Published in final edited form as:

Neuromuscul Disord. 2012 June ; 22(6): 483–491. doi:10.1016/j.nmd.2012.01.003.

Cerebral and Muscle MRI Abnormalities in Myotonic Dystrophy

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Abstract

Pathophysiological mechanisms underlying the clinically devastating CNS features of myotonic dystrophy (DM) remain more enigmatic and controversial than do the muscle abnormalities of this common form of muscular dystrophy. To better define CNS and cranial muscle changes in DM, we used quantitative volumetric and diffusion tensor MRI methods to measure cerebral and masticatory muscle differences between controls (n=5) and adults with either congenital (n=5) or adult onset (n=5) myotonic dystrophy type 1, myotonic dystrophy type 2 (n=5). Muscle volumes were diminished in DM1 and strongly correlated with reduced white matter integrity and gray matter volume. Moreover, correlation of reduced fractional anisotropy (white matter integrity) and gray matter volume in both DM1 and DM2 suggests that these abnormalities may share a common underlying pathophysiological mechanism. Further quantitative temporal and spatial characterization of these features will help delineate developmental and progressive neurological components of DM, and help determine the causative molecular and cellular mechanisms.

Keywords

Myotonic dystrophy; DM; DM1; DM2; diffusion tensor imaging; magnetic resonance imaging; MRI; cerebral white matter; cerebral gray matter; craniofacial muscle; pterygoid; temporalis; masseter

Introduction

Much of the morbidity in myotonic dystrophy (DM), the most common form of muscular dystrophy in adults, results from CNS aspects of the disease. Two genetic causes of DM have been identified: a trinucleotide (CTG) repeat expansion in the 3'-untranslated region of a protein kinase gene causes type 1 (DM1) [1–3]; a tetranucleotide (CCTG) repeat expansion in intron 1 of the zinc finger protein 9 gene causes type 2 (DM2) [4; 5]. The fact

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that both DM1 and DM2 result from untranslated repeat expansions led to recognition of a novel dominant pathogenic RNA mechanism, in which the transcribed repeat expansions alter RNA processing for multiple downstream genes, resulting in at least those clinical features common to both genetic forms. Any clinical features that happen to be restricted to just one of the genetic forms of DM could theoretically result from either disease-specific modifications of this dominant RNA effect, or from separate superimposed mutation-specific mechanisms, which has led to controversy regarding the pathogenesis of the congenital phenotype associated with DM1, but not DM2.

A developmental disorder of cognition has only been definitively associated with DM1, consistent with the lack of a congenital form of DM2. In adult onset DM1, although IQ is within the normal range it has been observed to be lower than age-matched controls [6; 7], indicating either occurrence of a degenerative cognitive loss in adulthood, or possibly reflecting that some individuals with mild congenital DM1 can escape clinical detection until adulthood. In DM2, no causal association with developmental cognitive impairment has been established, and no global decrement in IQ has been reported, but visuospatial and executive function deficits in adults mirror similar changes in DM1 [8–12], indicating that at least those features are likely to result from progressive neuropathological effects of the dominant RNA mechanism.

MRI investigations have previously demonstrated muscle [13; 14] and CNS changes in DM. Gray matter volume has been reported in both DM1 and DM2 using qualitative [15], voxel-based [16–18], and total brain volume analyses [19; 20]. Cerebral white matter hyperintensities have been observed by FLAIR (Fluid Attenuation and Inversion Recovery) MRI of older DM1 and DM2 adults [15; 21–24]; the pathophysiology and significance of these abnormalities is unclear though in some studies they correlate with neurocognitive dysfunction [25]. Diffusion tensor imaging (DTI) provides a more sensitive assessment of white matter microstructural integrity than FLAIR [26; 27], allowing investigation of younger people with DM who are less likely to be affected by superimposed or secondary CNS changes [28]. Previous studies have shown focal DTI abnormalities in DM1 [17; 29], but global CNS DTI has not been reported or compared to gray matter volume or craniofacial muscle changes in DM.

