Parahippocampal gray matter alterations in Spinocerebellar Ataxia Type 2 identified by voxel based morphometry

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Abstract

Spinocerebellar Ataxia Type 2 (SCA2) is a genetic disorder causing cerebellar degeneration that result in motor and cognitive alterations. Voxel-based morphometry (VBM) analyses have found neurodegenerative patterns associated to SCA2, but they show some discrepancies. Moreover, behavioral deficits related to non-cerebellar functions are scarcely discussed in those reports. In this work we use behavioral and cognitive tests and VBM to identify and confirm cognitive and gray matter alterations in SCA2 patients compared with control subjects. Also, we discuss the cerebellar and non-cerebellar functions affected by this disease. Our results confirmed gray matter reduction in the cerebellar vermis, pons, and insular, frontal, parietal and temporal cortices. However, our analysis also found unreported loss of gray matter in the parahippocampal gyrus bilaterally. Motor performance test ratings correlated with total gray and white matter reductions, but executive performance and clinical features such as CAG repetitions and disease progression did not show any correlation. This pattern of cerebellar and non-cerebellar morphological alterations associated with SCA2 has to be considered to fully understand the motor and non-motor deficits that include language production and comprehension and some social skill changes that occur in these patients.

1. Introduction

Spinocerebellar Ataxia Type 2 (SCA2) is a genetic disorder caused by an expanded CAG trinucleotide repeat in the gene encoding ataxin-2 [60], which causes cerebellar degeneration primarily affecting Purkinje cells, pontine nuclei and inferior olives [29,70]. Symptoms typically initiate by the 3rd or 4th decade and include several motor and visuomotor disorders, such as ataxia, dysmetria, dysarthria, dysdiadochokinesia, ophthalmoplegia, and saccade slowing [24,48,66,88,89].

The use of neuroimaging techniques in recent decades has allowed whole brain structural analyses of different neurodegenerative diseases that were difficult to perform in neuropathological studies. For example voxel-based morphometry (VBM) is based on the analysis of high-resolution magnetic resonance images of the whole brain, and it can determine different atrophy patterns in gray matter associated with neurodegenerative disorders [4]. For example, VBM analyses on various types of SCAs have been able to identify gray matter loss in many CNS regions. For example, it has identified volume reductions in the pons for SCA3 patients, in the hemispheric cerebellar lobules and vermis in SCA6 [47], and in the posterior lobe in SCA7, this last also showing alterations in non-cerebellar regions, such as the precentral and postcentral gyri, inferior and medial frontal, and the inferior parietal, parahippocampal and occipital cortices [1,35].

Regarding SCA2 patients, VBM studies have shown reduced gray matter volumes in the pons, the cerebellar vermis and cerebellar hemispheres while sparing lobules I and II, and Crus II, VII, and X [8,20,93]. VBM analyses have also identified loss of gray matter in cortical regions including the orbitofrontal cortex, the middle frontal region, the primary somatosensory cortex, and temporomesial and insular cortices [7,8].

Other studies using VBM have compared the atrophy patterns across different SCAs. For example, it has been reported that SCA1, SCA2 and SCA3 result in atrophy of the cerebellum and brainstem in the three SCA types, although there are still some controversies regarding the relative degree of the neurodegeneration among the three mutations [21,30,36,42].

Although VBM is used to determine morphometric alterations, this technique can also be used to complement the discussion on some
behavioral and cognitive dysfunctions. For example, SCA2 involves alterations on motor and learning processes regulated by extracerebellar structures, such as the substantia nigra, striatum, pallidum, and motor cortex [86], and atrophies in the posterior and anterior cerebellar lobes associated with SCA2 are related with executive and coordinative dysfunctions, respectively [18]. Regarding the emotional domain, cerebellar lesions impair affective recognition [22,65] and the vermis has been proposed as the limbic cerebellum to regulate emotional expressions [71]. Observations in neurological patients with morphological and functional alterations in the vermis and diagnosed with ataxia or with cerebellar cognitive affective syndrome manifest emotional fragility and several affective disorders [72,73,81,82]. In addition, some functional neuroimaging studies indicate cerebellar activity when individuals watch pictures of faces with emotional content [56] and while feeling anger, sadness, happiness, and fear [19,32]. In social cognition, the role of the cerebellum has been observed along with the activation of the hippocampus while processing socially related spaces [43] and along with the activity of the prefrontal cortex, predicts autonomic responses associated with risky social decision-making [15,16].

