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## **Microstructure of the cerebellum and its afferent pathways underpins dystonia in MYC/DYT-SGCE**

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## **Abstract**

**Background:** MYC/DYT-*SGCE* motor phenotype associates subcortical myoclonus and dystonia. Dysfunction within the cerebellum and basal ganglia are presumed to underpin the expression of the clinical manifestations. However, the microarchitectural abnormalities within these structures and related pathways are poorly known.

**Objectives:** To investigate the microarchitectural brain abnormalities related to the motor phenotype in MYC/DYT-*SGCE*.

**Methods:** We used neurite orientation dispersion and density imaging (NODDI), a multicompartiment model of diffusion neuroimaging to compare microarchitectural neurites organization in MYC/DYT-*SGCE* patients and healthy volunteers (HV). Neurite density index (NDI), orientation dispersion index (ODI) and free water fraction (ISOVF) were derived. Fractional anisotropy (FA) and mean diffusivity (MD) derived from the classic diffusion tensor model were complementarily analyzed. We also investigated regions related to the severity of myoclonus and dystonia and, using a tractography analysis, we studied the pathways that correlated with severity of motor symptoms.

**Results:** 18 MYC/DYT-*SGCE* and 24 HV were included in the final analysis. MYC/DYT-*SGCE* patients showed an increase of ODI and a decrease of FA within their motor cerebellum. More severe dystonia was associated with a lower ODI, NDI and a higher FA indexes within motor cerebellar cortex, as well as with lower NDI and higher ISOVF and MD indexes within the cortico-ponto-cerebellar and spino-cerebellar pathways. No association was found between myoclonus severity and NODDI parameters.

**Conclusions:** In MYC/DYT-*SGCE*, we found microstructural reorganization of the motor cerebellum. Moreover, structural change in the cerebellar afferent pathways that relay inputs from the spinal cord and the cerebral cortex were specifically associated with the severity of dystonia.

## Introduction

Myoclonus dystonia due to pathogenic variant in *SGCE* (MYC/DYT-*SGCE*) is mainly characterized by childhood onset subcortical myoclonus and dystonia (1). The exact pathophysiology of different motor symptoms of MYC/DYT-*SGCE* remains poorly understood (2). As in other dystonic syndromes, the dystonia and myoclonus in MYC/DYT-*SGCE* have been associated with dysfunctions involving primarily the cerebellum (3–5,2), the basal ganglia (6–8) and the sensorimotor cortex (9–11) and thus are considered to result from the motor system dysfunction (12). However, it is unclear which structures and pathways of the cortico-basal ganglia-cerebellar motor network preferentially contribute to the expression of symptoms in various types of dystonias.

Diffusion-weighted imaging (DWI) is an effective *in vivo* tool to investigate properties and potential abnormalities of white matter tracts and grey matter microstructure (13). Previous imaging study of the motor pathways in MYC/DYT-*SGCE* showed increased white matter volume and fractional anisotropy with a decreased mean diffusivity in the subthalamic area of the brainstem and red nucleus and decreased mean diffusivity in the cortical sensorimotor areas (14), suggesting abnormalities in pathways connecting the cerebellum with the thalamus. However, such alteration in FA and MD might be the consequence of different developmental processes such as myelination (15) or deafferentation phenomena (Wallerian degeneration or diaschisis) (16). Indeed, classic diffusion tensor imaging approaches are limited in providing specific information with regards of the microarchitectural anomalies of grey and white matter (17), which might be a key pathophysiological feature in this disorder. Recently, study using the CRISPR/Cas9 editing of a human cell line with a *SGCE* heterozygous mutation, showed more complex neurite morphology in these neurons (11). Moreover, the location of the abnormalities within the motor network tracts has not yet been properly investigated in MYC/DYT-*SGCE*.

