Microstructural MRI basis of the cognitive functions in patients with Spinocerebellar ataxia type 2

G. Olivito[1,2], M. Lupo[1], C. Iacobacci[1,3], S. Clausi[1,4], S. Romano[5], M. Masciullo[6], M. Molinari[7], M. Cercignani[2,8], M. Bozzali[2,8], M. Leggio[1,4].

1. Ataxia Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy;
2. Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy;
3. PhD Program in Behavioral Neuroscience, Sapienza University of Rome, Rome, Italy
4.Department of Psychology, Sapienza University of Rome, Italy;
5. Department of Neurosciences, Mental Health and Sensory Organs (NESMOS), "Sapienza" University of Rome - Sant'Andrea Hospital, Rome, Italy ;
6. SPInalREhabilitation Lab, IRCCS Fondazione Santa Lucia, Rome, Italy;
7. Neurorehabilitation 1 and Spinal Center, Robotic Neurorehabilitation Lab, IRCCS Santa Lucia Foundation, Rome, Italy.
8. Clinical Imaging Science Center, Brighton and Sussex Medical School, Brighton, UK.

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Corresponding Author

Dr. Giusy Olivito, PhD
Email: g.olivito@hsantalucia.it
Ataxia Laboratory, IRCCS Santa Lucia Foundation, Via Ardeatina 306, 00179, Rome, Italy;
Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Via Ardeatina 306, 00179, Rome, Italy.
Telephone number: #39-06-51501547

Abstract

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative disease involving the cerebellum. The particular atrophy pattern results in some typical clinical features mainly including motor deficits. In addition, the presence of cognitive impairments, involving language, visuospatial and executive functions, has been also shown in SCA2 patients and it is now widely accepted as a feature of the disease. The aim of the study is to investigate the microstructural patterns and the anatomo-functional substrate that could account for the cognitive symptomatology observed in SCA2 patients. In the present study, Diffusion tensor imaging (DTI) based-tractography was performed to map the main cerebellar white matter bundles, such as Middle and Superior Cerebellar Peduncles, connecting cerebellum with higher-order cerebral regions. Damage-related diffusivity measures were used to determine the pattern of pathological changes of cerebellar white matter microstructure in patients affected by SCA2 and correlated with the patients' cognitive scores. Our results provide the first evidence that white matter (WM) diffusivity is altered in the presence of the cerebellar cortical degeneration associated with SCA2 thus resulting in a cerebello-cerebral dysregulation that may account for the specificity of cognitive symptomatology observed in patients.

Introduction

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant neurodegenerative disease characterized by a progressive cerebellar syndrome, typically affecting motor functions (Takahashi et al., 2010). The cognitive performances of SCA2 patients have been exhaustively investigated and it has been shown that patients affected by SCA2 present not only motor impairment but also a cognitive symptomatology (Klinke et al., 2010; Fancellu et al., 2013; Moriarty et al., 2016), mainly involving visuospatial and executive functions (LePira et al., 2002; Kawai et al., 2008).

From a neuropathological point of view, SCA2 present with a macroscopic pattern of olivopontocerebellar atrophy as well with a pattern of neuronal loss in several and in cerebellar cortex, and a diffuse damage of the brainstem and cerebellar white matter (WM) (Durr et al., 1995; Gilman et al., 1996; Iwabuchi et al., 1999; Estrada et al., 1999; Pang et al., 2002). These features have been depicted in vivo by Magnetic Resonance Imaging (MRI) studies using voxel based morphometry (VBM) and diffusion tensor imaging (DTI) (Mandelli et al., 2007; Della Nave et al., 2008a,b). Specifically, the cerebellar vermis and hemispheres show a pattern of extensive GM loss with sparing of the vermian lobules I,II (lingula) and X (nodulus) and of the hemispheric lobules I,II (lingula) and Crus II. Cerebellar WM damage has been shown to affect mainly the peridentate regions and middle cerebellar peduncle (MCP) (Della Nave et al., 2008b). Consistent with the hypothesis that cerebellar atrophy may affect also the regions connected with the cerebellum (Dayan et al., 2016), several supratentorial areas have been found to be altered in SCA2, such as the right orbito-frontal cortex, right temporo-mesial cortex, the primary sensorimotor cortex bilaterally, the right thalamus, the left precentral gyrus and inferior frontal operculum as well as inferior parietal and post-central gyri (Brenneis et al., 2003; Della Nave et al., 2008a). The supratentorial atrophy can be related to both a primary degenerative process associated to SCA2 disease and to secondary effect resulting from the cerebellar deafferentation (Brenneis et al., 2003). Furthermore, the interruption of cerebello-thalamo-cortical pathways has been reported as the