Behavioral and cognitive functions associated with SCA2 are diverse and alterations have been scarcely discussed in terms of non-cerebellar regions identified as atrophied. The aim of this work was to identify and confirm SCA2 brain degeneration using a VBM analysis, and to discuss the behavioral effects of the cerebellar and non-cerebellar degenerations caused by this disease. We hypothesized that the clinical feature in SCA2 patients will be correlated with a reduced brain volume, and a low performance on behavioral and cognitive tasks.

2. Materials and method

2.1. Participants

Fifteen patients with a molecular diagnosis of Spinocerebellar Ataxia Type 2 (9 women) and 15 control volunteers (7 women) without any neurological or psychiatric condition participated in this study. The Scale for Assessing and Rating Ataxia (SARA) was used as a semi-quantitative valuation comprising eight items related to gait, stance, sitting, speech, finger–chase test, nose–finger test, fast alternating movements and heel–shin test [74,92]. General characteristics of the participants are presented in Table 1. All the participants gave their informed consent after the nature of the study was explained. The research protocol was conducted according to the international standard laid down in the Declaration of Helsinki and all procedures were approved by the Health and Ethics Committees of the National Autonomous University of Mexico.

2.2. Cognitive and behavioral instruments

Both patients and control participants took the Mini-mental State Examination and the Montreal Cognitive Assessment, and performed three tasks included in the Cambridge Neuropsychological Test Automated Battery.

The Mini-mental State Examination (MMSE) is a well-known test to assess cognitive impairment in neurological patients. As the original test [26], the validated version used in Mexico is applied in 5–10 min and comprises 11 items to assess five areas: orientation, attention/concentration, immediate memory, language and visuospatial perception [59]. Scores indicate five levels of cognitive impairment: 30–27 = no impairment; 26–25 = possible impairment; 24–20 = low to moderate impairment; 9–6 = moderate to severe impairment; ≤6 = severe impairment.

The Montreal Cognitive Assessment (MoCA) evaluates mild cognitive impairment in several kinds of neurological and psychiatry patients and has been proposed as a complement for the MMSE [55]. The version in Spanish language used in Mexico (The Montreal Cognitive Assessment—MoCA®) consists in a 30-point test administered in approximately 10 min to assess visuospatial abilities, executive functions, attention/concentration/working memory, language, and orientation to time and place. A score ≥26 indicates normal cognitive functions.

The Cambridge Neuropsychological Test Automated Battery (CANTAB) [68] consists in a computerized system to assess cognitive performance in elderly, demented, and neurological patients. Also, it has been used to study cognitive functions in psychiatric patients [37] and in healthy people across different ages [3,46,83]. The CANTAB comprises a variety of executive and memory tasks administered through a touch-sensitive screen where feedback is given in a standardized manner for all the subjects. In this study three tasks were administered: Big/Little Circle, Spatial Span, and Intra/Extra-Dimensional Shift.

The Big/Little Circle task is administered in 2 min and evaluates comprehension and learning while training the participant to follow and reverse a rule. First, the participant touches the smaller of two circles displayed in the screen. After 20 trials, the participant must touch the larger circle for 20 further trials. The outcome measures considered here were the number of errors committed and the response latency.

The Spatial Span is administered in 6 min and evaluates working memory. Each trial starts with nine white boxes displayed in the screen, some of which change in color in a variable sequence. The participant must touch the boxes that changed color in the same order they were displayed by the computer. At the beginning the participant has to choose between two boxes, but the number of boxes progresses up to nine. The test is terminated if the participant fails three consecutive trials. The outcome measures used for our study were the span length (the maximum span or box sequence successfully recalled) and the response latency.

The Intra/Extra-Dimensional Shift is administered in 7 min and is analogous to the Wisconsin Card Sorting Test. It assesses visual discrimination, attentional maintenance, shifting and flexibility of attention. The participant starts watching two simple color-filled shapes and must learn which one is correct by touching it. The complexity of the shape increases and white lines are added behind the color-filled shapes. Feedback indicates if the participant chose the correct stimuli and if the rules are changed. These shifts are initially intra-dimensional (e.g. the color filled shapes remain the only relevant dimension) but then they become extra-dimensional (e.g. the lines become the only relevant dimension). Participants have to perform six consecutive correct responses. The measures considered for our study were the number of errors and the latency.

