DM1					
Variant repeats (+)		Variant repeats (-)		Control	
Sex, Age	**mMV**	**Sex, Age**	**mMV**	**Sex, Age**	**mMV**
F, 22	11,802.2	F,23	20,858.7	F, 26	19,253.6
M, 33	13,962.5	M,31	23,479.5	M, 33	26,821.8
M, 36	14,047.3			M, 37	29,692.3

As previously explained, eLBM is known to significantly affect masseter volume, univariate linear regression was repeated with an additional term, eLBM, or using cmMV to correct for it (Appendix F.1). In both instances the results were similar, with the most notable difference being a significant association found with MDHI myotonia subscale ($p = 0.014$, $p = 0.018$, respectively), which quantifies this hallmark symptom of DM1.

3.6.2. Multivariate linear regression model

In an attempt to explain the rest of the variability in mMV and to control for the other important factors, multivariate linear regression was performed. eLBM together with genetic and clinical measures of disease severity, which were found significant on univariate regression (Table 6), were used as explanatory variables, including different interaction terms (Table 7). Two plausible complex models were devised (Appendix G) and model selection was performed using a backward stepwise method (using 'step' function in R) based on Aikake information criterion (AIC) value. The resulting models are presented in detail in Table 7.

Table 6
Univariate linear regression results for relationships between mMV and genetic measures of disease severity and most relevant patient-reported measures. Extended results shown in appendix E.1. (+) - positive correlation, (-) – negative correlation.

Predictors	*p-value*	R^2 adjusted	Direction of relationship
Disease duration	**0.009**	0.147	–
Age	0.840	−0.026	+
Log(ePAL)	**0.001**	0.226	–
Log(MAL)	**4.34e-05**	0.350	–
Muscle Impairment Rating Scale (MIRS)	**0.008**	0.153	–
DM1-ActivC centile	0.131	0.035	+
MDHI Group 1: Short Form subscale	0.063	0.066	–
MDHI Group 2: Mobility subscale	0.081	0.055	–
MDHI Group 3: Upper extremity function subscale	0.215	0.015	–
MDHI Group 4: Ability to do activities subscale	0.052	0.074	–
MDHI Group 8: Fatigue subscale	0.052	0.074	–
MDHI Group 10: Myotonia subscale	0.079	0.056	–
MDHI Total	0.097	0.048	–
Intracranial volume (ICV) [l]	**0.002**	0.215	+
Total (brain) grey matter volume / ICV [l]	0.811	−0.0254	+
Total (brain) white matter volume / ICV [l]	0.691	−0.0226	+

Table 7
Details of the multivariate models of mMV obtained using backward stepwise method.

Model	*p*-value	R^2 adjusted	AIC	Predictors	Estimates	CI	std. Error	Statistic	p
mMV ~ eLBM + Age +	1.02E-05	0.596	744.6	(Intercept)	180,525.8	77,903.2 – 283,148.5	50,249.2	3.59	**0.001**
log(MAL) +				eLBM	153.4	−20.9 – 327.7	85.3	1.8	0.082
log(ePAL) + Sex + ICV +				Age	−94.9	−196.8 – 7.0	49.9	−1.9	0.067
*log(MAL) *				MAL	−65,229.6	−97,782.0 – −32,677.1	15,939.3	−4.09	**3.00E-04**
*log(ePAL) + Sex * ICV*				log(ePAL)	−76,481.0	−125,260.2 – −27,701.8	23,884.74	−3.2	**3.22E-03**
				Sex [Male]	−30,206.3	−73,447.1 – 13,034.5	21,172.9	−1.43	0.164
				ICV	4278.9	−12,642.1 – 21,199.8	8285.4	0.52	0.609
				MAL * log(ePAL)	28,195.9	11,920.3 – 44,471.6	7969.4	3.54	**0.001**
				Sex * ICV	21,015.6	−10,092.3 – 52,123.6	15,232.0	1.38	0.178
mMV ~	3.81E-05	0.534	749.5	(Intercept)	20,596.3	−6221.7 – 47,414.3	13,149.2	1.57	0.127
eLBM + Sex + ICV +				eLBM	159.2	−24.6 – 343.1	90.1	1.77	0.087
log(MAL) + Disease				MAL	−9355.4	−15,455.5 – −3255.3	2991.0	−3.13	**0.004**
*duration + Sex * ICV +*				Sex [Male]	−33,129.6	−79,805.7 – 13,546.4	22,885.9	−1.45	0.158
*log(MAL) * Disease*				ICV	8573.9	−9425.8 – 26,573.6	8825.5	0.97	0.339
duration				Sex * ICV	22,638.9	−11,040.6 – 56,318.5	16,513.5	1.37	0.180
				Disease duration	−1190.4	−2253.2 – −127.5	521.1	−2.28	**0.029**
				MAL * Disease duration	411.4	9.6 – 813.2	197	2.09	**0.045**