We hypothesized that microstructural abnormalities in the motor network are implicated in the *MYC/DYT-SGCE* pathogenesis. To test this hypothesis, we used a neurite orientation dispersion and density imaging (NODDI), which is an emerging accurate diffusion MRI method to probe microstructural complexity of dendrites and axons (13,18). This model is more successful compared to classical DTI models in characterizing microstructural changes (19) as was shown by histological analyses of spinal cord lesions in multiple sclerosis (20). NODDI separates the signal arising from different tissue compartments and estimates the density and fanning of neurites, as well as the partial volume contamination from cerebrospinal fluid (13). In the NODDI model, each voxel is assumed as a combination of three compartments: intracellular (modeled as restricted anisotropic non-Gaussian diffusion), extracellular (modeled as hindered anisotropic Gaussian diffusion), and cerebrospinal fluid (CSF; modeled as isotropic Gaussian diffusion). The common measures used are : i) the neurite density index (NDI) which reflects the density of neurites, ii) the neurite orientation dispersion index (ODI) which indicates the fanning of neurites when increasing and iii) the isotropic volume fraction (ISOVF) which reflects extracellular free-water (18). Thus, NODDI provides a qualitative indication of the brain tissue microarchitecture and allows to infer about the histopathology and mechanisms involved in pathophysiology (20). NODDI has been employed in various physiological and pathological conditions such as ageing (21), Parkinson's disease (22), stroke (23) or amyotrophic lateral sclerosis (13).

In this study, we applied NODDI analysis alongside classic diffusion tensor model analysis to examine microarchitectural abnormalities and their potential association with the motor phenotype in *MYC/DYT-SGCE*. Furthermore, we employed tractography analysis to precisely pinpoint and localize these potential abnormalities within white matter pathways.

## **Methods**

### ***Subjects***

All participants signed an informed consent. All the procedures contributing to this work comply with the ethical standards of the relevant national (CPP/AU-1360) committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Patients' inclusion criteria were i) an age between 15 and 60 years old, ii) no botulinum toxin injections for at least three months prior to participation, iii) no pharmacological treatment modification for at least one month prior to the study onset. Patients and HV were matched for gender, age, handedness, and educational level. Disease severity was assessed using the Burke-Fahn-Marsden scale (BFM) (24) for dystonia and Unified Myoclonus Rating Scale (UMRS) for myoclonus at rest (section 2) and with action (section 4) (25).

### ***Imaging acquisition***

All scans were acquired on the 3T MRI scanner (Siemens Prisma, Germany) with a 64-channel head coil at the ICM neuroimaging platform.

3D T1-weighted MP2RAGE sequence was acquired in sagittal orientation, at 1 mm isotropic voxels resolution, with an in-plane matrix of 232 x 256 and 176 slices, TR = 5000 ms, TE = 2.96 ms, TI = 700 / 2500 ms, flip angle = 4 / 5°, grappa acceleration factor 3.

A multi-shell diffusion tensor imaging (DTI) acquisition was also performed (TR = 3.5s, TE = 75ms, Multi-band of 3, isotropic voxel size 1.75mm, 60 direction with b = 2000 s/mm<sup>2</sup>, 32 direction with b = 1000s/mm<sup>2</sup> and 8 direction with b = 300s/mm<sup>2</sup>, one b = 0 was each 10 directions).

### ***Anatomical imaging preprocessing***

T1-weighted images were background denoised (26) segmented and normalized to the Montreal Neurological Institute (MNI) space using the Computational Anatomy Toolbox (CAT12 -

<http://www.neuro.uni-jena.de/cat/>) implemented in SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>).

### ***Diffusion-weighted MRI preprocessing***

Data pre-processing was performed using the FMRIB software library (FSL, <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) and included correction for motion and eddy currents, tensor reconstruction, non-linear registration, alignment to the MNI space and thresholding data at 0.2. The EDDY Quality Control tools calculated the average signal to noise ratio (SNR) across all voxels within the brain mask to give for each subject a summary measure of the overall quality of the data (27). Subjects exceeding 1.5 SD for the SNR or the absolute motion were removed. After preprocessing, diffusion-weighted volumes were used for NODDI and tractography analysis.

### ***Demographic and clinical data analysis***

Statistical analysis was performed using the Statistical Package for Science (SPSS) version 21 (SPSS Inc. Chicago, IL, USA). Comparability between groups was tested using independent t-test and Fisher's exact test.

