How genetics affects the brain to produce higher-level dysfunctions in myotonic dystrophy type 1

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Summary

Myotonic dystrophy type 1 (DM1) is a multisystemic disorder dominated by muscular impairment and brain dysfunctions. Although brain damage has previously been demonstrated in DM1, its associations with the genetics and clinical/neuropsychological features of the disease are controversial.
This study assessed the differential role of gray matter (GM) and white matter (WM) damage in determining higher-level dysfunctions in DM1. Ten patients with genetically confirmed DM1 and 16 healthy matched controls entered the study. The patients underwent a neuropsychological assessment and quantification of CTG triplet expansion. All the subjects underwent MR scanning at 3T, with studies including T1-weighted volumes and diffusion-weighted images. Voxel-based morphometry and tract-based spatial statistics were used for unbiased quantification of regional GM atrophy and WM integrity.
The DM1 patients showed widespread involvement of both tissues. The extent of the damage correlated with CTG triplet expansion and cognition. This study supports the idea that genetic abnormalities in DM1 mainly target the WM, but GM involvement is also crucial in determining the clinical characteristics of DM1.

KEY WORDS: cognition, diffusion imaging, DM1, genetics, VBM.

Introduction

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults (Harper, 1989; Emery, 1991). It is an autosomal dominant condition, caused by expansion of CTG triplet repeats within the 3' untranslated region of the myotonic dystrophy protein kinase (*DMPK*) gene, located on chromosome 19q13.3 (Brook et al., 1992). DM1 is a multisystemic condition that also involves the central nervous system (CNS), and neurological symptoms are currently recognized as frequent clinical features of the disease (Meola and Sansone, 2007). Several neuropsychological deficits have been reported in patients with DM1, including mental retardation and memory, executive and visuospatial dysfunctions (Meola et al., 1999; Modoni et al., 2008; Wozniak et al., 2011). Mental retardation is more frequently observed in the congenital form of DM1 (Harper, 2001). Conversely, adult forms are typically characterized by less severe cognitive deficits, dominated by executive and memory dysfunctions (Meola and Sansone, 2007). Although a direct association between the severity of the CNS involvement and CTG triplet expansion might be expected, previous literature has reported controversial results. Some authors reported associations between CTG triplet expansion and patients' level of global cognition, as measured by the Wechsler Adult Intelligence Scale or by the Mini-Mental State Examination (Jaspert et al., 1995; Perini et al., 1999; Marchini et al., 2000). In contrast, other authors were

unable to replicate these findings (Meola et al., 1999, 2003; Modoni 2004).

In recent years, quantitative magnetic resonance imaging (MRI), applied to neurological and psychiatric conditions, has shown the ability to detect, *in vivo*, microscopic brain tissue abnormalities and to associate them with the presence and severity of patients' clinical and neuropsychological features (Bozzali et al., 2011; Hazlett et al., 2012; Vigeveno et al., 2012). In the case of a genetic disease, such as DM1, MRI investigation may help to clarify the pathophysiological relationship between the presence and severity of genetic abnormalities and patients' cognitive/psychiatric symptoms. Two major mechanisms can be implicated in the production of neuropsychological and psychiatric manifestations, namely i) regional involvement of gray matter (GM) tissue, and ii) brain disconnection, due to white matter (WM) damage. In a recent study, Minnerop et al. (2011) employed unbiased brain volumetric and diffusion imaging data analysis methods to assess, respectively, GM atrophy and microscopic WM damage in a group of patients with DM1. These authors reported prominent involvement of the WM tissue and its association with CTG triplet expansion. In contrast, they did not find any evident association between patients' neuropsychological deficits and measures of either GM or WM brain tissue abnormalities.

The aim of the current MRI study was to investigate, in a group of patients with genetically confirmed DM1, the pathophysiological effect of CTG triplet expansion on brain tissue, and its ability to account for patients' cognitive and behavioral characteristics.

Materials and methods

Subjects

A cohort of 10 genetically confirmed DM1 patients was recruited from the Neuromuscular and Neurological Rare Diseases Center of San Camillo Forlanini Hospital (Rome, Italy), and investigated at the Neuroimaging Laboratory of Santa Lucia Foundation (Rome, Italy). The diagnosis of DM1 was genetically confirmed in all the patients and, as detailed below, expansion of the CTG triplet in the *DMPK* gene was quantified in each individual. Patients were classified according to the nomenclature proposed by the International Myotonic Dystrophy Consortium (IDMC, 2000), which is based on CTG triplet expansion. One of the 10 patients (10%) was classified as the E1 type (<150 CTG triplet expansion), eight (80%) were classified as the E2 type (150-1000 CTG triplet expansion), and one (10%) as the E3 type (>1000 triplet expansion). In eight of the 10 patients the clinical onset occurred in adulthood (age range: 18-30 years), while one patient had onset in childhood (aged 13 years), and one patient had a congenital form of DM1. In all the patients, neuromuscular impairment was assessed using the Muscular Impairment Rating Scale (MIRS) (Mathieu et al., 2001). Table I summarizes the principal genetic and clinical characteristics of the DM1 patients. Sixteen sex- and age-matched healthy subjects (HSs) were also recruited and served as controls. In order to reduce any potential source of variability due to hemispheric dominance, all the participants (patients and controls) had to be right-handed.