mechanism responsible for crossed cerebello-cerebral diaschisis (Broich et al., 1987; Boni et al., 1992; Komaba et al., 2000).

Thus, it is possible to hypothesize that a disruption of cerebello-cerebral pathway is responsible for structural and functional alteration of cortical areas. Middle (MCP) and Superior cerebellar peduncles (SCP) are respectively the feedback and feedforward limbs of the cerebello-cortical system through which the cerebellum receives information from cerebral regions and then sends back the cerebellar-processed information to accomplish functions successfully. Therefore it is reasonable that white matter alterations of the peduncles may reflect alteration in the cerebello-cortical interactions and may be responsible for patients' cognitive symptomatology.

DTI has proven to be a valuable tool for investigating brain WM since it can probe tissue microstructure by assessing the displacement of water molecules within specific WM tracts (Basser et al., 1994). In the brain the motion of water molecules is hindered by the local microstructure, as they tend to diffuse in preferred directions corresponding to white matter fiber bundles orientation. The diffusion tensor (DT) model is a simplistic diffusion MRI (dMRI) model which assumes only one fiber direction per voxel. It is commonly used to quantify the diffusion process with DT-derived metrics such as fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD), which relate to the tendency of water molecules to move in a particular direction (Alexander et al., 2007; Feldman et al., 2010). The 3D connectivity patterns of WM could be investigating by using WM tractography, a well-established approach which follows coherent spatial patterns in the major eigenvectors of the diffusion tensor field (Alexander et al., 2007) thus providing a model of brain connectivity, through which brain disconnection can be studied.

Using different tracing methods, tractography algorithms are capable of generating anatomically plausible estimates of WM trajectories in the human brain (Alexander et al., 2000, 2007). In particular, more advanced diffusion imaging methods that allow multiple fiber directions to be estimated, such as QBall Imaging (QBI) (Tuch et al., 2004) and the persistent angular structure (PAS) (Jansons and Alexander, 2003) may be particularly suitable in the case of the cerebellum

since they are able to better resolve intersecting crossing WM regions, such as MCP and SCP (Alexander et al.,2007; see also Dayan et al., 2016). Alterations of MCP and SCP dMRI metrics (Della Nave et al, 2008b, 2011; Vieira Karuta et al., 2015) have been demonstrated in degenerative ataxia patients, but no studies specifically used tractography to reconstruct these tracts and assessed voxel-wise diffusivity alterations and their impact on cognitive outcomes of SCA2 patients.

The aim of this work is to investigate the microstructural organization of MCP and SCP, reconstructed via tractography and to investigate the relationship between dMRI metrics and cognitive subscores of SCA2 patients thus providing a novel contribution to understanding the relationship between cerebello-cerebral disconnection likely to be associated with SCA2 neurodegenerative processes and the specific cognitive symptomatology.

Experimental Procedures

Participants

Nine patients affected by SCA2 [F/M=6/3; mean age ± SD = 47,6± 10.2 years], were recruited from the Ataxia Lab of Foundation Santa Lucia Hospital. Both in-patients (admitted for rehabilitation) and out-patients (followed up at the clinic) were included. At the time of assessment, all patients had more than 6 months of illness from the genetically confirmed diagnosis. T2-weighted MRI scans, acquired as part of this research study, were visually inspected by an expert neuroradiologist to ensure the absence of any extra-cerebellar lesion.

