Structural and functional neuroimaging in Myoclonus-Dystonia

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Severity of dystonia is correlated with putaminal gray matter changes in Myoclonus-Dystonia

R J. Beukers¹, J.N. van der Meer², S.M. van der Salm¹, E.M. Foncke¹, D. J. Veltman³, M.A.J. Tijssen¹

¹Academic Medical Centre, University of Amsterdam, department of Neurology.
²Academic Medical Centre, University of Amsterdam, department of Clinical Neurophysiology.
³Academic Medical Centre, University of Amsterdam, department of Psychiatry.

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Abstract

Background: Myoclonus-Dystonia (M-D) is an autosomal dominantly inherited movement disorder characterized by myoclonic jerks and dystonic postures or movements. Morphometric studies have been performed in other, mainly heterogenous, types of dystonia producing conflicting results. However, all these studies agree on abnormalities in sensorimotor structures, mainly in the basal ganglia. We aimed to study gray matter volumes in sensorimotor brain structures with magnetic resonance imaging (MRI) in a genetically homogeneous form of dystonia; Myoclonus-Dystonia.

Methods: Twenty-five clinically affected DYT-11 mutation carriers (MC), and 25 matched control subjects were studied using T1-weighted 3D anatomical images of the entire brain, obtained with a 3.0 Tesla MRI. MC were clinically scored using the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) and the Unified Myoclonus Rating Scale (UMRS). Gray matter volumes in sensorimotor cortices and basal ganglia of patients and controls were compared and multiple regression analyses were used to correlate the gray matter volumes of patients with the clinical rating scales BFMDRS and UMRS.

Results: No significant differences were found between groups, but dystonia severity in mutation carriers was strongly correlated with increased gray matter volume in bilateral putamina.

Conclusions: This study provides further evidence for the involvement of putamina as important motor structures in the pathophysiology of (Myoclonus-) Dystonia. Changes in these structures are associated with the severity of dystonia.
Changes in putaminal gray matter

Introduction

Myoclonus-Dystonia is a rare movement disorder, clinically characterized by the presence of myoclonic jerks and dystonic postures or movements.\(^1\) Inheritance is autosomal dominant, frequently caused by mutations in the epsilon-sarcoglycan gene (SGCE) on chromosome 7q21 (DYT-11), the function of which is incompletely understood.\(^2,3\) Penetrance of M-D is highly dependent on the parental origin of the gene, most likely caused by the mechanism of maternal imprinting.\(^4\) Patients inheriting the gene from their father have the M-D phenotype in more than 90% of cases. Only a few cases inheriting the gene from their mother with the M-D phenotype have been described.\(^5\) The pathophysiology of M-D is largely elusive, it is considered a dystonia-plus syndrome with the basal ganglia being thought to play a major role in dystonia.\(^6,7\) Neuronal models of dystonia have postulated hyperactivity of the direct putamino-pallidal pathway with reduced inhibitory output of the internal segment of the globus pallidus (GPi), with subsequently increased thalamic input to the (pre-) motor cortex, resulting in excessive motor cortex excitation.\(^7\) A relatively new idea is that this altered function could lead to (micro)anatomic changes through cortical plasticity.\(^8\) Morphometric brain imaging studies investigating these structural changes have been published regarding various types of dystonia, i.e. primary generalized dystonia, cervical dystonia, focal hand dystonia and blepharospasm, or a combination of these types, producing conflicting results.\(^9-15\) All these studies agree on abnormalities in sensorimotor structures, mainly in the basal ganglia.

Based on current neuronal models and these previous studies in different types of dystonia, we hypothesized that gray matter volume in motor structures, i.e. basal ganglia, cerebellum and sensori-motor cortex, are altered in M-D patients when compared to control subjects. We also hypothesized that these structural changes are associated with clinical parameters for dystonia and myoclonus severity.