Major systemic, psychiatric and neurological (other than DM1 for the patient group) illnesses were carefully investigated and excluded in all subjects.

Approval by the Ethics Committee of the Santa Lucia Foundation and written informed consent (either from the subjects or, if they were incapable of giving consent, from their legal guardians) were obtained before the start of this study.

Genetic assessment

Normal and proto-mutated alleles were analyzed using the "touch down" polymerase chain reaction (PCR) technique on DNA obtained from peripheral blood leukocytes (PBL DNA). Briefly, 50 pg of PBL DNA was amplified in a 20 µl volume with fluorescent dye-labeled primer 101 and primer 102. Reactions were cycled through eight rounds at 94°C - 30'', 68°C - 30'' (-1° C per cycle) and 72°C - 30'', followed by 30 rounds at 94°C - 30'', 60°C - and 72°C - 30''. PCR products were analyzed using an Abi-Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Detection of expanded alleles was performed on 10 pg of PBL DNA, which underwent extra-

Table I - Principal demographic and clinical characteristics of the studied subjects.

	DM1 patients n=10	Healthy subjects n=16	p-value [a]
Mean (SD) age [years]	41.8 (9.6)	42.8 (12.9)	ns
Gender (F/M) [%]	40.0/60.0	43.7/56.3	ns
Mean (SD) years of formal education	11.9 (2.1)	15.7 (2.3)	<0.05
Mean (SD) number of CTG triplet expansion	534.4 (539.3),	-	-
on *DMPK*, [range]	[54- >2000]	-	-
Mean (SD) MIRS score	2.7 (0.9)	-	-
Mean (SD) BDI (normal cutoff ≤ 14.0)	5.3 (3.5)	-	-
Mean (SD) HAM-A (normal cutoff ≤ 17)	9.2 (5.6)	-	-
Mean (SD) PSQI	4.4 (3.3)	-	-

[a] One-way ANOVA. Abbreviations: DM1=Myotonic dystrophy type 1; *DMPK*=mytonic dystrophy protein kinase gene; MIRS=Muscular Impairment Rating Scale score; BDI=Beck Depression Inventory score; HAM-A=Hamilton Anxiety Rating Scale score; PSQI=Pittsburgh Sleep Quality Index score; ns=not significant

long PCR (Cheng et al., 1996) and 1% agarose gel electrophoresis. PCR products were analyzed by Southern blotting with subsequent hybridization to a P32 radiolabeled (CTG) 7 oligonucleotide probe and detected by autoradiography.

Neuropsychological and behavioral assessments

All the patients underwent an extensive neuropsychological battery including: a) the Mini-Mental State Examination (MMSE) (Folstein et al., 1975; Measso et al., 1993) and Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1981; Orsini and Laicardi, 1997) as measures of global cognition; b) Rey's 15-Word List (Immediate and 15-min Delayed recall and Recognition) (Carlesimo et al., 1996) and Short Story Test (Immediate and 20-min Delayed recall) (Carlesimo et al., 2002), to assess verbal episodic long-term memory; c) Rey's Complex Figure (20-min Delayed recall) (Carlesimo et al., 2002), to assess visuospatial episodic long-term memory; d) Digit span and Corsi Block Tapping task forward and backward (Orsini et al., 1987; Lezak, 2004;) as measures of short-term memory; e) Naming objects subtests of the BADA ("Batteria per l'Analisi dei Deficit Afasici", Italian for "Battery for the analysis of aphasic deficits") (Miceli et al., 1991) to assess language abilities; f) Raven's Colored Progressive Matrices (Carlesimo et al., 1996) to assess reasoning; g) Copy of drawings with and without landmarks (Carlesimo et al., 1996) and Copy of Rey's Complex Figure (Carlesimo et al., 2002), to evaluate constructional abilities; h) Semantic and Phonological Word Fluency (Spinnler and Tognoni, 1987; Carlesimo et al., 1996), Modified Card Sorting Test (Nocentini et al., 2002), Trail-Making Test (parts A and B) (Giovagnoli et al.,1996); Frontal Assessment Battery (FAB) (Apollonio et al., 2005), to explore executive functions. The HSs underwent a shorter neuropsychological assessment battery (Table II) to exclude the presence of any cognitive impairment. For all the tests employed, we used Italian normative data for both score adjustment (sex, age and education) and to define cutoff scores of normality, determined as the lower limit of the 95% tolerance interval for a confidence level of 95%. For each test, normative data are reported in the corresponding references. A series of non-parametric ANOVAs (Kruskal-Wallis) was used to test for between-group differences in cognitive performances. The statistical threshold, with Bonferroni's correction for multiple comparisons, was set at $p \leq 0.003$. In the patients only, partial correlations (controlled for education) were tested between measures of general cognitive efficiency (MMSE scores and the three WAIS-R IQ scores) and CTG triplet expansion size, and MIRS scores. In this case, the threshold with Bonferroni's correction for multiple comparisons was set at $p<0.01$. In addition, a series of Spearman's coefficients was used to test for associations between the measures of general cognitive efficiency (MMSE and three WAIS-R IQ scores) and the performances obtained by patients on the following tests: Rey's 15-Word List Delayed recall, Digit span and Corsi Block Tapping task backward, Raven's Colored Progressive Matrices, Copy of drawings and FAB. The statistical threshold with Bonferroni's correction for multiple comparisons was set at $p<0.004$.