2.3. Statistical analysis

Since demographic information, disease characteristics, and scores for the cognitive test are variable in patients and control participants

<table>
<thead>
<tr>
<th>Table 1 General characteristics of the participants.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
</tr>
<tr>
<td>M ± S.D.</td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>Controls</td>
</tr>
</tbody>
</table>

Note. M = Mean; S.D. = standard deviation; SARA = Scale for the Assessment and Rating of Ataxia.
we used the Shapiro–Wilks test to verify the data normal distribution. Only the scores for the MMSE and the MoCA for all the participants and the SARA ratings and CAG repetitions for patients presented a $p > 0.05$ indicating a normal distribution. Parametric or non-parametric test was run depending on the normal distribution of the data. Data bases and statistical analysis were performed using SPSS 17.0 software.

A Student’s t-test for independent samples was used to compare the patients’ and controls’ MMSE and MoCA scores. A Mann–Whitney U test was used to compare the patients’ and controls’ scores for the CANTAB tasks. A Spearman’s rank correlation coefficient was applied to evaluate the relation between the years of education and the MMSE, the MoCA and the CANTAB tests. A Mann–Whitney U test was applied comparing the women’s and men’s scores to analyze the effect of gender in each cognitive and behavioral task. The Spearman’s coefficient was used to correlate the CAG repetitions with disease progression and scores of the CANTAB tests. The Pearson parametric quotient was used to correlate the CAG repetitions with scores for the MMSE, the MoCA and the SARA. Spearman’s coefficient was also used to analyze the correlations between the SARA ratings with the patients’ age, the time of disease evolution, and the scores for the CANTAB test. The Pearson coefficient was applied to analyze the correlation between the SARA ratings and the scores for the MMSE and the MoCA.

2.4. Image acquisition and analysis

Images were acquired using a 3 Tesla Philips Achieva MRI scanner (Philips Medical Systems, Eindhoven, Netherlands) at the National Institute of Psychiatry, Mexico City. A high-resolution 3D volume consisted of a T1 Fast Field-Echo sequence, with TR/TE = 8/3.7 ms, FOV 256 × 256 mm² and an acquisition and reconstruction matrix of 256 × 256, resulting in an isometric resolution of 1 × 1 × 1 mm³.

To reduce patients’ movements and prevent motion artifacts participating in motion cases. Brain images for each participant were checked by an expert radiologist to verify the lack of artifacts classically observed in motion cases.

To analyze gray matter changes we used VBM [31] implemented on FSL (FMRIB Software Library) [78]. The original DICOM files were converted into NIFTI format using dcm2nii (Chris Rorden’s MRIcron, copyright 2012). Voxels belonging to the neck and other non-brain tissues were eliminated using BET software and gray matter was segmented before being registered to the MRI 152 standard space using non-linear registration [2]. The images were averaged and flipped on the x-axis to create a symmetric, study-specific gray matter template. Original gray matter images were then non-linearly registered to this specific template and modulated and corrected for local expansion or contraction. The modulated images were softened by applying an isotropic Gaussian 3 mm kernel. The voxel-wise GLM was applied using a two-sample t-test corrected for multiple comparisons across space. We used a randomize permutation testing ($n = 5000$) for inference in VBM-style analysis and we chose the appropriate smoothing (sigma = 3 mm) for the TFCE (Threshold-Free Cluster Enhancement) based analysis. The family-wise error (FWE) rate was controlled and only corrected $1 - p$ values $\leq 0.005$ were accepted. Cluster centroid coordinates of brain activation were estimated on the MNI template and its anatomical location and proximate Brodmann’s area were identified by using the Talairach Deamons Client system [44] and fslview.

To obtain the values of the patients’ brain tissue to be correlated with cognitive, behavioral and clinical measures, the brain tissue volume was normalized for each subject head size and was estimated with SIENAX which is a tool incorporated in the FSL software [77–79]. The SIENAX analysis extracts brain and skull images from the single whole-head input data [76]. The brain image is then affine-registered to the MNI152 space [38,39] using the skull image to determine the registration scaling and to obtain the volumetric scaling factor to be used as a normalized head size. Next, tissue-type segmentation with partial volume estimation was carried out [95] to calculate the total volume of brain tissue as well as estimations of gray matter volume and white matter volume (see Table 2).