### ***DTI models analysis***

DWI NODDI models were fitted using Accelerated Microstructure Imaging via Convex Optimization (AMICO) toolbox. We used the usual NODDI measures: NDI, ODI and ISOVF indexes. Diffusion tensor models were fitted using DTIFIT, implemented in FSL with default parameters, to generate maps of fractional anisotropy (FA) and mean diffusivity (MD). Individual subjects' maps were smoothed (FWHM = 5 mm) and masked with subject's brain template (i.e union of their grey and white matter) derived of their anatomical imaging.



Voxel-wise statistical analysis of NODDI (NDI, ODI and ISOVF maps) and diffusion tensor (FA and MD maps) data were performed using the general linear model approach of SPM12. We used independent-sample t-tests to compare MYC/DYT-*SGCE* and controls. For patients, we implemented UMRS and BFM scales as covariable of interest to identify clusters associated with these variables. The peak statistical threshold was set to  $p < 0.001$  with a cluster level threshold of  $p < 0.05$  corrected with false discovery rate (FDR) method for all these analyses.

### ***Tractography analysis***

To specify the pathways concerned by the white matter clusters derived from the DTI models analysis, we performed a probabilistic tractography analysis. We investigated the main inflow and outflow pathways of the cerebellum and basal ganglia, namely: the cerebello-thalamic (CTP), cortico-ponto-cerebellar (CPCP), spino-cerebellar (SCP), motor cortico-striatal (mCSP) and motor thalamo-cortical (mTCP) pathways. We used FSL 6.0 with de default parameters at each step of the procedure. First, we used “bedpostx” implemented in FSL to model crossing fibers within each voxel of the brain (28). Three fibers were modelled in each brain voxels and we set the number of iterations before starting the sampling to 1000. Then, we used ProtrackX (‘probtrackx2’) (29) to reconstruct the fibers of the CTP, CPCP, SCP, mCSP and mTCP with the default 0.5mm step length, 5000 samples and 2000 steps and enabled loopcheck settings. These tractography analysis were performed following the physiological directions of these pathways of interest and for each side separately (accounting for decussation when appropriate) and guided using waypoint, termination, and exclusion masks according to the NODDI and diffusion tensor results and the neuroanatomical knowledges (see supplementary materials). The masks used were: a merged mask of pre-SMA and primary motor cortex, thalamus, putamen derived from the automated anatomical labeling atlas (AAL) (30). According to the NODDI and diffusion tensor results, we choose the cerebellum VI (AAL) as cerebellar seed.

We built masks (using FSLeves) of the upper spinal cord, as well as the bilateral superior and middle-inferior cerebellar pedunculi. For each subject, individual tractography maps were normalized on their total waypoints. Then, these normalized maps were averaged over the group. The resulting pathway mask of the group was thresholded at 20% of robust range of non-zero voxels and then binarized.

For white matter clusters identified through NODDI and diffusion tensor analysis, we determined the proportion of their voxels located in each pathway as follow:

$$(N_{\text{voxels}_{\text{cluster} \cap \text{pathway}}}) / N_{\text{voxels}_{\text{cluster}}}$$

$N_{\text{voxels}_{\text{cluster} \cap \text{pathway}}}$  represents the count of voxels that belong to both the cluster and the specific pathway, while  $N_{\text{voxels}_{\text{cluster}}}$  indicates the total voxel count within that cluster.

### ***Data Sharing***

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

## **Results**

### ***Participants***

Twenty-one MYC/DYT-SGCE and 25 HV were enrolled in the study. Three patients and one HV were identified as outliers due to excessive movement and/or disease severity and were removed from the analysis. Consequently, the final analysis was performed on 18 MYC/DYT-SGCE and 24 HV. As presented in Table 1, groups were comparable for age, sex, handedness, and level of education (all  $p > 0.05$ ). The overall quality of the DTI dataset (SNR) was comparable across groups (mean  $\pm$  SEM; MYC/DYT-SGCE:  $20.82 \pm 0.74$ ; HV:  $21.32 \pm 0.74$ ;  $t_{40} = -0.47$ ,  $p = 0.64$ ).