The neurological examination of the patients showed that they all presented with a pure cerebellar syndrome, except CA-3 (see Table 1) that presented the Babinski sign. A quantification of cerebellar motor signs was also performed using the International Cooperative Ataxia Rating Scale (ICARS, Trouillas et al., 1997), whose global score ranges from 0 (absence of any motor deficit) to

100 (presence of motor deficit at the highest degree). Demographic and clinical characteristics of the patients are reported in Table 1.

A group of 25 healthy subjects (HS) [F/M=19/6] ranging from 40 to 60 years of age [mean age ± SD = 53.8 ± 5.9 years] with no history of neurological or psychiatric illness were also recruited as control group for the MRI protocol.

This research study was approved by the Ethics Committee of Santa Lucia Foundation, according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from each subject.

Neuropsychological Assessment

SCA2 patients underwent a neuropsychological evaluation according to the evidence that in this pathology specific cognitive domain are mainly involved, in particular visuospatial, executive and attentional abilities (LePira et al., 2002; Kawai et al., 2008). For the cognitive assessment, the following tests were used and then grouped by the functional domains that were measured: (Lezak, 1995):

- Wechsler Adult Intelligent Scale–revised Intelligent Quotient (Wechsler, 1981; Orsini and Laicardi, 1997, 2003) and Raven'47 progressive matrices test (Raven, 1949) to analyze the Intellectual level;

- Rey-Osterrieth Complex Figure Test (recall and copy) (Caffarra, 2002), forward and backword Corsi (Corsi, 1972), and Wechsler Adult Intelligent Scale -revised block design subtest (Wechsler, 1981; Orsini and Laicardi, 1997, 2003) to analyze the visuospatial ability;

- Stroop Test ("time effect" and "error effect") (Caffarra, 2002), semantic, phonological and verbal fluency (Borkowskyet al., 1967), Wisconsinn Card Sorting Test (WCST) (Heaton et al., 2002), and Tower of London procedure (TOL) (Krikorian et al., 1994), to analyze the executive functions;

-Trail Making Test B-A (Giovagnoli et al., 1996) to analyze the attention abilities.

The results of the neuropsychological assessment are reported in Table 2.

MRI acquisition protocol

The MRI examination was performed by using a 3T scanner (Magnetom Allegra, Siemens, Erlangen, Germany) and the following scans were acquired: 1) dual-echo turbo spin echo [TSE] (TR = 6190 ms, TE = 12/109 ms); 2) fast-FLAIR (TR = 8170 ms, 204TE = 96 ms, TI = 2100 ms); 3) 3D Modified Driven Equilibrium Fourier Transform (MDEFT) scan (TR = 1338 ms, TE = 2.4 ms, Matrix = 256 × 224×176, in-plane FOV=250×250mm2, slice thickness=1 mm); 4) diffusion weighted Spin-Echo Echo Planar Imaging (SE EPI) along 61 non-collinear directions (TR=7 s, TE=85 ms, b factor=1000 s/mm^2, 45 contiguous slices volumes with a 2.3mm^3 isotropic reconstructed voxel size). Nine volumes without diffusion weighting (b=0) were also acquired.

MRI imaging and data analyses

DTI processing

Affine registration to the first non-diffusion weighted volume using FSL was done on DTI volumes to correct for eddy currents and small head movements (Smith et al., 2004). After brain segmentation with the BET utility (Smith, 2002), the diffusion tensor (DT) coefficients were computed in Camino (Cook et al., 2006) to generate whole brain maps of the dMRI metrics, including FA and MD. Additionally, to better characterize the tissue microstructure changes AD and RD were also analyzed. Each FA volume was registered to the native space MDEFT volume with a linear registration first, followed by a non-linear transformation. The target for the linear registration was the skull-stripped MDEFT, while the original volume (including skull). was the target for the non-linear transformation. The registration was achieved using the tools FLIRT (Jenkinson et al., 2002) and FNIRT (Andersson et al., 2008) from FSL. This “FA to MDEFT” transformation was combined with each individual “MDEFT to MNI” transformation, obtained by non-linear registration of the MDEFT to the ICBM152 MNI template. This resulted in the final transformation from each participant’s DTI space to the ICBM152 MNI template.