Brain volume estimations had a normal distribution as indicated by the Shapiro–Wilks test. Pearson product–moment correlation was used to correlate the volume estimations of the total brain, gray matter and white matter with the CAG repetitions and with scores for the SARA, the MMSE and the MoCA test.

3. Results

Student’s t-test showed that SC2A patients have significantly lower scores than control participants in the MMSE (control: $M = 29.06 \pm S.D. = 2.05$; patients: $M = 25.80 \pm S.D. = 3.42$; $t_{28} = 3.16$, $p = .004$) and the MoCA (control: $M = 28.06 \pm S.D. = 1.62$; patients: $M = 21.06 \pm S.D. = 6.12$; $t_{28} = 4.27$, $p < .001$). Analysis of the CANTAB results showed that patients executed more incorrect responses in the Intra/Extra-Dimensional Shift ($U_{30} = 3.28$, $p \leq 0.001$) and passed less correct spans in the Spatial Span task ($U_{30} = -3.97$, $p \leq 0.001$). Also, patients took more time to choose the correct response while executing the Big Little Circle task ($U_{30} = -3.96$, $p \leq 0.001$, the Intra/ Extra-Dimensional Shift ($U_{30} = -3.83$, $p \leq 0.001$) and the Spatial Span task ($U_{30} = -2.71$, $p = 0.007$) (see Table 3).

Spearman’s coefficient indicated a positive correlation between years of education and MMSE scores ($r_{30} = .784$, $p > .001$), for the MoCA ($r_{30} = .686$, $p > .001$) and span length in the Spatial Span task ($r_{30} = .692$, $p > .001$). Negative correlations were observed with the latency for the Big/Little Circle task ($r_{30} = -0.439$, $p = .015$), the number of errors ($r_{30} = -0.730$, $p > .001$) and latency ($r_{30} = -0.626$, $p = .007$) (see Table 3).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCL</strong> # incorrect responses</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCL latency (s)*</td>
</tr>
<tr>
<td>Patient</td>
<td>1751.8 (1090.3)</td>
</tr>
<tr>
<td>Control</td>
<td>981.28 (229.2)</td>
</tr>
<tr>
<td><strong>IED</strong> # incorrect responses*</td>
<td></td>
</tr>
<tr>
<td>IED latency (s)*</td>
<td>2374.5 (1386.2)</td>
</tr>
<tr>
<td></td>
<td>1410.1 (832.1)</td>
</tr>
<tr>
<td><strong>SSP</strong> passed span*</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Control</td>
<td>7 (2)</td>
</tr>
<tr>
<td><strong>SSP</strong> latency (s)*</td>
<td></td>
</tr>
</tbody>
</table>

Note: BCL # incorrect responses = number of incorrect responses when executing the 40 trials for the Big Little Circle task; BCL latency (s) = time in seconds to choose the correct response when executing the Big Little Circle task; IED # incorrect responses = number of incorrect responses when executing the trials for the Intra/Extra-Dimensional Shift task; IED latency (s) = reaction time in seconds to respond on the trials for the Intra/Extra-Dimensional Shift; SSP passed span = last passed span for the Spatial Span task; SSP latency (s) = time in seconds to choose the correct stimuli until the last passed span.

* Represents significant statistical differences between patients and controls when applying the nonparametric Mann–Whitney U test at $p \leq 0.001$. 

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The Big Little Circle task; BCL latency = time to choose the correct response when executing the Big Little Circle task; IED incorrect responses = number of incorrect responses when executing the 59 trials for the Intra/Extra-Dimensional Shift task; IED latency = reaction time to respond to the trials for the Intra/Extra-Dimensional Shift; SSP passed span = last passed the Talairach Daemon System [44].

Note: The indicated value corresponds to the rho Spearman’s coefficient. N = 15 for all cases. BCL incorrect responses = amount of incorrect responses when executing the 40 trials for the Big Little Circle task; BCL latency = time to choose the correct response when executing the Big Little Circle task; IED incorrect responses = number of incorrect responses when executing the 59 trials for the Intra/Extra-Dimensional Shift task; IED latency = reaction time to respond to the trials for the Intra/Extra-Dimensional Shift; SSP passed span = last passed span for the Spatial Span task; SSP latency = time to choose the correct answers until the last passed span; MMSE = score for the Mini-mental State Examination; MoCA = score for the Montreal Cognitive Assessment; SARA = scale for the Scale for the Assessment and Rating of Ataxia.