Finally, patients underwent behavioral evaluations to assess the presence of depression, anxiety and sleep disorders. These were performed using the Beck Depression Inventory (BDI) (Beck and Stee, 1997), the Hamilton Anxiety Rating Scale (HAM-A) (Hamilton, 1959) and the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989). In the HSs, behavioral abnormalities were excluded by clinical interviews.

MRI acquisition

All the subjects underwent an MRI examination at 3T (Magnetom Allegra, Siemens, Erlangen, Germany), including the following acquisitions: i) dual-echo turbo spin echo [TSE] (TR=6190 ms, TE=12/109 ms); ii) fast-fluid attenuated inversion recovery (FLAIR) (TR=8170 ms, TE=96 ms, TI=2100 ms); iii) 3D modified driven equilibrium Fourier transform (MDEFT) scan (TR=1338 ms, TE=2.4 ms, Matrix=256x224, number of slices=176, thickness=1 mm); iv) Diffusion tensor imaging (DTI) (TR=7000 ms, TE=85 ms, 61 diffusion directions, maximum b factor=1000 smm^{-2}, isotropic resolution 2.3 mm^3).

MRI data analyses

First, conventional MRI scans were used to assess, in every participant, the presence of macroscopic WM lesions. Then, T1-weighted volumes were used for regional GM volumetric studies, according to the optimized voxel-based morphometry (VBM) protocol (Ashburner and Friston, 2001, 2005), while diffusion data were processed according to the tract-based spatial statistics (TBSS) protocol (Smith et al., 2006) to quantify microscopic WM abnormalities. Both VBM and TBSS are operator-independent image analysis techniques that allow an unbiased assessment of GM and WM damage, as well as correlations with clinical, genetic and cognitive variables.

White matter lesion assessment

Similarly to previous studies (Romeo et al., 2010; Minnerop et al., 2011), TSE and FLAIR scans were reviewed by an expert radiologist, while the age-related WM change scale (ARWMC; Wahlund et al., 2001) was used to evaluate the severity of WM lesions (WMLs) in both hemispheres. WML volumes were also calculated, in all subjects, by using semi-automated local thresholding contouring software (Jim 4.0, Xinapse System, Leicester, UK, http://www.xinapse.com/). A one-way ANOVA was used to test for between-group differences in WMLs. In the patient group only, Spearman's coefficients were employed to investigate the potential asso-

Table II - Performance of DM1 patients and healthy subjects on neuropsychological testing.

Domain Test/subtest	DM1 patients n=10	Healthy subjects n=16	p-value [a]
Cognitive efficiency			
MMSE (normal cutoff ≥ 23.8)	28.3 (2.1) [28.2]	-	-
WAIS-R			
- Verbal IQ (mean 100; SD 15) {range}	96.3 (22.4) {57-121} [101.5]	-	-
- Performance IQ (mean 100; SD 15) {range}	105.4 (27.7) {51-125} [111.5]	-	-
- Total IQ (mean 100; SD 15) {range}	100.1 (26.4) {50-124} [103.5]	-	-
Verbal episodic long-term memory			
15-Word List:			
- Immediate recall (cutoff ≥ 28.5)	42.2 (4.7) [42.6]	46.2 (11.2) [46.5]	ns
- Delayed recall (cutoff ≥ 4.6)	7.7 (2.1) [7.7]	10.8 (3.2) [10.5]	ns
- Recognition: *hit rates*	14.6 (0.5) [15.0]	-	-
- Recognition: *false*	0.6 (1.1) [0.0]	-	-
Visuospatial episodic long-term memory			
Rey's Complex Figure:			
- Delayed recall (cutoff ≥ 6.3)	15.3 (6.9) [18.0]	19.4 (5.6) [19.7]	ns
Verbal short-term memory			
Digit span (cutoff ≥ 3.7)	5.1 (1.9) [4.7]	6.1 (1.1) [6.2]	ns
Visuospatial short-term memory			
Corsi span (cutoff ≥ 3.5)	4.1 (0.7) [3.8]	5.6 (0.9) [5.3]	0.002
Language			
Naming of objects (cutoff ≥ 22)	28.5 (1.6) [29.0]	27.7 (1.3) [27.5]	ns
Reasoning			
Raven's Colored Progressive Matrices (cutoff ≥18.9)	27.6 (8.1) [30.5]	33.6 (2.5) [33.8]	ns
Constructional praxis			
Copy of drawings (cutoff ≥ 7.1)	10.0 (3.1) [11.2]	12 (0.0) [12.0]	ns
Copy of drawings with landmarks (cutoff ≥ 61.8)	65.1 (11.4) [68.8]	68.1 (2.5) [69.6]	ns
Copy of Rey's Complex Figure (cutoff ≥ 23.7)	28.2 (10.4) [32.2]	35.6 (0.9) [36.0]	<0.001
Executive functions			
Phonological Word Fluency (cutoff ≥ 17.3)	29.2 (11.0) [29.0]	38.2 (10.0) [38.9]	ns
Semantic Word Fluency (cutoff ≥ 7.2)	17.7 (2.5) [14.0]	-	-
Trail-Making Test			
- A	48.8 (36.4) [36.5]	44.8 (15.5) [43.5]	ns
- B	121.4 (100.5) [93.0]	106.1 (42.2) [105.0]	ns
- B-A	71.8 (60.1) [59.5]	61.5 (40.5) [57.0]	ns
Modified Card Sorting Test			
- Criteria achieved (cutoff ≥ 4.2)	5.6 (1.3) [6.0]	6.0 (0.0) [6.0]	ns
- Perseverative errors	3.2 (5.5) [1.0]	6.5 (2.1) [6.0]	ns
Frontal Assessment Battery (FAB)			
- FAB-Total score (cutoff ≥ 13.5)	15.4 (2.7)	-	-
- FAB-Similarities (range 0-3)	2.7 (0.7)	-	-
- FAB-Lexical fluency (range 0-3)	2.8 (0.6)	-	-
- FAB-Motor series (range 0-3)	3.0 (0.0)	-	-
- FAB-Conflicting instructions (range 0-3)	2.9 (0.3)	-	-
- FAB-Go no Go (range 0-3)	2.3 (1.0)	-	-
- FAB-Environmental autonomy (range 0-3)	2.7 (0.9)	-	-