### ***NODDI analysis results***

As presented in Figure 1A and Table 2, group comparison showed an increase of ODI index in patients with MYC/DYT-*SCGE* within a cluster encompassing the left and the right grey matter of the cerebellum IV, V and VI as well as their associated vermis. There was no difference between groups for NDI and ISOVF indexes.

Regression analysis (Figure 2A) exhibited an associations between BFM and NODDI parameters mainly in the cerebellum and in the brainstem. NDI signal in the white matter of the left middle cerebellar pedunculus, the brainstem (one cluster extended from mesencephalon to the lower pons and a second in the medulla oblongata) was negatively associated with BFM. NDI signal in the grey matter of the right cerebellum VIII and IX was also negatively associated with BFM. We found a negative association between BFM and grey matter ODI signal in the right cerebellum VI and VIII, the vermis VI, VII, VIII, the right temporal lobe and the right occipital lobe. White matter ISOVF signal in the brainstem at the junction between pons and mesencephalon was positively associated with BFM. There was no association of the UMRS parts 2 and 4 with NODDI parameters.

### ***Diffusion tensor analysis results***

As depicted in Figure 1B and Table 2, group comparisons revealed a reduction in the FA index among patients with MYC/DYT-*SCGE* within the gray matter regions of the right cerebellum IV, V, VI, and VIII, the left cerebellum IV, V, VIIb, and VIII, and the vermis IV, V, VI. This pattern resembled the clusters observed between groups with ODI index. No significant differences between groups were observed for the MD index.

Regression analysis (Figure 2B) demonstrated associations between the BFM score and diffusion tensor parameters, primarily in the cerebellum and brainstem as observed with the NODDI model. Specifically, FA signal in the gray matter of the right cerebellum VI exhibited

a positive association with the BFM. Additionally, a positive association between FA and the BFM was found in the right occipital cortex. MD signal in the white matter of the brainstem (similar to regions negatively associated with BFM and NDI index) displayed a positive association with the BFM. Furthermore, MD was positively associated with the BFM in the left temporal cortex. No association were observed between UMRS parts 2 and 4 and FA or MD indexes.

### ***Tractography analysis results***

The masks of the CTP, CPCP, SCP, mCSP, mTCP are presented in Supplementary figure 1. As presented in Figure 2, white matter clusters obtained with the NODDI analysis were overlaid with these pathways' masks. Importantly, voxels could potentially belong to multiple pathways (31).

For NDI index, white matter clusters obtained from the regression analysis with BFM presented for the extended brainstem cluster (maximal intensity at  $x = -4$ ,  $y = -18$ ,  $z = -20$ ), 81% of its voxels were in the CPCP, 31% in the SCP and 17% in the CTP; for the medulla oblongata cluster (at  $x = 2$ ,  $y = -38$ ,  $z = -51$ ), 68% of its voxels were in the SCP, 9% were in the CTP and 1% were in the CPCP, and for the left middle cerebellar pedunculus (at  $x = -20$ ,  $y = -51$ ,  $z = -39$ ), 48% of its voxels were in the CPCP, 35% in the SCP and 16% in the CTP.

For ISOVF index, the brainstem cluster (at  $x = 14$ ,  $y = -21$ ,  $z = -27$ ) obtained from the regression analysis with BFM had 95% of its voxels in the CPCP, 15% in the CTP and 14% in the SCP.

For MD index, the brainstem cluster (at  $x = 12$ ,  $y = -30$ ,  $z = -34$ ) obtained from the regression analysis with BFM had 61% of its voxels in the CPCP, 32% in the SCP and 2% in the CTP.

## **Discussion**

We used the histologically supported neurite orientation dispersion and density imaging (NODDI) model alongside diffusion tensor model, to investigate the brain microarchitecture in *MYC/DYT-SGCE*. Compared to controls, they exhibited an increase of orientation dispersion index (ODI) and decrease fractional anisotropy (FA) within the motor cerebellar cortex. Greater dystonia severity was linked to lower ODI and Neurite Density Index (NDI) and higher FA within the motor cerebellar cortex. Furthermore, increased dystonia severity was associated with reduced NDI and with a rise in the Isotropic Volume Fraction (ISOVF) and the mean diffusivity (MD) within the cerebellar afferent pathways, specifically the cortico-ponto-cerebellar and spino-cerebellar pathways. Altogether, these findings suggest that the severity of dystonia in *MYC/DYT-SGCE* was primarily related to microstructural abnormalities in the cerebellum and its afferent pathways, which relay inputs from the spinal cord and the cerebral cortex.