Consistent with univariate analysis, the strongest associations were found for genetic determinants of DM1 severity (MAL, SI and ePAL) and they remained significant despite many other determinants of masseter volume included in the models (Table 7). MIRS was omitted in the optimal model suggesting that the other variables explain more of the variability in mMV.

3.6.3. Holm-Bonferroni correction

P-values for correlation of mMV were corrected using the Holm-Bonferroni method, which revealed that only complex models and univariate models with log(MAL) and log(SI) remained significant (Appendix H).

3.6.4. Sex differences

None of the strongest correlations observed in the whole DM1 cohort were replicated in female DM1 patients when the cohort was divided by sex, while the correlations were maintained or even strengthened for males in comparison to the DM1 cohort overall (Fig. 3A–G). Although DM1 males had a slightly larger absolute decrease in mMV when compared to controls than females, the larger variability (Table 4) in their cohort makes it unlikely that this is the sole explanation for these stark differences.

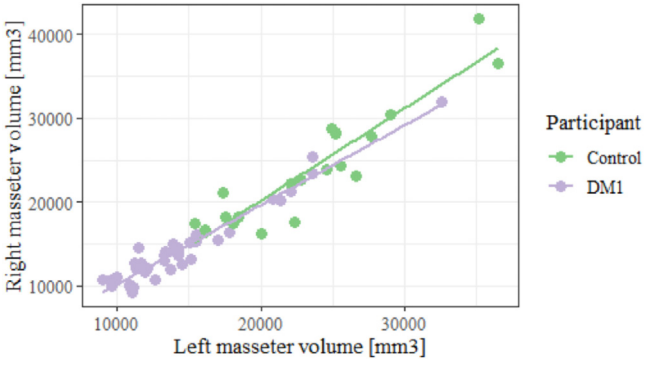

Fig. 3. Relationships between mMV and genetic and clinical determinants of DM1 disease severity
Relationships between mMV and genetic and clinical determinants of disease severity shown for both female (in green) and male (in brown) DM1 patients. Trend lines are fitted based on linear regression. (A) mMV vs MDHI scores of activity, mobility and myotonia (B) mMV vs disease duration (C) mMV vs MAL (D) mMV vs log(ePAL) (E) mMV vs MIRS (F) mMV vs ICV.

4. Discussion

Advances in the understanding of molecular pathophysiology of DM1 have led to the development of the first targeted therapies [11]. In the advent of clinical trials, there is a pressing need to develop and evaluate objective and reliable outcome measures for relevant DM1 symptoms. However, this must be balanced against the number of different assessments a patient can reasonably be exposed to in a research context, which may be limited both by study budget and tolerability to participants [21]. The latter may be particularly relevant to DM1 cohorts, in whom the constellation of physical limitations and CNS symptoms can be

particular barriers to research participation [56]. In line with this, this study investigated the potential value of masseter volume, estimated from an MR brain imaging, in serving as a marker of the disease process and muscle involvement, and therefore, as a potential outcome measure in clinical trials.

Masseter muscle has been described as being physiologically comparable to other peripheral skeletal muscles [57]. In-keeping with this, we found that in a moderate-sized cohort of DM1 patients, who are affected by generalized muscle atrophy, masseter volume was significantly lower than in control participants ($p < 0.001$, Table 4). From previous publications we know that the observed mMV for unaffected participants (Table 4) were broadly similar to those previously reported in control populations (Appendix E.1–2), although there is marked variability stemming predominantly from different age and sex of the participants. For DM1 patients, the single study investigating exclusively the masseter muscle reports the corrected masseter volumes with a mean of 132.3 mm^3/kg (+/- 37.82 SD) [45], which is lower than observed in our sample (Table 4). However, this study was limited to just four DM1 patients. Comparing both sides, the difference in mean volume of the muscle between left and right in controls was less than 1 cm^3 in controls (Appendix D), which is similar to values reported in some previous MRI studies [58–60], although others report a mean difference of almost 2 cm^3 [61,62]. In DM1 patients, the difference was even smaller (Appendix D).