[a] Kruskal-Wallis ANOVA. Abbreviations: DM1=myotonic dystrophy type 1; WAIS-R=Wechsler Adult Intelligence Scale-Revised; ns=not significant.
The table shows the performance scores obtained on neuropsychological testing in each group of studied subjects. The values are expressed as mean (SD) [median]. For each administered test appropriate adjustments for gender, age and education were applied according to the Italian normative data. Available cutoff scores of normality (≥ 95% of the lower tolerance limit of the normal population distribution) are also reported for each test.

ciation between WML volumes and genetic and clinical variables (CTG triplet expansion, MIRS scores and MMSE scores). The statistical threshold with Bonferroni's correction for multiple comparisons was set at p<0.02.

VBM analysis for gray matter volumetric assessment

T1-weighted (MDEFT) volumes were first visually inspected to exclude the presence of macroscopic artifacts. They were then pre-processed for the optimized VBM protocol (Ashburner and Friston, 2001, 2005) implemented in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/), which consists of an iterative combination of segmentations and normalizations to produce a GM probability map (Ashburner and Friston, 2001, 2005) in standard space (Montreal Neurological Institute, or MNI coordinates) for every subject. In order to compensate for compression or expansion, which can occur during warping of images to match the template, the GM was "modulated" by multiplying the intensity of each voxel in the final images by the Jacobian determinant of the transformation, corresponding to its relative volume before and after warping (Ashburner and Friston, 2001). GM, WM and CSF volumes were computed from these probabilistic images for every subject. All the data were then smoothed using a 12-mm FWHM Gaussian kernel. Statistical analyses were performed on smoothed GM maps within the framework of the general linear model. A two-sample t-test was used to assess between-group differences in regional GM volumes. The intracranial volume (ICV) (obtained by summing the WM volume, GM volume and CSF volume) was entered as a covariate of no interest. Results were considered significant at p values <0.05, after family-wise error (FWE) cluster-level correction.
For the patient group only, associations were investigated between patients' genetic CTG triplet expansion and regional GM volumes. For the between-group comparisons, the multiple regression model included the patients' ICV as a covariate of no interest. The statistical threshold was set at p<0.05 FWE corrected at cluster level.

Microscopic white matter assessment by tract-based spatial statistics

Diffusion data were corrected for misalignment between volumes according to the following steps: i) b0 images were realigned to the first volume with a rigid body transformation computed using the FMRIB linear image registration tool (FLIRT) (Jenkinson and Smith, 2001), and averaged; ii) the 61 diffusion-weighted (DW) volumes were averaged and co-registered to the scalp-stripped mean b0 image, to yield an average transformation (Tx1), matching the mean DW image to the mean b0; iii) each DW volume was realigned to the mean DW image (with a rigid body transformation, described by Tx2), and the transformation matching each DW volume with the b0 image was obtained by combining Tx2 with Tx1. The b matrices were rotated accordingly (Leemans and Jones, 2009). All the remaining processing was done using the Camino toolkit (www.camino.org.uk), if not otherwise specified. The diffusion tensor was estimated in every voxel (Basser and Pierpaoli, 1996), and maps of fractional anisotropy (FA) were obtained. FA maps were fed into TBSS (Smith et al., 2006) to obtain a projection of all the subjects' FA data onto a mean FA tract skeleton. TBSS involves the following steps: i) alignment of FA data into a common space by non-linear registration (Rueckert et al., 1999); ii) averaging of all subjects' FA data and thinning of the mean FA image to create a mean FA skeleton, followed by projection of each subject's FA data onto the skeleton; iii) voxel-wise statistics within the skeleton only. Differences in FA between DM1 patients and HSs were assessed using a permutation-based statistical analysis (randomized). The resulting statistical maps were thresholded at p<0.05, corrected for multiple comparisons using the threshold-free cluster enhancement method (Smith and Nichols, 2009). In the patient group only, correlations between FA, clinical parameters (CTG triplet expansion and MIRS scores) and MMSE scores were also investigated.