Our study suffers from several limitations. Firstly, we have not identified any relation between the NODDI indexes and the severity of myoclonus, which is also the most disabling manifestation of the disease. Indeed, head movement during acquisitions could affect our findings and explain for instance the absence of the structural association with myoclonus. However, the overall quality of the imaging dataset as measured with the SNR was similar between the groups and our findings with regards of dystonia in *MYC/DYT-SGCE* pointed to specific involvement of motor cerebellum (32) and its afferent pathways. Thus, it is unlikely that a systemic bias such as excessive movements may significantly affect our findings. Alternative explanation might be that the structural abnormalities we found do not explain the entire neural activity underpinning myoclonus. Furthermore, the coupling between neuronal activity and neuronal microstructure, as assessed with DWI imaging, may have certain limitations. For instance, the diffusion magnetic resonance signals acquired from organotypic

cortical brain cultures in rats did not show a correlation with the neuronal activity observed through intracellular calcium imaging using fluorescence (33). Further studies using additional imaging modality such as functional MRI are warranted to investigate the anatomical correlates of myoclonus.

Secondly, no structural alterations in the motor cortex have been detected, as previously identified using cell biology techniques (11). It is possible that these alterations are of a subtle nature, making their detection challenging when using diffusion imaging techniques. Future neuroimaging studies, utilizing a higher magnetic field strength, such as 7T, may potentially improve the model's sensitivity. Lastly, the overall mild severity of the disease might also constrain the capacity to identify subtle structural changes. Nevertheless, we had to include patients with mild motor phenotype, thereby ensuring the quality of the imaging dataset.

The ODI metric increased, while the FA metric decreased in the motor cerebellar cortex of patients compared to healthy volunteers (HVs), reflecting likely morphological changes to neurites (34). ODI represents the alignment and coherence of the neurites in a voxel and an increased ODI indicates a higher dispersion of neurites (35). FA characterizes the directionality of water diffusion (36) and, despite the need for caution in interpreting FA in grey matter (37), a decrease in FA during neurodevelopment may indicate an increase in dendritic elongation and branching orthogonal to cortical columns (38). In summary, abnormalities observed in ODI and FA metrics could reflect a higher degree of complexity of the grey matter dendritic trees (39). Indeed, a recent observation reported an increased number of branches and longer branch lengths in dendrites in cortical glutamatergic cells in subjects with *SGCE*-mutation (11). Our results suggest that other cellular lines such as cerebellar neurons can also be affected by these dendritic changes. A first interpretation of these findings may be that structural abnormalities reflect an alteration in the functioning of the cerebellar cortex. In neurodevelopmental disorders, changes in neurite morphology have also been associated with diminished synaptic

efficiency and may result from alterations in neuronal homeostasis (40–44). On the other hand, we observed that the greater the magnitude of these abnormalities in the cerebellar cortex, the lower the severity of dystonia. We also found that a lower severity of dystonia was associated with a higher density of neurite (i.e. NDI) in the motor cerebellar cortex. The direction of these associations might rather indicate that this higher level of organization complexity in the cerebellar cortex reflect a compensatory mechanism, as suggested in X-linked dystonia-parkinsonism (45). However, this interpretation should be considered with caution.

We also found an association between the severity of dystonia in *MYC/DYT-SGCE* and the microstructure of white matter, as reflected by NDI, ISOVF and MD indexes, in both the cerebellum and its afferent pathways, namely the cortico-ponto-cerebellar and the spino-cerebellar pathways. The SGCE protein is expressed within the cortex, cerebellum, and spinal cord (11,46). Dysfunction of these structures can participate in dystonia pathogenesis (47). A recent mouse model featuring *TOR1A* deletion primarily within the spinal cord and dorsal root ganglia had a dystonia phenotype (48). Consistent with these findings, it is plausible that the pathways connecting these anatomical regions could also undergo structural changes associated with the manifestation of the dystonia in patients with *SGCE* mutation.