Interestingly, the sex difference in masseter volume evident in controls was diluted in our DM1 cohort (Fig. 2A). This is unlikely to be explained by greater disease severity in males, since ePAL and MAL were actually, on average, larger in female participants (Table 2). Instead, this observation could suggest that the muscle volume tends to the same minimum, regardless of sex. Most preceding studies of muscle MRI in DM1 have been too small to investigate sex differences, and do not attempt to analyse them. However, in one example, there were no differences in the pattern of lower limb muscle involvement between the sexes, although total volume was not compared [63]. In another study, males were shown to lose more muscle strength over time than females [64].

Previous studies have been inconsistent in demonstrating correlations between imaging markers of muscle involvement and disease duration or allele length, with some reporting no relationship [65,66], while others reporting a strong relationship [30,67]. A particular strength of our study was the use of small-pool PCR to measure the CTG repeat length, which takes account of the age-dependant nature of somatic mosaicism and has been shown to improve correlations with clinical measures compared with traditional methods [68]. Using this approach, we detected significant correlations between masseter volume and CTG repeat length (ePAL: $p = 0.001$, adjusted $R^2 = 0.226$ and MAL: $p < 0.001$, adjusted $R^2 = 0.350$) Together with a significant correlation with disease duration ($p = 0.009$, adjusted $R^2 = 0.147$), these results are consistent with masseter muscle volume being a valid marker of the primary disease process in skeletal muscle. Importantly, association for modal allele length remained significant even following a conservative correction for multiple testing, while other associations did not reach the required p-value (Appendix H).

A strong, significant association was found between masseter volume and a clinical measure of muscle impairment: the MIRS score ($p = 0.008$, adjusted $R^2 = 0.153$). This implies that masseter volume is a representative marker of more general peripheral muscle function. Several trends were observed between mMV and self-reported symptoms measured by MDHI, which is a DM1-specific patient-reported measure of disease symptoms and therefore burden, though several did not meet statistical significance [69]. Lower mMV was broadly associated with lower ability to do activities ($p = 0.052$) and mobility ($p = 0.081$) suggesting a possible correlation of mMV with impairment that is of relevance to patients' daily life. Lower mMV was also associated with more severe myotonia ($p = 0.08$, improved to $p = 0.014$ when corrected for eLBM) which is a hallmark muscle symptom of DM1. In contrast, no correlations were found with different neuropsychological assessments including the trail 5 of the D-KEFS Trailmaking test ($p = 0.764$), which involves a simple motor task [70]. Another significant correlation observed was the positive relationship of mMV with ICV ($p = 0.02$, Table 6), which was also significant among control participants ($p = 0.023$, Appendix F.2).

Dysphagia is a common feature occurring in DM1 patients [71] and, if severe, can lead to the need for modification of the texture of the patient's diet with some patients ingesting a liquid or soft diet [72]. It has been shown that the consumption of such diet can cause decreased weight, atrophy and/or smaller cross-sectional area of the masseter muscle in various mammals including mice [73,74], rats [75], minipigs [76], rabbits [77] and ferrets [78]. Therefore, it is possible that the type of diet can influence masseter volume in DM1 patients. We did not have data on this available, therefore it would be interesting to investigate this in future studies. Additionally, the only measure of the swallowing function that we had data available for in our cohort, was the MDHI 12: Swallowing subscale, which is a subjective patient-reported measure. There was no significant correlation between this measure and (c) mMV (Appendix F.1 and Appendix H). However, future studies could explore such correlations with objective measures of swallowing, e.g., using volume-viscosity swallow test [72].While preceding, similar-sized studies have investigated whole DM1 cohorts together [29], our study further investigated the effect of sex. None of the relationships found significant in the whole cohort held true for female DM1 patients alone, while most were strengthened when only males were considered (Table 6). Sex differences do occur in DM1, including a tendency for higher BMI in females and higher rates of severe myotonia and muscle weakness in males [54]. It is plausible that masseter volume might only prove to be a useful marker in male DM1 patients. However, our results could equally simply reflect an artefact of the limited sample size (n = 22 female and n = 17 male DM1 patients), and should be replicated in larger studies before firm conclusions are drawn.

There is a growing body of evidence that the presence of sequence variations within the *DMPK* CTG repeat array ('variant repeats') may have an ameliorating effect on the disease phenotype [79,80]. In our cohort, three patients from the same extended family had variant repeats, all of whom had comparatively mild muscle weakness (MIRS 1, 2 and 3, respectively). Perhaps surprisingly, these subjects had lower masseter volumes than the mean and aged-matched DM1 patients without variant repeats. Again, this could simply reflect sampling bias given the small numbers of such patients identified.