To better delineate CNS abnormalities common to DM1 and DM2, which are presumably caused by the dominant RNA mechanism, and to compare CNS and muscle abnormalities in the same individuals, we used quantitative DTI and volumetric MRI methods to study masticatory muscle and cerebral structural integrity of adults with either congenital-onset DM1, adult-onset DM1, or DM2.

Methods

Participants

Young and middle-aged adult subjects aged 25–45 years with either congenital onset DM1, adult onset DM1, or DM2 were recruited from the University of Minnesota Muscular Dystrophy Clinic (n=5 for each group: congenital-onset DM1, adult-onset DM1, DM2 and controls). All affected individuals were genetically confirmed to have DM1 or DM2 (average repeat lengths for each group are in Table 1). Adult onset DM1 subjects had no history of symptoms attributable to DM1 prior to age 10 and none of the craniofacial stigmata of early onset disease (prominent forehead, tapered chin or highly arched palate). Congenital onset DM1 subjects had weakness in early childhood, often noted at birth, and the typical craniofacial dysmorphic features. The adult-onset DM1 and congenital-onset DM1 groups were age-matched to controls. DM2 subjects were somewhat older than the control group (n=5 for each group, Table 1). Participants were excluded if they had history

of brain injury or if MRI was contraindicated. All procedures were approved by the University of Minnesota Institutional Review Board.

MRI data acquisition

Imaging data were acquired on a Siemens 3 Tesla Trio scanner (Erlangen, Germany) at the University of Minnesota's Center for Magnetic Resonance Research. Axial DTI was performed using a typical dual spin echo, single shot, echo planar, diffusion weighted sequence (TR=8100ms, TE=84ms, 64 slices, $2.5 \times 2.5 \times 2$ mm voxel, FOV=320mm, 12 directions with b value=1000s/mm² and 1 volume with b=0s/mm², 3 averages) Structural T1 images were acquired using a coronal 3D MP-RAGE sequence (TR=2530ms, TE=3.65ms, TI=1100ms, 240 slices, $1 \times 1 \times 1$ mm voxel, flip angle=7 degrees, FOV=256mm). PD-weighted images were acquired using turbo spin echo (TSE) axial sequence (TR=8550ms, TE=14ms, 80 slices, $1 \times 1 \times 2$ mm voxel, flip angle=120 degrees, FOV=256mm). Total scan time was approximately 35 minutes.

DTI data processing

Diffusion-weighted images were processed using tools from the FMRIB Software Library (FSL) [30]. Eddy current-correction of the individual diffusion-weighted EPI images was performed using a standard affine transformation method [31] and magnetic field susceptibility effects were corrected using a B_0 fieldmap acquired on the subject. Structural and DTI images were aligned to the subject's own T1 acquisition using the FSL linear registration tool (FLIRT), CSF, white and gray matter classes were segmented using the FMRIB Automated Segmentation Tool (FAST) as demonstrated in Figure 1. The diffusion tensor was calculated using the 12 diffusion images and the b=0s/mm² image, and fractional anisotropy (FA) maps were created.

A semi-automated regional ROI method computed average FA from segmented white matter of compartments delineated by specific planes (the genu, splenium and superior edge of the corpus callosum, and a plane connecting the anterior and posterior commissures) that defined superior frontal, inferior frontal, supra-callosal and occipital regions (Figure 2).

Additionally, a region of interest including the entire cerebrum was calculated. Although this method does not assess specific tracts, it does have the advantage of sampling entire regions, thus avoiding sampling problems inherent to analyzing smaller, localized ROIs. To account for variation in total head size, proportional gray matter volumes were determined for each compartment by normalizing to the total average intracranial volume for all subjects. No consistent differences in total brain size were observed between groups. The Statistical Package for the Social Sciences (SPSS, Inc., Chicago, Ill.) was used to test for group differences in FA, and non-parametric methods were used for the subsequent statistical analyses given the relatively small sample sizes and non-normal distributions in the data.