We found that a higher lower severity of dystonia was associated with a decrease in NDI while increase in ISOVF and MD within the cerebellar afferent pathways. The neurite density index (NDI) metric reflects the space within axons, the Intracellular Volume Fraction (ISOVF) corresponds to the volume fraction of unhindered water (cerebrospinal fluid), while the mean diffusivity (MD) reflects the mean rate of water diffusion within tissues (18,20). It suggests that a lower density of axons in these pathways may underlie a higher severity of dystonia in *MYC/DYT-SGCE*. Indeed, when referring to the histological validation of the NODDI model, chronic multiple sclerosis lesions affected by axonal loss were associated with a tendency toward decreased NDI and FA along with an increased ISOVF and MD (20). In patients with

multiple sclerosis or cerebral palsy, these abnormal NODDI changes were interpreted as reflecting axonal loss, potentially arising from Wallerian degeneration (49–51). However, in *MYC/DYT-SGCE*, earlier observations have indicated an increase in FA and a decrease in MD within the brainstem, suggesting a contrary enhancement of directional axonal growth (14). Taken together, these prior findings, along with our own, might suggest that a higher axonal density in the afferent cerebellar pathways is associated with a lower severity of dystonia, potentially indicating a compensatory mechanism. However, this assumption should be approached with caution. The correlational nature of the analysis prevents us from making definitive inferences about the pathological or compensatory nature of the observed association. Furthermore, the interpretation of the tensor diffusion model in the white matter can be challenging since it is jointly influenced by demyelination, neuroaxonal loss and even changes in the glial component (20). Finally, we did not observe such group differences in these pathways, as previously reported (14). We attribute this discrepancy to some methodological differences. Firstly, we conducted a whole-brain analysis and did not restrict it to the sensorimotor system or perform small volume correction. Secondly, the patients included in our study were younger (mean = 29.33 vs 45.75), which may also contribute to the differences in results.

Within the cerebellum, the highest expression of the *SGCE* is found in the Purkinje cells (52). Thus, the microstructural change we found in the cerebellar grey matter may mostly reflect reorganization of the Purkinje cells neurites. The Purkinje cells send GABA inhibitory projections to the dentate nucleus which in turn displays a disynaptic excitatory connection through the thalamus to the contralateral motor cortex (53–55). The *SGCE* mutation is supposed to primarily underpin a GABAergic deficit due to Purkinje cell dysfunction (5) as demonstrated in other dystonic syndromes (56–58). As discussed above, the microstructural changes in the cerebellar cortex may underlie the dysfunction of Purkinje cells or the compensatory



mechanisms addressing this dysfunction. Alcohol or zonisamide which both enhance the GABAergic transmission improve *MYC/DYT-SGCE* symptomatology including dystonia (2,5,12,59) and thus may help restore the GABA deficiency (5,12,2). Purkinje cells abnormal functioning may result in release the inhibition of the dentate nucleus and enhance its excitatory projections to the motor cortex (5). The increase of metabolism in the cerebellar nuclei was previously found in *MYC/DYT-SGCE*, supporting this assumption (4). Importantly, projections from the cortex and from the spinal cord have an excitatory influence on the Purkinje cells (60,61). Disruption of these cerebellar afferent pathways may alter the Purkinje cells excitatory inputs and reinforce their inhibitory release. Contrary, enhancing these cerebellar inflow pathways may help to compensate the inhibitory release of the Purkinje cell. Involvement of cerebellar afferent pathways in the emergence of dystonia has been previously suggested (62). Notably, silencing of olivocerebellar synapses which constitutes a major Purkinje cells excitatory input, causes dystonia-like movements in mice (63).

The resulting loss of cerebellar inhibition on motor cortex may be a key feature leading to the emergence of dystonia in general (55,64). Moreover, in *MYC/DYT-SGCE*, the effect of this cerebellar inhibitory release on the motor cortex may also be potentialized by an abnormal neuronal intrinsic excitability of their cortical cells as recently demonstrated (11).