Subjects and Methods

Subjects

25 DYT-11 mutation positive manifesting carriers (MC) (13 women, median age 50 years old, range 22-75) and 25 age- and gender matched healthy control subjects
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(13 women, median age 50 years old, range 18-78) were studied. Twenty-one MC inherited the mutation from their father, 4 inherited the mutation from their mother. These 4 patients only showed mild dystonia without myoclonus. All subjects were right handed. The severity of myoclonus and dystonia in patients were assessed using the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS)\(^{16}\) and the Unified Myoclonus Rating Scale (UMRS).\(^{17}\) Psychiatric history was positive in 17 of the MC, diagnosed by a psychiatrist using Diagnostic and Statistical Manual (DSM-IV) criteria. None of the control subjects had a psychiatric history. Subject characteristics of MC are summarized in Table 1. All MC and control subjects gave written informed consent and the study was approved by the local medical ethics committee.

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<th>BFMDRS</th>
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Image acquisition and processing

T1-weighted 3D FFE anatomical images of the entire brain were obtained with a 3.0 Tesla MRI system (Philips Intera, Best, the Netherlands) using the the following pulse sequence parameters: field of view 256x256 mm²; scanning matrix, 256x256; 170 slices; slice thickness, 1 mm; sagittal slice orientation; TE = 4.6 ms; TR = 25 ms; SENSE-factor, 2.5. All images were converted to Analyze format and processed with SPM8 (Wellcome Department of Cognitive Neurology, London, UK) and the DARTEL toolbox, in MATLAB version 7.3.0 (2006b) (The Mathworks, MA, USA). Several methods of volumetry have been used in volumetric studies; of these, the fairly recent development of voxel-based morphometry (VBM) using Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) allows reliable detection of differences in gray matter through spatial normalization and warping onto a template created specifically for the study population, instead of warping to standard (MNI) space. First, segmentation into white matter (WM) and gray matter (GM) maps was performed using default priors. A group-specific GM template was created and all GM maps were subsequently warped to this template. Finally, the warped GM images were smoothed using an 8 mm Gaussian filter. To avoid edge-effects, implicit masking was performed using an absolute threshold of 0.05.

Statistical analysis

All statistical analyses were performed on the whole group of 25 DYT-11 mutation positive manifesting carriers and 25 age- and gender matched healthy control subjects. Because of the lack of myoclonus in the four patients who inherited the mutation from their mother and the discussion of maternal imprinting we also performed the analysis without these 4 patients. Groups were compared using one-way ANOVA, with age, gender, and total WM/GM , as well as presence of depression and anxiety as covariates to remove regional differences between groups due to these potential confounders. Secondly, multiple regression analyses were performed to assess correlations of the clinical parameters myoclonus and dystonia severity (UMRS and BFMDRS) and gray matter volumes. In these analyses, age, gender, and total GM- and WM-volume as well as presence of depression and/ or anxiety were similarly included as additional regressors. To rule out myoclonus severity as a potential confound in the regression analysis assessing the effect of dystonia on GM volume, myoclonus
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severity as measured with the UMRS was added as a regressor in the analysis regarding dystonia. Also, to rule out dystonia severity as a potential confound in the regression analysis assessing the effect of dystonia on GM volume, severity of dystonia as measured by the BFMDRS was added as a regressor in the analysis regarding myoclonus. We set an initial height threshold of $p<0.001$ uncorrected, with a voxel extent threshold of 20. Based on our a-priori hypothesis regarding involvement of motor structures, a further small volume correction ($p<0.05$ corrected for multiple comparisons) was performed using a sphere with a radius of 10 mm at regions of interest. This method has been used previously in a number of VBM studies.\textsuperscript{12,20}

**Results**

No significant differences in age ($p=0.626$), total volume of gray ($p=0.313$) or white ($p=0.808$) matter were observed between groups. When patients were compared to healthy control subjects, no significant differences in grey matter volume were detected between groups in either analysis (with and without MC inheriting the mutation from their mothers). In the multiple regression analyses, dystonia severity as measured by the BFMDRS was strongly associated with increased gray matter volume in the bilateral putamina of all MC after correction for confounding variables such as age, gender, and total WM/GM, as well as presence of depression, anxiety and severity of myoclonus (Figure 1, Table 2).
Changes in putaminal gray matter

**Figure 1:** Regression analysis of all mutation carriers (n=25). Correlation of bilateral putaminal gray matter volume with severity of dystonia rating scale.