Masticatory muscle measurements

Medial and lateral pterygoid muscle volumes were measured on T1 structural scans, which were also used to calculate masseter and temporalis muscle cross-sectional areas. These muscles were manually segmented using the FMRIB Software Library region of interest (ROI) tool and an MRI neuroanatomy atlas [32; 33]. Total lateral and medial pterygoid muscle volumes were determined using axial slices with primary landmarks at the superior medial aspect of the mandible, the tuberosity of the maxilla and the lateral pterygoid plate, outlining each muscle on each slice to determine overall volume. The masseter cross-sectional maximal area was determined on the axial slice with the greatest anterior-posterior masseter muscle width, outlining the muscle on that single slice. Temporalis area was similarly determined by measuring muscle cross-section in the most superior axial slice that

included the mandibular condyloid process. Figure 3 demonstrates methods on a typical subject. All measurements were performed bilaterally, and when performed in a blinded fashion by two trained analysts showed excellent reproducibility with an inter-rater reliability calculated to have a Cohen's *kappa* statistic of 0.83.

Results

Diffusion Tensor Imaging

A significant difference of fractional anisotropy (FA) in each brain compartment among all four groups ($P < 0.003$) was revealed by an all-inclusive Kruskal-Wallis non-parametric analysis of variance test. More specifically, when average FA within the brain compartments was compared between each of the three groups of DM subjects to controls using the non-parametric Wilcoxon-Mann-Whitney U test with sequential Bonferroni correction [34], significant pair-wise differences were observed. The adult-onset DM1 and congenital-onset DM1 groups had significantly lower FA than controls in the inferior frontal, supra-callosal and occipital regions ($p < 0.05$), while the trend toward differences between DM2 and controls in the same regions did not reach statistical significance (Table 2, Figure 4).

Gray Matter Volume

A significant difference in average gray matter volumes in the supra-callosal and superior frontal brain compartments was observed among all clinical groups compared to controls ($p < 0.05$), using the Kruskal-Wallis analysis of variance test to assess differences in gray matter volumes among all four groups. Significantly lower gray matter volumes were subsequently observed in the adult-onset DM1 group as compared to the control group in the cerebral, superior frontal, and supra-callosal compartments ($p < 0.05$, Wilcoxon-Mann-Whitney U test after sequential Bonferroni correction) (Table 3). Although average cerebral, superior frontal and supra-callosal compartment gray matter volumes in both congenital-onset DM1 and DM2 were decreased compared to controls, these differences did not reach clinical significance after Univariate Wilcoxon-Mann-Whitney U testing.

Correlations between compartment FA and gray matter volume across all groups were made for each using Spearman's nonparametric correlation coefficient. High correlations were found in the total cerebrum (0.544), superior to corpus callosum (0.696), and superior frontal (0.728) compartments. A weaker correlation between FA and gray matter volume was found in the occipital compartment (0.466).

Masticatory Muscle Size

Volumes of medial and lateral pterygoid muscles, as well as maximal areas for temporalis and masseter muscles (Table 4), differed significantly from controls for congenital-onset DM1 subjects (Univariate groupwise analyses using Wilcoxon-Mann-Whitney U tests and sequential Bonferroni corrections). The temporalis and masseter maximal areas and pterygoid volumes also differed significantly from controls for adult-onset DM1 subjects. Masticatory muscle volumes in DM2 did not differ from controls.

Muscle volumes and DTI measures of FA were significantly correlated across the four groups for the above-described brain compartments (> 0.5 , Spearman's nonparametric correlation coefficient), demonstrating a positive correlation between FA and muscle size (Table 5). The correlation between the combined medial and lateral pterygoid muscle volume and total cerebral FA is plotted in Figure 5; the Spearman's nonparametric correlation coefficient between cerebral FA and the volume across all groups was measured to be 0.654.

Discussion

DTI and volumetric MRI measurements showed significant differences of white matter integrity and gray matter volume in DM as compared to controls, microstructural white matter abnormalities being conspicuous by DTI in congenital-onset DM1, adult-onset DM1, and DM2, but gray matter changes being more pronounced in both forms of DM1 than in DM2. Moreover, this study showed white matter pathology throughout the cerebrum rather than preferentially in the frontal lobes as has previously been observed [15], suggesting that a generalized pathophysiological process may underlie the neuropsychological deficits involving multiple cognitive domains [18,28]. Previous studies have emphasized the loss of executive function as well as frontal lobe blood flow and structural changes in DM subjects [11–12, 21–25]; the finding we now report are not in conflict with these previous results, but rather indicate that the underlying pathophysiological changes are more diffuse although the functional consequences may be more focal. Ongoing studies will need to clarify the relationship between the molecular and cellular abnormalities, which appear to be more generalized within the CNS, and the clinical phenotype, wherein more limited or focal abnormalities are observed.