Results

Demographic, clinical, neuropsychological and behavioral characteristics of the studied subjects

The patients with DM1 did not differ from the controls in age ($F_{1,24}$=0.04, p=ns) or gender (chi-square=0.04, p=ns) distributions, while, as expected, the level of formal education was significantly lower in the patients ($F_{1,24}$=16.5, p=0.000) (Table I). According to the MIRS disease classification, one of the 10 patients was at the first stage of disease, three were at the second disease stage, four were at the third stage, one was at the fourth stage, and one was at the fifth stage. The patients' CTG triplet expansion was not significantly associated with age (r=-0.05, p=ns) or MIRS score (r=0.06, p=ns), while it was inversely associated with the level of formal education (r=-0.76, p=0.01). Moreover, partial correlations revealed that the patients' CTG triplet expansions correlated with their MMSE scores (r=-0.95, p=0.001).
No significant association was found between patients' MIRS scores and levels of general cognitive efficiency. None of the patients had any remarkable symptom suggestive of depression, anxiety or sleep disorders. The patients' CTG triplet expansions did not correlate with their BDI (r=-0.16, p=ns), HAM-A (r=0.9, p=ns) or PSQI (r=0.37, p=ns) scores.
From a neuropsychological viewpoint (Table II), the patients with DM1 showed a normal level of general cognitive efficiency, as shown by their IQs and performances on the MMSE. Only one patient showed a

condition consistent with mild mental retardation (IQs of about 50) as classified by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) criteria (APA, 2000). When exploring each single domain, the patients with DM1 were found to perform worse than the controls on tests exploring visuospatial short-term memory (Corsi test; chi-square=9.3, df=1, p=0.002) and constructional apraxia (Copy of Rey's Complex Figure; chi-square=12.8, df=1, p<0.001).
The correlation analysis performed in the patient group showed that a test of constructional apraxia (Copy of drawings) correlated with some measures of cognitive efficiency (Total IQ: r=0.91, p=0.001; Verbal IQ: r=0.91, p=0.001).

White matter lesions

There was no significant difference between the DM1 patients and the HSs in the mean total volume of WMLs [mean (SD) 2991.2 (5466.8) mm^3 in the DM1 patients and 404.8 (463.5) mm^3 in the HS group] ($F_{1,24}$=3.15, p=ns).
A significant direct correlation was found between the total volume of WMLs and CTG triplet expansion (r=0.93, p<0.001) and a significant inverse correlation between the total volume of WMLs and MMSE score (r=-0.82, p=0.003). No other significant correlation was found with WMLs.
As reported in table III, the patients with DM1, compared with the HSs, showed significantly higher ARWMC scores in the frontal ($F_{1,24}$=12.2, p=0.002) and temporo-insular regions ($F_{1,24}$=10.2, p=0.004) .

Voxel-based morphometry

Cross-sectional analysis

Compared with the controls, the patients with DM1 showed GM loss in the left posterior cingulate, in the left supramarginal gyrus, in the left pre- and post-central gyrus, in the right cerebellum (lobules VIII, VII and Crus), and, bilaterally, in the precuneus (Table IV and Fig. 1).

Correlations between patients' genetics and regional gray matter volumes

A significant correlation was found in the patients between CTG triplet expansion and GM volumes in the cingulate gyrus, orbitofrontal cortices (BA25 and BA12), frontal pole (BA10) bilaterally, and left pre-central gyrus (Fig. 2).

Tract-based spatial statistics

Cross-sectional analysis

As shown in figure 3, TBSS analysis revealed widespread reductions of FA in patients with DM1 as compared with the HSs. The main WM regions showing involvement were the whole corpus callosum, the fronto-occipital fasciculus, the anterior cingulate gyrus, the inferior and superior longitudinal fasciculus, and the anterior limb of the internal capsule bilaterally; unilateral areas included the right uncinate fasciculus (frontal part), corona radiata, external capsule, and left anterior thalamic radiation.

Table III - Severity of macroscopic white matter damage in the studied subjects.

Anatomical distribution	DM1 patients n=10	Healthy subjects n=16	p-value [a]
Frontal	1.0±0.67	0.21±0.42	0.001
Temporo-insular	0.75±0.72	0.11±0.21	0.004
Parieto-occipital	0.85±0.58	0.28±0.42	ns
Infratentorial	0.0±0.0	0.0±0.0	ns
Basal ganglia	0.20±0.63	0.0±0.0	ns

[a] One-way ANOVA. Abbreviations: DM1=myotonic dystrophy type 1; ns=not significant.
The severity of white matter hyperintensities was rated according to the ARWMC scale (Wahlund et al., 2001). Scores reflect both lesion number and extent. The values in the table are the mean (±SD) ARWMC scores for each anatomical localization. See text for further details.

Table IV - Regions of GM atrophy in patients with DM1.

Brain region	Side	Size	Coordinates (mm)			Peak Z-score
			x	y	z	
Pre- and post-central gyrus/Cingulate cortex/Supramarginal gyrus/Precuneus	L	3588	-50	2	50	4.32
Cerebellum	R	608	38	-58	-60	3.92

Abbreviations: DM1=myotonic dystrophy type 1; R=right; L=left.
The size of each region is expressed in number of voxels. Only those regions surviving correction for multiple comparison (p<0.05, family-wise error corrected at cluster level) were considered statistically significant. See text for further details.