![Regression analysis of all mutation carriers](image)

Figure Legend: Correlation of dystonia with putaminal gray matter, corrected for the effect of myoclonus, projected on the average gray matter template of all participants (height threshold p 0.005 for graphical purposes, voxel extent threshold 40).

**Table 2:** Multiple regression analysis. Montreal Neurological Institute (MNI) coordinates and significance values of changes in gray matter density.

<table>
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<tr>
<th>Patients</th>
<th>N</th>
<th>Scale</th>
<th>side</th>
<th>structure</th>
<th>MNI (x, y, z)</th>
<th>Z</th>
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<td>-</td>
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<td>putamen</td>
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<td>-</td>
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<tr>
<td>from their father</td>
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<td>putamen</td>
<td>-21, 5, 12</td>
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<td>26, -16, 7</td>
<td>4.36</td>
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</table>


Similar results were found in the 21 MC inheriting the mutation from their fathers, with slightly lower Z-values (**Table 2**, not shown in **Figure**). A graph of fitted responses was plotted for relative gray matter density in the right putamen as a function of BFM dystonia rating scale, see **Figure 2**. The y-axis represents the
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percentage of relative gray matter density increase (0.01 = 1%) for that particular voxel relative to the average gray matter density of all subjects (0 on the y-axis). No correlations were found in any other of the sensorimotor structures.

Figure 2: Correlation of dystonia severity with gray matter density in the putamina.

Figure Legend: Fitted responses for gray matter density in both putamina as a function of Burke-Fahn-Marsden dystonia rating scale (BFMDRS). The y-axis represents the percentage of relative gray matter density increase (0.01 = 1%) for that particular voxel relative to the average gray matter density of all subjects (0 on the y-axis).

No significant correlations were found between myoclonus severity and gray matter volumes.

Discussion

In the present VBM study, we demonstrated a clear relationship between severity of dystonia and bilateral putaminal gray matter volume. A strong point of this study is the genetically homogeneous study population and the relatively large number of participants. A possible limitation to this study is the fact that there was only slight dystonia present in a relatively large number of M-D patients, this could also be an explanation for why no significant differences were found in the group comparisons between patients and healthy control subjects.

Two previous morphometric studies, in writer’s cramp and focal hand dystonia, reported conflicting results, i.e., a decrease and an increase in somatosensory and motor cortex volume.9,10 Two studies in cervical dystonia reported an increase
Changes in putaminal gray matter

in gray matter (GM) volume predominantly in the GPi\textsuperscript{11,12}, whereas a third study found a GM increase in the caudate nuclei, thalamus and right cerebellum.\textsuperscript{13} In this latter study, a decrease in GM was found in bilateral putamina. Two morphometric studies regarding blepharospasm found GM increases in both putamina.\textsuperscript{14,15} Morphometric changes in the GPi have been noted in two studies regarding generalized-, cervical- and focal hand dystonia, although these studies reported an increase rather than a decrease of gray matter in patients.\textsuperscript{11,12} Despite these conflicting results, the studies agree on abnormalities in sensorimotor structures, mainly in the basal ganglia and, to a lesser extent, in the cerebellum and sensory and motor cortices.

Two plausible explanations can be put forward for the phenomenon of altered gray matter volumes in dystonia. First, these increases in gray matter could be primary and hence underlie the involuntary movements through altered neuronal activity patterns. Alternatively, this increase could be secondary to excessive involuntary movement, causing reactive changes in gray matter due to neuronal plasticity. Physiological neuronal plasticity has been demonstrated in healthy subjects learning a complex motor skill (i.e. juggling) and has been shown to be reversible.\textsuperscript{21} Aberrant plasticity of the sensorimotor circuitry is considered to be an integral part of the pathophysiology of dystonia\textsuperscript{22} and has been clearly demonstrated in patients with focal hand dystonia\textsuperscript{23}, although it is as yet unclear whether these changes might also be reversible. Either way, these results provide additional evidence of motor structure involvement in the pathophysiology of dystonia.