Indicates significant correlation at p ≤ .05.
** indicates significant correlation at p ≤ .005.

The number of CAG repetitions and the scores for the MMSE and the MoCA passed the Shapiro–Wilk test of normality. However, none of these measures correlated with the patients’ grey matter, white matter or total brain volume when using the Pearson product–moment correlation. Regarding the motor impairment, SARA scores negatively correlated with the grey matter volume (r(15) = −.804, p ≤ .001), with the white matter volume (r(15) = −.593, p = .020) and with the total brain volume (r(15) = −.761, p = .001). Furthermore, a linear regression model analysis showed that SARA scores predicted the patient’s three volume measurements (see Table 6).

4. Discussion and conclusions

The results of the VBM analysis showed significant bilateral degeneration in the parahippocampal gyrus. Our analyses also corroborated previous findings including significant degeneration in the cerebellar vermis and brainstem, as well as in insular, precentral, frontal, parietal and temporal cortices [17,30] as discussed below.

The most important finding of our study is the gray matter reduction in the parahippocampal gyrus, which has not previously been reported in VBM studies on SCA2. Two previous sets of results support our finding. First, previous studies in other spinocerebellar ataxias including SCA7 and SCA17 have found grey matter reductions in the parahippocampal gyrus [1,35,64]. And second, a previous finding using voxel-based FDG-PET showed a severe metabolic impairment also in the parahippocampal area in SCA2 patients when compared with SCA3 and SCA6 patients [90]. The parahippocampal gyrus has been linked to memory formation and spatial analysis of social environments that may be required to integrate incoming information with.

\[ p > .001 \] for the Intra/Extra-Dimensional Shift, and with the latency for the Spatial Span task (\( r_{(20)} = - .429, p = .018 \)). No gender differences were observed for any test.

Regarding the clinical characteristics of the patients, Spearman’s coefficient did not show any correlation between the CAG repetitions and any behavioral or cognitive test.

As shown in Table 4 several correlations were observed between the behavioral and cognitive tests. Scores for the SARA correlated with most of the tests with the exception of the MoCA and the number of incorrect responses for the Intra/Extra-Dimensional Shift of the CANTAB. Scores of the tests with the exception of the MoCA and the number of incorrect responses for the Intra/Extra-Dimensional Shift of the CANTAB correlated with most of the tasks included in the CANTAB with the exception of the number of incorrect responses for the Intra/Extra-Dimensional Shift.

Positive correlations were observed between the patients’ age and the latency for the Spatial Span task (\( r_{(20)} = .592, p = .010 \) and between the disease evolution and the latency of the three tasks included in the CANTAB test: Big Little Circle (\( r_{(15)} = .541, p = .037 \)), Intra/Extra-Dimensional Shift (\( r_{(15)} = .611, p = .016 \)), and Spatial Span (\( r_{(15)} = .679, p = .005 \). Also, disease progression was negatively correlated with the MMSE scores (\( r_{(15)} = - .566, p = .028 \)) and with the MoCA scores (\( r_{(15)} = - .749, p = .001 \)), but positively correlated with the SARA scores (\( r_{(15)} = .547, p = .035 \)).

The VBM analysis showed significant loss of gray matter in the SCA2 group cerebellar vermis. Significant gray matter loss was also found in the pons of the brainstem, in the parahippocampal gyrus and insula bilaterally, and in a variety of cortical regions including the precentral, inferior frontal, middle frontal, inferior parietal and middle temporal gyri (see Table 5 and Figs. 1 and 2).