We have not come across a strong association between the severity of dystonia and abnormalities in the cerebellar outflow pathway and namely of the cerebello-thalamic pathway. It does not rule out the potential contribution of the cerebellar efferent pathway to the expression of the symptoms, but might indicate a lesser significance in the context of dystonia expression in *MYC/DYT-SGCE*.

Interestingly, microarchitecture of the basal ganglia and their connecting pathways did not exhibit any difference between groups or relation with disease severity. Involvement of the striato-pallido-thalamo-cortical network in the emergence of motor symptoms of *MYC/DYT-*

*SGCE* has been previously suggested (12). Especially, pallidal neuronal activity recordings and the dramatic motor symptoms improvement with deep brain stimulation of the internal globus pallidus support this assumption (7,8). However, it has been well established that bidirectional direct connections exist between cerebellum and basal ganglia (65). Recent mice model of acute knockdown of *SGCE* produced dystonia and jerking movements when applied in the cerebellum but not in the basal ganglia (2). A current model of dystonia suggests that basal ganglia abnormalities could result from abnormal signals arising from the cerebellum (66). Our findings, further support that dysfunction of the cerebellum and cerebellar afferent pathways might be primarily involved in the *MYC/DYT-SGCE* dystonic phenotype.

Finally, we found that a decrease in ODI and an increase in FA in the right occipital lobe, a decrease in ODI the right temporal and, an increase of MD in the left temporal lobe were associated to a higher severity of dystonia. Structural abnormalities of these cortical areas have been occasionally found in focal dystonia (67,68). In *MYC/DYT-SGCE*, a reduced regional cerebral blood flow in the temporal lobe using single photon emission computed tomography has been described (69). The structural abnormalities in occipital cortex with relationship to sensory information processing has been also described in *MYC/DYT-SGCE* (70). Taken together, these results suggest that non-motor cortical areas might contribute to the motor phenotype of the *MYC/DYT-SGCE*, but their exact role remains to investigate. Interestingly, although we found here that an association with the severity of dystonia, these multimodal cortical areas are also involved in high order cognitive functions (71,72). We could suppose that they also contribute to the severity of non-motor symptoms which are part of the *MYC/DYT-SGCE* phenotype (73).

## **Conclusion**

In *MYC/DYT-SGCE*, we showed microstructural changes in the motor cerebellum indicating a higher complexity of its neurite organization. Moreover, the structural alterations of the

cortico-ponto-cerebellar and spino-cerebellar pathways were associated with the severity of dystonia. Our findings further support the role of the cerebellum and afferent cerebellar pathways in *MYC/DYT-SGCE* and refine our understanding of its pathogenesis.

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### **Authors' Roles**

Design: YW, ER, CG; execution: All authors; analysis: CT, CAC, RV; writing: CT, YW, ER, CG; editing of final version of the manuscript: All authors.

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## Figure legends

### Figure 1. Result of NODDI analysis

**A.** Results of the comparison of orientation dispersion index between groups with a contrast patients > healthy volunteers. The peak statistical threshold was set to  $p < 0.001$  with a cluster level threshold of  $p < 0.05$  corrected with FDR method for all these analyses. **B.** Results of the regression analysis of NODDI parameters by Burke-Fahn-Marsden Dystonia Rating Scale in patient groups. The characters “plus” and “minus” represent respectively a positive and a negative association with BFM. The peak statistical threshold was set to  $p < 0.001$  with a cluster level threshold of  $p < 0.05$  corrected with FDR method for all these analyses. HV = Healthy volunteers ; BFM = Burke-Fahn-Marsden Dystonia Rating Scale ; ODI = orientation dispersion index ; NDI = neurite density index ; ISOVF = isotropic volume fraction.

### Figure 2. Cluster localization using tractography

Significant white-matter clusters of the NODDI analysis (in yellow-red color) are overlaid with the cortico-ponto-cerebellar pathway (CPCP) in green, cerebello-thalamic pathway (CTP) in yellow, and spinocerebellar pathway (SCP) in blue. For a better readability, the cortico-striatal and motor thalamo-cortical pathways are not displayed.

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