Correlations between patients' genetic, clinical and neuropsychological features and regional white matter fractional anisotropy

As shown in figure 4, the patients' FA values were, in most of these WM regions, inversely associated with their CTG triplet expansions (panel A) and MIRS scores (panel B). Finally, FA values in most of the cerebral, but not cerebellar, WM were directly associated with patients' MMSE scores (Fig. 4, panel C).

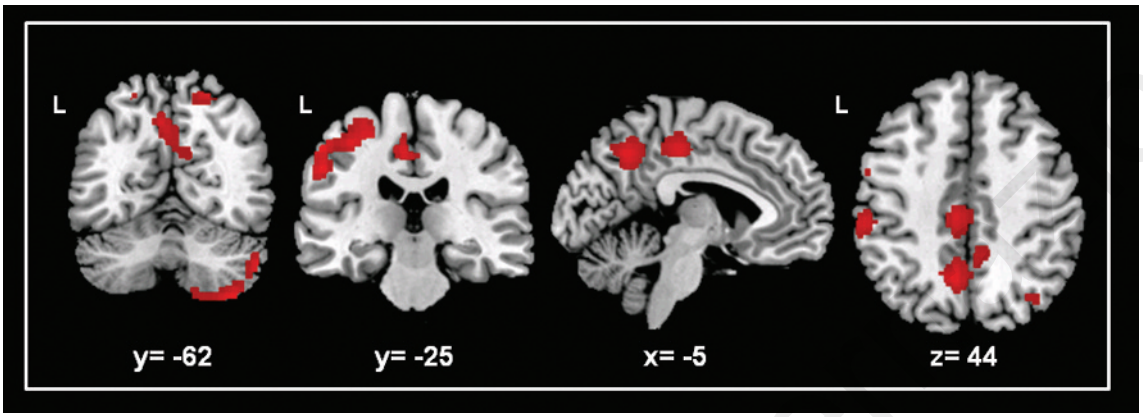

Figure 1 - Distribution of regional gray matter atrophy in DM1 patients.
The figure illustrates the pattern of regional GM loss (in red) observed in DM1 patients as compared to healthy controls. The description of the regions corresponds to that summarized in table IV. Areas are overlaid on a T1-weighted template. Statistical threshold: p<0.05, family-wise error corrected at cluster level. The opposite contrast (healthy subjects less than DM1 patients) was not significant. See text for further details.

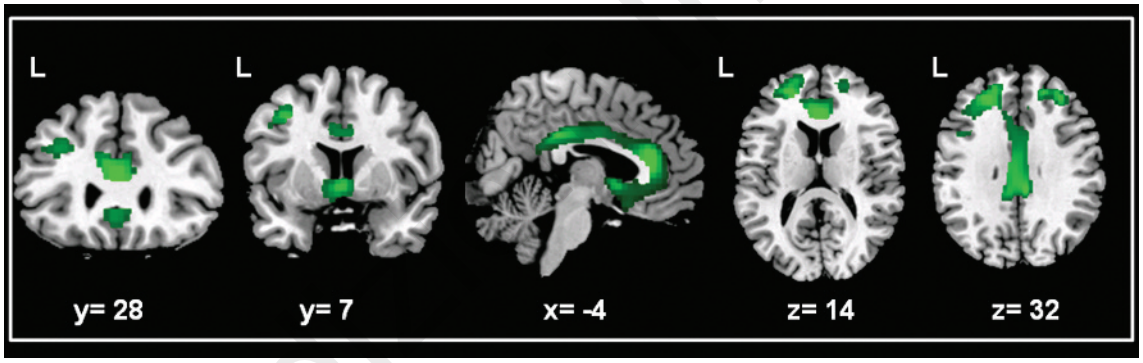

Figure 2 - Associations between DM1 patients' genetic characteristics and regional GM volumes.
The green areas represent the brain regions in which patients' gray matter volumes are directly associated with CTG triplet expansion. These areas are overlaid on a T1-weighted template. Statistical threshold: p<0.05, family-wise error corrected at cluster level. See text for further details.

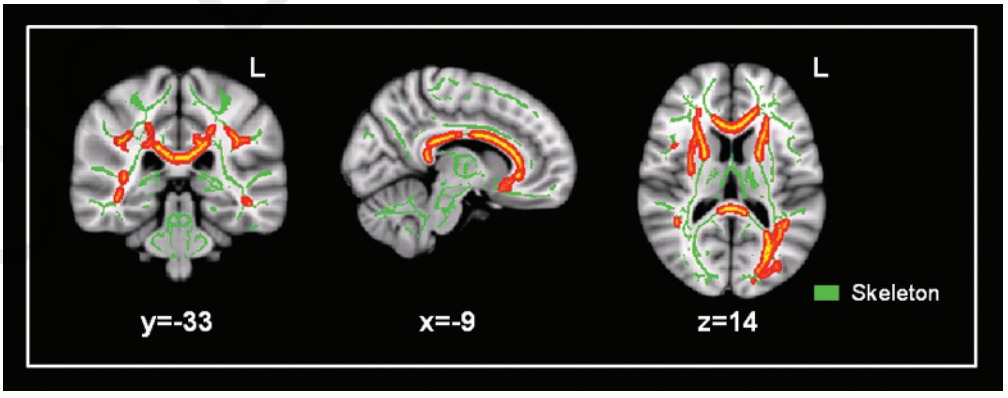

Figure 3 - Pattern of microscopic white matter damage in patients with DM1.
The yellow-orange areas represent the white matter regions in which DM1 patients' fractional anisotropy (FA, an index of tissue integrity) was significantly lower than in healthy controls. Statistical threshold: p<0.05, family-wise error corrected at cluster level for multiple comparisons. These areas are overlaid on a T1-weighted template. The FA skeleton resulting from the tract-based spatial statistics analyses is shown in green. The opposite contrast (healthy subjects less than DM1 patients) was not significant. See text for further details.