diffusion MRI based Tractography

It has been pointed out previously (Ye et al., 2013) that tractography based on DTI is not able to adequately segment the SCP, and particularly the decussation of the SCP. Here MCP and SCP were reconstructed using tractography based on two multi-fiber models implemented in Camino. Specifically, QBall (Tuch, 2004) was used for MCP, as it provides less false positive fiber components while PAS MRI (Janson and Alexander, 2003) was used for left (LSCP) and right (RSCP) SCP, as it deals more effectively than QBall with fiber crossing. This procedure was optimized in a previous study from our group (Dayan et al., 2016).

Once the multi-fiber directions were estimated, probabilistic tractography was carried out based on these data using the PICo algorithm. N = 10000 tracking iterations were performed from each voxel of the seed Region of Interest (ROI) with stopping criteria of FA $\leq$ 0:1 and curving angle $\leq$ 80°. Five ROIs were manually drawn on the FA map images for MCP tracking. Cerebellar tract reconstruction was performed using the same approach as in Dayan and colleagues (2016). The SCP was segmented separately for each cerebellar hemisphere and two endpoint ROIs were chosen so as to select all the fibers that continue posteriorly to the seed ROI, centered in the dentate nucleus, and to include both the red nucleus and its medial area, contralaterally, where the SCP is known to decussate (see Dayan et al., 2016).

In order to obtain a binary map of the "average tract", every subject's reconstructed MCP, LSCP and RSCP maps were binarised using a probability threshold for probability index of connectivity (PICo) maps computed by in-house software to minimize the amount of tract volume variation with PICo threshold. These images were then warped into standard space using the FA to ICBM152 MNI space transformation previously calculated, and averaged. The resulting maps were thresholded to retain only those voxels that were common to at least 50% of subjects.

Statistical Analysis

Neuropsychological assessment

For each tests, individual raw scores were converted to obtain a mean Z-score for each functional domain. For the tests that lacked published normative data, individual z-scores were calculated with reference to specific control groups using the following formula: (subject raw score – population mean)/population standard deviation (SD). Demographic and performance data of control groups are reported in Table 3.

Published normative data were used for the following tests: Rey-Osterrieth Complex Figure Test, (recall and copy versions), Raven'47 progressive matrices and Trail Making Test. No control subject had history of neurological or psychiatric illness, and all controls were well matched with regard to age and education (independent-sample t-test: p= not significant).

Voxel-wise analysis on white matter tracts

A voxel-wise analysis was performed in order to compare FA and MD ~~changes~~ differences in the white matter between SCA2 patients and HS, restricting the comparison to the voxels of the MCP and SCP, based on the average tract masks obtained as described above. T-contrasts were evaluated with voxel significance set at $p < 0.001$ and corrected for family-wise error (FWE) at cluster level with significance level chosen for $p < 0.05$. In order to better characterize the tissue microstructure both FA and MD were used, while AD and RD were analyzed to help the interpretation of changes to FA and MD (Alexander et al., 2007).

To remove the effect of confounding variables, the analysis was adjusted for age, since statistically significant difference was found between patients and controls as assessed by the t-test analysis ($T=-2.23422$; $p=0.03$). Although there was no difference in gender distribution between groups (chi-square= 0.2962, df=1, $p=0.58$), sex was also set as covariate.

In order to investigate the relationship between WM damage and cognitive impairment Spearman rank-order correlation coefficient was used to analyze possible correlations between individual

values of WM diffusivity, extracted using FSL command line from the FMRIB software library (FSL, www.fmrib.ox.ac.uk/fsl/) and the correspondent neuropsychological scores.

Results

Subjects with cerebellar damage had negative Z-scores for all cognitive domain except for Attention (0.14) (Fig 1).