While our preliminary findings with respect to muscle volume in masseter have yielded encouraging results, it should also be borne in mind that muscle volume represents only a single dimension of muscle involvement. One MRI study of lower limbs in DM1 patients showed muscle fat fraction to be the measure with the strongest correlation with function ($p = 0.005$, $R^2 = 0.288$), compared with contractile volume [29]. This emphasises the need to consider additional quantitative imaging methods, such as measures of fatty infiltration or diffusion tensor imaging, in future work on masseter muscle in DM1.

Manual segmentation of MR images is a laborious and time-consuming process, which therefore limits the total number of patients that can be assessed within reasonable time constraints [81]. In the last two decades many semi-automated and automated methods of segmentation have been developed, most of which are specifically used for brain imaging analysis [82–85]. While

those for muscles also exist, they are predominantly validated only in the limbs [81,86–91]. Multiple commercial software options (e.g., sliceOmatic® Tomovision, Inc., ANALYSE, Simpleware ScanIP, Synopsis®) and services (AMRA Medical AB, Linköping, Sweden) are also available on the market. None of the above methods have been validated in assesing the masseter muscle. The only automated method which has been developed specifically for masseters [92] is not available for public use. Once those methods are accessible, it would be worthy to study a larger cohort of patients and assess not only masseter, but also other facial muscles, including the frontalis and temporalis muscles. Recently, a year-long observational study of another form of muscular dystrophy, Duchenne muscular dystrophy, showed that MRI markers (weighted mean fat fraction in muscle) were more sensitive in detecting disease progression than clinical scores of muscle involvement, hence showing promise as a potential outcome measure in clinical trials [93] and are currently being validated [94]. Similar longitudinal studies assessing the changes in muscle MRI markers are warranted in DM1 patients, and one such study is ongoing [95], though plans for facial muscle assessment are not specifically acknowledged. In fact, despite facial weakness being currently included as part of the DM1 Disease Severity Index, no explicit recommendations are made on how it should be assessed in clinical trials [13–15]. Given its early involvement and its severe consequences, it would be advisable to clarify the optimal way of measuring its function, potentially using MRI data, in future studies. They would ideally also aim to automate the segmentation process, both to estimate the volume and extent of fat infiltration. This would make the interpretation of the imaging data less laborious and could ensure higher reproducibility of results (despite the high inter-rater reliability of manual segmentation achieved in this study). Additionally, strategies could be introduced to keep the jaw movement to a minimum to ensure highest quality of the images. Finally, new studies should also seek to include congenital and juvenile-onset DM1 cohorts, since this study was limited to adult-onset patients.

5. Conclusions

In conclusion, the masseter muscle exhibits volume loss in DM1 patients and its volume is comparable in female and male patients. In our exploratory study, masseter volume was strongly inversely correlated with disease duration and CTG repeat length, suggesting promise as a marker of the disease process and disease severity. It was also inversely related to muscle impairment rating scale, implying that its involvement is representative of skeletal muscle function more generally. Additionally, sex differences may play a role in these associations, which should be explored in future studies using sufficiently powered studies.

This is the largest study of masseter muscle involvement in DM1 to date. It provides encouraging results for masseter volume as a potential imaging biomarker of the disease process and hence an important outcome measure for DM1 clinical trials. Priorities for future work include development of more automated method to derive muscle volume and data relating to fat infiltration, large sample size and a longitudinal study design.

Abbreviations

cmMV	corrected mean masseter volume
eLBM	estimated Lean Body Mass
ePAL	estimated Progenitor Allele Length
ICV	intracranial volume
MAL	modal allele length
mMV	mean masseter volume
MR(I)	Magnetic Resonance (Imaging)
SI	somatic instability

Funding

Collection of the data on which this analysis is based was supported by grants from Muscular Dystrophy UK (Ref: MC3/1073) and Chief Scientist Office, Scotland (Ref: CAF/MD/15/01).

Declaration of Competing Interest

AO, CH, MJH, JMcL, SC, BB, RJ, CL and MEF declare no conflict of interest.

DGM: Within the last three years Professor Monckton has been a scientific consultant and/or received an honoraria/stock options from AMO Pharma, LoQus23, Triplet Therapeutics and Vertex Pharmaceuticals. Professor Monckton has also had research contracts with AMO Pharma and Vertex Pharmaceuticals.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2022.09.005.

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