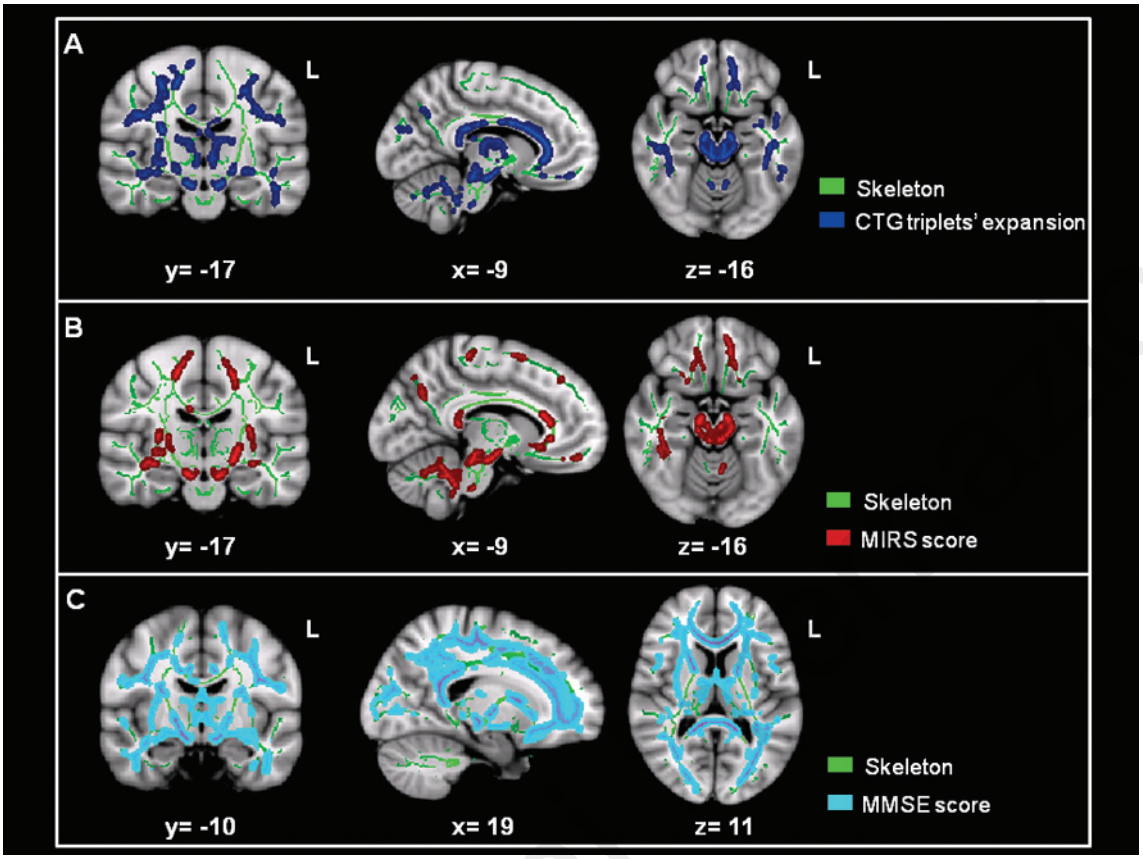

Figure 4 - Associations between patients' microscopic white matter integrity and genetic, clinical and neuropsychological features. DM1 patients' fractional anisotropy (FA, an index of tissue integrity) in widespread white matter areas was found to correlate with CTG triplet expansions (panel A; shown in dark blue), MIRS scores (panel B; shown in red), and MMSE scores (panel C; shown in light blue). In all cases, the statistical threshold was set at $p<0.05$ cluster-level corrected for multiple comparisons. Areas of association are overlaid on a T1-weighted template. In all cases, the FA skeleton resulting from the tract-based spatial statistics analyses is shown in green. See text for further details.

Discussion

In this study, using quantitative MRI techniques, we investigated the presence and extent of brain tissue damage in patients with DM1, considering both the WM and the GM. Furthermore, we assessed potential relationships between patients' brain tissue abnormalities and their genetic, clinical and neuropsychological characteristics. The majority of our patients had an adult onset of the disease; from a clinical point of view, most of them had reached the second or third MIRS stage. Consistently with previous results reported by others (Minnerop et al., 2011), we did not find any significant correlation in the patients between CTG triplet expansion and MIRS scores, age, depression, anxiety or sleep disorders. In contrast, we found an interesting association between the patients' severity of genetic abnormalities and years of formal education.