The results of tractography were visually evaluated in every participant. In order to be deemed successful, the segmentation had to fulfill the following criteria: the MCP included the transverse pontine fibers both posterior and anterior to the corticospinal tracts and the SCP decussation was visible at the level of the midbrain, as expected from known anatomy (see also Dayan et al., 2016). Based on this procedure, MCP and SCPs were successfully reconstructed in all patients and HS. Fig 2 shows the 3D fiber reconstruction for the average MCP and SCP of both groups of subjects. Voxel-wise comparisons between patients and HS were performed for each diffusion metric separately in each tract (MCP, LSCP, RSCP). WM analysis showed a widespread pattern of WM diffusivity alterations to affect MCP, LSCP and RSCP. Indeed, when compared to controls, SCA2 patients showed a significant decrease of FA and a significant increase of MD in all tracts examined. When compared to controls, SCA2 patients also showed a significant increase of both AD and RD to affect both the MCP and SCP, bilaterally.

Results are illustrated in Fig 3 and detailed statistics are reported in Table 4.

Regarding the relationship between WM damage and patients' cognitive scores, Spearman's correlation analysis showed mean LSCP MD to be negatively correlated with the Visuospatial

ability (*R*= -0.67; p=0.05) and mean RSCP MD to be negatively correlated with Executive function (*R*= -0.67; p=0.05).

No correlation was detected between FA and cognitive impairment.

Discussion

In the present study we aimed to investigate the pattern of WM changes associated with cerebellar degeneration in SCA2 patients. dMRI based tractography was used to reconstruct the main cerebellar WM tracts, namely MCP and SCP, and then to evaluate DTI metrics within those tracts. Specifically, FA decrease and MD increase were found bilaterally in MCP and SCP of SCA2 patients compared to controls. This pattern is consistent with the presence of microstructural white matter damage, although at this stage we can only speculate on the underlying pathology. Since the examination of multiple DTI measures may provide more specific information about the tissue microstructure (Alexander et al., 2007), AD and RD were also examined, in order to investigate the combination of AD and RD changes that may underlie the decreased FA in the examined tracts. In the present study, an increase of both AD and RD was also found to affect bilaterally MCP and SCP of SCA2 patients.

FA is widely recognized as a marker of so-called white matter integrity and thus it is used as the primary measure of tissue microstructural damage. Both demyelination and axonal loss can result in reduced FA (Beaulieu, 2002; Song et al., 2002): indeed, when axons packing is not sufficiently dense, more intercellular water will result in a less restriction of diffusion and therefore lowering FA (Feldman et al., 2010). Although FA is a highly sensitive measure of tissue microstructure, it is a non-specific biomarker of neuropathology. Further insight can be gained from the evaluation of MD. Conversely to FA, MD has been shown to negatively correlate with fiber integrity (Beaulieu, 2002; Song et al., 2002). Although the exact pathological correlates of increased MD cannot be established, it has been proposed that it predominantly reflects wallerian degeneration. Present

findings of MD increase thus might reflect fiber degeneration within the cerebellar peduncle as often reported in SCA2 patients (Durr et al., 1995; Gilman et al., 1996; Iwabuchi et al., 1999; Estrada et al., 1999; Pang et al., 2002).

While RD increase is largely accepted to correlate with myelin disruption (Alexander et al., 2007; Feldman et al., 2010), human studies found increased AD in association with axonal degeneration (Hasan et al., 2008; Roosendaal et al., 2009; Metwalli et al., 2010). Consistently, our data suggest an increase in AD of SCA2 patients, which may reflect fiber degeneration. Similar results were observed in patients affected by Friedriech's ataxia (FRDA) (Della Nave et al., 2011). Indeed, it has been postulated that the RD values increase with loss of myelin integrity in chronic pathological conditions while the AD values increase in areas with reduced axonal density or caliber (Hasan et al., 2008; Kumar et al., 2008, 2010; Della Nave et al., 2011). Further support to this interpretation comes from DTI studies on murine models of acute lesions (Song et al., 2003; Budde et al., 2007, 2009; Kim et al., 2010) suggesting that AD changes are characterized by a time-dependent course with initial decrease during the acute phase followed by normalization or increase in more advanced stages as axon fragments are cleared (Concha et al., 2006). Consistently, increases of AD have been shown in chronically degenerated white matter bundles in humans (Pierpaoli et al., 2001; Glenn et al., 2003). Thus, a significant increase of AD may feature either in direct WM damage, i.e stroke and Multiple Sclerosis, (Bammer et al., 2000; Pierpaoli et al., 2001) or in the more advanced stage of wallerian degeneration (Mandelli et al., 2007). DTI metrics modifications have been already reported in the inferior, middle and superior cerebellar peduncles of SCA2 patients (Della Nave et al., 2008b; Hernandez-Castillo et al., 2015). Those studies, however, were based on tract-based spatial statistics (TBSS) (Smith et al., 2006), thus investigating voxel-wise differences of the WM along the core of tracts. By contrast, we evaluated the DTI Metrics for the whole tract, restricting the analysis to the fibers of interest tracked in each subject (see also Roine et at., 2015).