All subjects who participated in this study were young or middle-aged adults, avoiding older individuals who are more likely to be affected by superimposed or secondary disease processes that alter CNS MRI results. The greater sensitivity of DTI than FLAIR methods was demonstrated by the fact that even though these younger adult subjects had normal routine MRIs, with only a minority of subjects demonstrating any subcortical FLAIR hyperintensities, the DTI investigations nonetheless clearly revealed marked fractional anisotropy abnormalities. Furthermore, this quantitative measure allows objective comparisons between brain regions and between disease groups; longitudinal characterization of reduced white matter integrity may clarify the distribution of static developmental as compared to progressive or degenerative CNS abnormalities in DM. Interestingly, the adult-onset DM1 group had lower measures of white matter integrity than the congenital-onset DM1 group, a difference that may diminish with study of more individuals or may reflect the slight age difference between the congenital-onset and adult-onset DM1 groups in this study. It is also possible that neurodevelopmental abnormalities responsible for congenital-onset DM1 do not affect white matter microstructural integrity in the same way, in the same distribution, or to the same extent as the changes that develop in all DM adults, but additional longitudinal and cross-sectional investigations in multiple age groups are needed to explore that possibility. Our observation that white matter is somewhat less affected in DM2 than in DM1 is consistent with other aspects of DM2 [35], in which the CCTG expansion within intron one of the cellular nucleic acid binding protein gene (*CNBP*, previously *ZNF9*) has been associated with less devastating somatic and cognitive effects.

Masticatory muscle sizes were measured given their stereotypic involvement in DM. Similarly to the white and gray matter results, muscle volumes were significantly reduced in congenital-onset DM1 and adult-onset DM1 compared to controls, but DM2 muscle volumes were indistinguishable from control even though some craniofacial muscles can be affected by DM2 [35]. A correlation analysis within all DM groups showed a high correlation between DTI measures in each brain compartment and muscle volumes, suggesting common mechanisms may underlie the severity of both muscle and cerebral pathology in DM.

The cellular pathophysiology of DM white and gray matter changes remains unexplained. In general, loss of white matter integrity as measured by fractional anisotropy can result from either axonal, myelin, or extracellular abnormalities [36], and reduced gray matter volume reflects cortical neuronal loss [37]. Postmortem studies of DM1 have suggested the

possibility of neuronal loss in cortical and subcortical cerebral regions, and increased cerebral perivascular Virchow-Robin space, but the cause and consequences of these findings remains unclear [38; 39], and their relationship to the FA changes is unknown. Ribonuclear inclusions containing the CUG or CCUG expansions have been reported in neurons and glia of multiple brain regions on DM post mortem examination, including cerebral white matter [40], demonstrating that the CNS is affected by the same molecular mechanisms that occur in muscle. As in muscle, multiple CNS transcripts have been shown to be mis-spliced in DM, resulting in abnormal isoforms of potentially pathogenic proteins, including tau [41] and amyloid precursor proteins [42], though the pathophysiological consequences of these alterations and any causal role in MRI abnormalities remain uncertain.

Abnormalities of white matter integrity and gray matter volume occur concurrently and with correlated severity in all DM groups, consistent with a possibility that a common pathophysiological process may underlie white and gray matter changes, as has also been described in normal subjects [43]. By refining the spatial and temporal aspects of white matter, gray matter and muscle changes in DM1 and DM2, and assessing whether each follows a time course indicative of single or separable pathophysiological processes that are progressive or static will expand our understanding of this complex multisystemic disease. Furthermore, quantitative imaging of CNS and muscle with methods established in this study will allow the comparison of changes in patients before and after treatments, and will allow direct comparison of human subjects to transgenic animals as multisystemic models of myotonic dystrophy are developed [44–46].