From a neuroimaging perspective, we found a widespread distribution of GM atrophy in the DM1 patients, which was strongly associated with their clinical and cognitive features. Analyzing the anatomical distribution of the patients' GM loss, this was found to involve brain areas that are implicated in either cognitive (i.e., cingulate and supramarginal gyri, precuneus, lobules VII-VIII and Crus of the cerebellum) or motor functions (i.e., pre- and postcentral gyri). This latter aspect may extend pathophysiological knowledge of motor dysfunction in DM1, which is traditionally dominated by muscular impairment, but might also in some respects depend on involvement of motor networks (Koch et al., 2010). Assessment of the cognitive profile of our DM1 patients showed that most were suffering from mild cognitive deficits. Just one patient met the DSM-IV-TR criteria for mental retardation (APA, 2000). Even though the cognitive impairment in our patient population was found to be mild, they were nevertheless found to show associations between cognitive efficiency, CTG triplet expansion, and, more importantly, regional GM volumes in brain areas critical for cognition. These findings provide support for the still debated theory that there exists a strict relationship between CTG triplet expansion and level of cognitive impairment in myotonic dystrophy (Jaspert et al., 1995; Perini et al., 1999; Marchini et al., 2000), and also suggests that the mechanism through which genetic abnormalities may produce neuropsychological deficits is mediated by changes in specific GM areas. In the cross-sectional analysis, the DM1 patients were found to be more atrophic than the con-

trols in the posterior cingulate cortex and precuneus and, consistently, they performed poorly on tests assessing visuospatial short-term memory and constructional apraxia. It has previously been proposed that the "contents" of visuospatial short-term memory are stored in the precuneus (Christophel et al., 2012; Mok, 2012), while the posterior cingulate cortex plays a critical role in successful retrieval of short-term memory information (Herron et al., 2004; Koch et al., 2006). Moreover, in a study focusing on another model of neurodegeneration, we recently demonstrated the critical role of the posterior cingulate cortex and precuneus in constructional abilities (Serra et al., 2014). The pattern of regional GM loss that we observed in the present study might therefore account for the patients' short-term memory and visuospatial deficits, which have already been reported by others as typical features of DM1 (Meola et al., 1999; Modoni et al., 2008). In addition we also demonstrated an association between patients' levels of general cognitive efficiency and their ability in constructional apraxia as assessed by Copy of drawings.

In the present study, we extensively investigated the WM of patients with DM1. From a macroscopic point of view, we were unable to find significant differences in WML volumes between the DM1 patients and the HSs. Nonetheless, the WML volumes in the patients correlated with both CTG triplet expansion and MMSE scores, thus suggesting that they might contribute to DM1 pathophysiology. The lack of between-group differences in WML volumes might be merely due to a large inter-subject variability in these DM1 patients, as suggested by their wide standard deviations. Studies including larger populations of DM1 patients are needed to better clarify this issue. We also documented that DM1 patients, compared with the HSs, have prominent WM lesion sizes in frontotemporo-insular regions. This anatomical distribution is, at least partially, consistent with the DM1 patients' cognitive profile.

The TBSS analysis method, based on diffusion imaging data, is able to provide indirect information on the microscopic integrity of the WM tissue, even in the absence of macroscopic lesions detectable on conventional MRI. Consistent with Minnerop et al. (2011), our analysis revealed a widespread reduction of FA in most WM tracts of both hemispheres, including the corpus callosum, fronto-occipital fasciculus, anterior cingulum, and inferior and superior longitudinal fasciculi. All these tracts are involved in the connections between major association areas, and their structural damage is likely to participate in determining cognitive deficits in DM1 patients. In support of this interpretation, our analysis revealed, in the patients, significant associations between FA in the major fiber tracts and genetic, clinical and cognitive characteristics. Comprehensively, all these findings indicate that DM1 can, in several ways, be regarded as a disconnection syndrome. Unfortunately, diffusion imaging is unable to provide information on the specific pathological substrates of the brain tissue damage and on the possible causal relationships between GM and WM involvement (Bozzali and Cherubini, 2007).

Considering our results together with those reported by Minnerop et al. (2011), we speculate that DM1 probably first targets the WM tissue, which produces secondary degeneration of the GM tissue. As previously shown in predominantly degenerative conditions, too, brain disconnection may itself induce GM degeneration (Gili et al., 2011). To better address this issue, future longitudinal studies involving patients at early disease stages are needed. With respect to the pathological substrates underlying the tissue damage occurring in DM1 brains, which remains a controversial issue (Wozniak et al., 2011; Franc et al., 2012), future studies employing other quantitative MR techniques, such as magnetization transfer (Giulietti et al., 2012), might help to increase our understanding.

The main limitation of the present study, which has to be regarded as explorative, is the small sample size. Future investigations on larger populations of patients are needed to confirm and extend our findings. On the other hand, the results presented here are consistent with previous literature data (Minnerop et al., 2011), and were obtained in a completely data-driven fashion. However, the strong significance of the effect we detected in this small sample (10 patients only) suggests it may be rather strong.

In conclusion, this study confirms that DM1 is characterized by a prominent involvement of brain tissue, as indicated by correlations between genetic and neuroimaging data. The WM tissue is widely involved, showing macro- and microscopic abnormalities that strongly suggest a role of disconnection mechanisms in DM1. Nevertheless, the GM is also affected by disease, and the regional distribution of the GM damage fits well with the cognitive features observed in the DM1 patients. This is an aspect also relevant to clinical neurology, and should increase awareness among clinicians of the need to look in greater depth at the cognitive aspects of DM1 in terms of diagnosis as well as neurorehabilitation.

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