A further element of novelty in this study is the observed relationship between cerebellar microstructural damage and cognitive abilities in SCA2 patients, which was not investigated previously. Interestingly, we found that cognitive functions typically altered in SCA2, i.e executive and visuospatial functions (LePira et al., 2002; Kawai et al., 2008), correlate with damage of right and left SCP respectively. This datum is in line with cerebellar lateralization of functions (Stoodley and Schmahmann, 2008). According to the crossed cerebello-cerebral projections, negative Z-scores of visuospatial functions negatively correlated with increased MD values in the left SCP, the main cerebellar output tract connecting the cerebellum with right cerebral cortex, known to be predominantly involved in visuospatial processing (Baillieux et al., 2010). The correlation between executive functions and MD values in the right SCP is consistent with the evidence that left prefrontal cortex plays a crucial role in many critical aspects of executive processing such as strategy (Grafman et al., 2005; Vallesi et al., 2012), hypothesis generation (Reverberi et al., 2005), and manipulations of goal hierarchies (Kaller, et al., 2011; Reverberi et al., 2005; Crescentini et al., 2011; Langdon and Warrington, 2000).

Overall, our results show a specific pattern of white matter microstructural damage, likely to be associated with SCA2 neurodegenerative processes, as expressed by the decrease of FA and the increase of MD affecting bilateral MCP and SCP. This suggests that in SCA2 the cerebellar atrophy also affects the diffusivity of the main cerebellar white matter tracts thus resulting in a cerebello-cerebral dysregulation that may account for the cognitive symptomatology of SCA2 patients. Consistent with this interpretation, a previous DTI study in FDRA individuals (Zalensky et al., 2014) has specifically shown that the interruption of the cerebellar afferent and efferent connections leads to secondary functional effects in distant cortical and subcortical regions, a phenomenon referred to as reverse cerebellar diaschisis, thus resulting in the cerebellar cognitive affective syndrome (Schmahmann and Sherman 1998).

Furthermore, the fact that we found a precise lateralization of cognitive functions and structural alterations indicating significant correlations of visuospatial functions with RSCP MD and executive functions with LSCP MD, provides additional support to our conclusions.

In light of our results, we hypothesize a combination of demyelination, as expressed by increased RD, and axonal changes, as expressed by AD increase, to affect the tracts examined. Taken together our data suggest some heterogeneity of microstructural WM damage in SCA2 as suggested also by Della Nave and colleagues (2008b).

However, it has to be considered that in the case of more complex diseases, where a combination of demyelination, axon loss, gliosis, and inflammation may affect brain regions, the use of integrated approaches with other imaging measures (e.g., T1, T2, magnetization transfer, perfusion, fast/slow diffusion, spectroscopy) could improve the DTI information on the neuropathology and the interpretation of diffusivity changes (Alexander et al., 2007). According to this evidence, our interpretation of combined diffusivity changes in SCA2 patients needs further investigation and should be confirmed in future studies.