Acknowledgments

We are grateful for ongoing involvement of many families and patients in these studies, and for support from the National Registry of FSHD and DM patients and Families at University of Rochester (NIAMS: N01AR52274-7-0-1) and the Myotonic Dystrophy Foundation for help in subject recruitment, the NIH/NINDS (R01NS056592-03; P01-NS058901) for direct support of this research, NIH for core muscle laboratory support (P30 AR057220), and core support for the Neuroscience Magnetic Resonance Research Centers (P30 NS057091 and P41 RR008079) and the MDA for core clinical support.

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Figure 1. Automated segmentation of white and gray matter and cerebrospinal fluid

Images from a single control subject illustrate the automated segmentation technique (FMRIB's Automated Segmentation Tool or FAST) used in the calculation of cerebral volumes by tissue type: White matter is yellow, grey matter is blue, and CSF is red. This segmentation method was used for volumetric measurements, and to identify white matter in diffusion tensor imaging studies, which was then subsequently evaluated for fractional anisotropy as a measure of tissue integrity.

Figure 2. Cerebral compartments for white and gray matter analyses

Regions of interest (ROIs) defined by the investigators for the purpose of comparing cerebral volumes and regional white matter integrity across subjects. The pre-defined ROIs are shown overlaid on a T1-weighted image of a control subject for reference. The ROIs are as follows: superior frontal (SUP), inferior frontal (INF), superior to corpus callosum (ACC) and occipital (OCC).

Figure 3. Sample segmentation of lateral and medial pterygoid, masseter and temporalis muscles
Manual segmentation of lateral and medial pterygoid muscles (left), masseter (center), and temporalis (right) muscles in a sample subject.

Figure 4. Pairwise analysis of FA in four cerebral compartments

Significantly lower FA was observed in the DM1 adult onset and DM1 congenital onset groups in the cerebral, supra-callosal, inferior frontal and occipital compartments.

Figure 5. Scatterplot of FA and combined medial and lateral pterygoid muscle volumes for all subjects

The correlation between the combined medial and lateral pterygoid volume and total cerebral FA across all subjects; the Spearman's rho statistic is 0.654 over all subjects.

Table 1

Demographic characteristics of Myotonic Dystrophy and Control groups

	CTL (n=5)	DM1 adult (n=5)	DM1 congenital (n=5)	DM2 (n=5)
Age Range	33–43	30–43	24–34	38–49
Mean age	35.8	38.3	29.5	43.1
Age of onset	N/A	19.5	<1	29.5
Average repeat length	N/A	317	792	3217

Table 2

Pair-wise Differences in Specific Brain Compartment Fractional Anisotropy between DM Subgroups and Controls

	DM1 adult (n=5)	DM1 congenital (n=5)	DM2 (n=5)
Total cerebral compartment [Ctrl Mean (SD) = 0.380 (0.017)]			
Mean (SD)	0.299 (0.011)	0.330 (0.029)	0.358 (0.012)
p-value	0.001 *	0.023 *	0.057
effect size	5.71	2.10	1.50
Mean % difference	-23.7	-22.0	-5.7
Supra-callosal compartment [Ctrl Mean (SD) = 0.378 (0.013)]			
Mean (SD)	0.315 (0.022)	0.321 (0.029)	0.356 (0.015)
p-value	0.001 *	0.004 *	0.048
effect size	4.75	4.25	1.60
Mean % difference	-16.7	-14.9	-5.6
Superior frontal compartment [Ctrl Mean (SD) = 0.362 (0.032)]			
Mean (SD)	0.277 (0.020)	0.322 (0.029)	0.331 (0.021)
p-value	0.001 *	0.077	0.115
effect size	2.61	1.22	.94
Mean % difference	-23.4	-10.9	-8.4
Inferior frontal compartment [Ctrl Mean (SD) = 0.377 (0.024)]			
Mean (SD)	0.274 (0.008)	0.319 (0.042)	0.360 (0.012)
p-value	<0.001 *	0.026 *	0.230
effect size	4.37	2.48	.74
Mean % difference	-27.5	-15.6	-4.7
Occipital compartment [Ctrl Mean (SD) = 0.404 (0.015)]			
Mean (SD)	0.332 (0.014)	0.358 (0.027)	0.386 (0.005)
p-value	<0.001 **	0.010 *	0.031
effect size	4.83	3.13	1.22
Mean % difference	-17.6	-11.4	-4.5

* Significant groupwise (<0.05) difference after Wilcoxon-Mann-Whitney U tests with sequential Bonferroni corrections.