In dystonia, the hypothesis of hyperactivity of the putamina is supported in M-D by an \textit{SGCE} knockout mouse model, in which a clinical phenotype of M-D was coupled with a marked increase in striatal dopamine levels.\textsuperscript{24} Furthermore, an IBZM-SPECT study in M-D patients by our group showed decreased striatal D2 receptor availability, possibly reflecting increased endogenous dopamine, again consistent with this theory.\textsuperscript{25} On the other hand, abnormalities of sensory input processing in dystonic patients have also been reported, which are reflected by the ‘geste antagoniste’.\textsuperscript{26} Several observations strongly support the idea that sensorimotor integration is impaired in (myoclonus)-dystonia.\textsuperscript{6,27} Findings of an fMRI study in M-D by our group are consistent with this hypothesis, showing altered activation patterns in sensori-motor cortices and cerebellum.\textsuperscript{28} Regarding the hypothesis that this altered function may lead to (micro)anatomic changes
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through cortical plasticity, altered cortical excitability has been found using TMS studies in DYT-1 dystonia, although, interestingly, this observation could not be reproduced in M-D.

A number of volumetric studies are available in adult psychiatric study populations, which is important because the psychiatric co-morbidity in our study population may constitute a possible confound in our analyses. However, we corrected for this using depression and anxiety as additional regressors in all analyses. In a morphometric study in OCD, neither motor cortex nor the basal ganglia showed changes in gray matter when compared to control subjects, although “contamination/washing” behaviour severity was negatively correlated with right caudate GM in a multiple regression analysis. A VBM study in acute depression showed a decrease in hippocampal gray matter in patients, but no differences in motor structures. A study performed in panic disorder found differences in gray matter volumes in the left insula of patients, left superior temporal gyrus, the midbrain, the pons and right anterior cingulate cortex, but again no changes in any of the known motor structures is reported. To summarize, none of these VBM studies in psychiatric disorders reported any changes in motor structures, rendering it highly unlikely that our results should be attributed to psychiatric symptoms in our patients.

Another possible confounder to our results could be medication use of our patients, especially neuroleptics. A recent systematic review regarding the effect of antipsychotic medication on brain structure included 33 VBM studies and reported an association between use of typical neuroleptics and increase of basal ganglia gray matter volume. This relationship was not found with usage of atypical neuroleptics. It remains unclear if there is a causal relationship between the found changes and medication usage. Because none of our patients use typical neuroleptics, we feel the neuroleptics usage in our patient group (only patient 19) is unlikely to have influenced our results. Regarding antidepressant usage, a VBM study in depressed patients found no differences in patients continuing their medication during the 3 year study period vs. those who stopped taking medication. It therefore seems unlikely that antidepressant usage in our patient group influenced our results. No studies were found regarding benzodiazepines and brain morphometry, a possible confounding effect of their usage on our results can therefore not be excluded.
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Apparently, myoclonus does not contribute to the observed results. Little is known about the myoclonus in M-D, other than that its origin appears to be subcortical, because of the lack of stimulus sensitivity and the absence of giant potentials in somatosensory evoked potential studies. Further studies are needed to elucidate the structure(s) generating myoclonus in M-D, for instance using event related combined EMG-fMRI studies.

In conclusion, this study provides further evidence for the presence of structural abnormalities of motor structures in myoclonus and dystonia in general, and inherited Myoclonus-Dystonia in particular, in addition to all previously found evidence to date, summarized and reviewed by Kinugawa et al in early 2009. Dystonia severity was strongly correlated with abnormal volume of putamina, suggesting that M-D is correctly classified as one of the dystonia-plus syndromes. Future studies should aim to investigate whether these changes are causally related to M-D or secondary, and whether these changes are specific for M-D.

Acknowledgements: The authors thank dr. Fiorella Contarino and dr. Hans Speelman for clinical assessment of all patients.
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