It is also important to discuss some methodological limitations. First of all, it is important to reiterate that typically diffusion MRI data are acquired with a resolution of 2-3 mm^3, which is too coarse to capture fine anatomical details. In addition, all tractography algorithms are at high risk of both false positives and false negatives. Furthermore, reconstructing the SCP is notoriously difficult because of the crossing occurring at their decussation. Several tractographic reconstructions have failed (Zhang et al., 2008; Ye et al., 2012). Ye and colleagues (2013) have proposed a method based on random forest classification, which can be trained to segment the SCPs based on the shape of the diffusion tensor in every voxel. Instead, we used two sophisticated models of diffusion, namely Q-ball and PAS MRI to rely exclusively on tractography for the segmentation. Overall, this approach allowed us to independently analyze which voxels along the tracts presented with DTI metrics changes.

The present findings are largely consistent with MRI studies that have investigated dMRI metrics in patients affected by cerebellar atrophy of different etiology. Indeed, patterns of diffusivity WM changes to affect FA and MD of MCP and/or SCP have been evidenced in SCA1, SCA2 (Mandelli et al., 2007) and SCA6 (Ying et al., 2006; Ye et al., 2013) patients. With the exception of the study from Ye and colleagues (2013), that examines the FA and MD in SCA6 within the whole volumes of the MCP and SCPs, all these studies measured dMRI metrics of the MCP and SCPs in a Regions of Interest (ROI) limited to a single slice. The use of single slice can introduce important bias and variations in the metric averaged over that region, depending on the particular ROI position (Dayan et al., 2016). The use of tractography allows the whole bundle to be taken into account, overcoming this limitation.

Furthermore, the present study demonstrates the sensitivity of dMRI to detect the microstructural alterations linked to cognitive symptomatology in SCA2 patients. The additional correlation with cognitive scores further support the idea that DTI metrics may serve as clinical imaging biomarker to differentiate between different kinds of cerebellar neurodegenerative diseases, as previously suggested in another study of our group with mixed cerebellar ataxias (Dayan et al., 2016).

In conclusion, we advance the hypothesis that microstructural WM damage in cerebellar peduncles may account for the specificity of the cognitive impairment observed in SCA2 patients.

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Authors' Contribution

Giusy Olivito, Maria Leggio, Marco Molinari and Marco Bozzali contributed to the study conception and design and supervised development of work;

Marcella Masciullo and Silvia Romano contributed to recruitment and enrollment of patients;

Michela Lupo, Claudia Iacobacci and Silvia Clausi contributed to the acquisition and interpretation of neuropsyhological data;

Michela Lupo contributed to the analysis of neuropsychological data;

Mara Cercignani contributed to the implementation of MRI protocol and analysis;

Giusy Olivito contributed to acquisition of MRI protocol, preprocessing and analysis of MRI data.

Giusy Olivito contributed to the writing of the original manuscript;

All co-authors contributed to final editing and critical revision of the original manuscript.

Conflict of Interest

Conflicts of interest: none'.

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Figure Captions

Fig.1 Neuropsychological assessment. Mean and Standard Error of cognitive functions in the SCA 2 group expressed in Z-scores (mean Z-scores are reported). Neuropsychological functions are grouped according to the cognitive domains assessed.

Fig.2 DTI-based tractography of middle and superior cerebellar peduncles. 3D reconstruction of the average tract of MCP (red) LSCP (green) and RSCP (blue) with voxels belonging to at least 50% of the subjects. Reconstructed tracts are superimposed on the Spatially Unbiased Template of the Cerebellum and Braistem (SUIT) (Diedrichsen et al., 2009). The decussation of the SCPs at level of the ventral brainstem is clearly visible. A: anterior view; P: posterior view; S: superior view.

Fig.3 Voxel-wise analysis of white matter tracts. Regions showing altered Fractional Anisotropy (FA), Mean Diffusivity (MD) , Axial Diffusivity (AD) and Radial diffusivity (RD) in patients compared to controls. Only clusters of significant diffusivity changes that survived after correction for multiple comparisons are reported. Results are shown in different colors for the middle cerebellar peduncle (red), left superior cerebellar peduncle (green), and right superior cerebellar peduncle (blue), Clusters are superimposed on MNI (the Montreal Neurological Institute) template in coronal, sagittal and axial slices.
*FWE uncorrected at $p<0.001$ at cluster level