Table 3

Pair-wise Differences in Specific Brain Compartment Gray Matter Volumes between DM subgroups and Controls

	DM1 adult (n=5)	DM1 congenital (n=5)	DM2 (n=5)
Cerebral compartment [Ctrl Mean (SD) = 629.5 cm³ (30.86 cm³)]			
Mean (SD)	521.6 (39.9)	587.8 (75.9)	577.2 (71.5)
p-value	<0.001 *	0.517	0.033
effect size	4.35	.54	1.79
Mean % difference	-14.3	-13.6	-6.30
Supra-callosal compartment [Ctrl Mean (SD) = 201.9 cm³ (15.7 cm³)]			
Mean (SD)	145.5 (6.2)	172.7 (26.1)	167.6 (14.4)
p-value	0.003 *	0.181	0.008
effect size	4.03	1.08	2.63
Mean % difference	-27.8	-25.2	-14.9
Superior frontal compartment [Ctrl Mean (SD) = 65.7 cm³ (5.8 cm³)]			
Mean (SD)	48.0 (4.0)	58.9 (10.0)	61.7 (10.6)
p-value	0.012 *	0.215	0.550
effect size	2.76	.796	.420
Mean % difference	-26.4	-23.7	-4.07

* Significant groupwise (<0.05) difference after Wilcoxon-Mann-Whitney U tests with sequential Bonferroni corrections.

Table 4

Pair-wise differences in Masticatory Muscle sizes between DM subgroups and Controls

	DM1 adult (n=5)	DM1 congenital (n=5)	DM2 (n=5)
Medial pterygoid volume [Ctrl Mean (SD) = 16.1 cm³ (2.7 cm³)]			
Mean (SD)	12.1 (4.1)	10.3 (2.9)	16.5 (3.8)
p-value	0.08	<0.01 *	0.91
Effect size	1.60	2.35	-0.18
Mean % difference	0.43	0.37	-0.03
Lateral pterygoid volume [Ctrl Mean (SD) = 18.9 cm³ (3.2 cm³)]			
Mean (SD)	11.6 (3.4)	12.5 (2.2)	21.1 (5.2)
p-value	<0.01 *	0.02 *	0.35
Effect size	4.88	3.45	1.31
Mean % difference	0.40	0.60	0.24
Temporalis area [Ctrl Mean (SD) = 835.4 mm² (161.6 mm²)]			
Mean (SD)	183.2 (52.9)	284.0 (104.8)	637.0 (99.9)
p-value	<0.01 *	<0.01 *	0.10
Effect size	4.88	3.45	1.31
Mean % difference	0.40	0.60	0.24
Masseter area [Ctrl Mean (SD) = 1098.8 mm² (113.1 mm²)]			
Mean (SD)	716.0 (106.0)	555.8 (72.1)	1073.0 (128.1)
p-value	<0.01 *	<0.01 *	0.31
Effect size	3.05	6.03	0.27
Mean % difference	0.56	0.47	0.02

* Significant groupwise (<0.05) difference after Wilcoxon-Mann-Whitney U tests with sequential Bonferroni corrections.

Table 5

Correlations between Brain Compartment Fractional Anisotropy and Masticatory Muscle sizes across DM subgroups

	Medial Pterygoid	Lateral Pterygoid	Temporalis	Masseter
Superior frontal FA	0.531	0.623	0.678	0.624
Inferior frontal FA	0.597	0.680	0.839	0.783
Occipital FA	0.592	0.653	0.844	0.702
Supra-callosal FA	0.520	0.608	0.794	0.656
Cerebral FA	0.602	0.690	0.845	0.750