### Table 4
Spearman’s correlations between the patients’ scores obtained for the cognitive and behavioral test.

<table>
<thead>
<tr>
<th></th>
<th>IED incorrect responses</th>
<th>BCL incorrect responses</th>
<th>IED latency</th>
<th>BCL latency</th>
<th>SSP passed span</th>
<th>SSP latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MoCA</td>
<td>1.000</td>
<td>0.176</td>
<td>0.042</td>
<td>0.296</td>
<td>-0.452</td>
<td>0.389</td>
</tr>
<tr>
<td>MMSE</td>
<td>1.000</td>
<td>-0.270</td>
<td>-0.485</td>
<td>-0.212</td>
<td>-0.427</td>
<td>-0.287</td>
</tr>
<tr>
<td>SARA</td>
<td>1.000</td>
<td>0.305</td>
<td>-0.368</td>
<td>-0.504</td>
<td>-0.433</td>
<td>-0.444</td>
</tr>
<tr>
<td>1.000</td>
<td>-0.506</td>
<td>-0.418</td>
<td>0.226</td>
<td>0.877**</td>
<td>-0.381</td>
<td>0.551**</td>
</tr>
</tbody>
</table>

Note: The indicated value corresponds to the rho Spearman’s coefficient. N = 15 for all cases. BCL incorrect responses = amount of incorrect responses when executing the 40 trials for the Big Little Circle task; BCL latency = time to choose the correct response when executing the Big Little Circle task; IED incorrect responses = number of incorrect responses when executing the 59 trials for the Intra/Extra-Dimensional Shift task; IED latency = reaction time to respond to the trials for the Intra/Extra-Dimensional Shift; SSP passed span = last passed span for the Spatial Span task; SSP latency = time to choose the correct answers until the last passed span; MMSE = score for the Mini-mental State Examination; MoCA = score for the Montreal Cognitive Assessment; SARA = scale for the Scale for the Assessment and Rating of Ataxia.

Indicates significant correlation at p ≤ .05.
** indicates significant correlation at p ≤ .005.

### Table 5
Neuroanatomical regions indicating a significant decrease of gray matter volume in SCA2 patients.

<table>
<thead>
<tr>
<th>Lat.</th>
<th>Brain region</th>
<th>Anatomical location</th>
<th>Brodmann’s area</th>
<th>Cluster size</th>
<th>Coordinate (x, y, z)</th>
<th>1 - p (corrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Frontal lobe</td>
<td>Precentral gyrus</td>
<td>4</td>
<td>40</td>
<td>(−30, −28, 64)</td>
<td>0.982</td>
</tr>
<tr>
<td>L</td>
<td>Frontal lobe</td>
<td>Middle frontal gyrus</td>
<td>6</td>
<td>222</td>
<td>(−24, −6, 48)</td>
<td>0.989</td>
</tr>
<tr>
<td>R</td>
<td>Frontal lobe</td>
<td>Inferior frontal gyrus</td>
<td>45</td>
<td>285</td>
<td>(34, 24, 8)</td>
<td>0.996</td>
</tr>
<tr>
<td>R</td>
<td>Parietal lobe</td>
<td>Inferior parietal gyrus</td>
<td>40</td>
<td>4</td>
<td>(−52, −28, 28)</td>
<td>0.985</td>
</tr>
<tr>
<td>L</td>
<td>Temporal lobe</td>
<td>Middle temporal gyrus</td>
<td>38</td>
<td>32</td>
<td>(36, −4, −38)</td>
<td>0.980</td>
</tr>
<tr>
<td>R</td>
<td>Sub-lobar</td>
<td>Insula</td>
<td>13</td>
<td>681</td>
<td>(−40, −32, 22)</td>
<td>0.992</td>
</tr>
<tr>
<td>L</td>
<td>Sub-lobar</td>
<td>Insula</td>
<td>13</td>
<td>991</td>
<td>(−34, 24, 8)</td>
<td>0.998</td>
</tr>
<tr>
<td>R</td>
<td>Limbic lobe</td>
<td>Parahippocampal gyrus</td>
<td>28</td>
<td>181</td>
<td>(−20, −14, −14)</td>
<td>0.995</td>
</tr>
<tr>
<td>L</td>
<td>Limbic lobe</td>
<td>Parahippocampal gyrus</td>
<td>28</td>
<td>181</td>
<td>(−20, −22, 32)</td>
<td>0.977</td>
</tr>
<tr>
<td>L</td>
<td>Brainstem</td>
<td>Pons</td>
<td>*</td>
<td>6</td>
<td>(−20, −22, 32)</td>
<td>0.977</td>
</tr>
<tr>
<td>L</td>
<td>Post. Cerebellum</td>
<td>Uvula of vermis. lob. IX</td>
<td>*</td>
<td>20,680</td>
<td>0 − 66 − 34</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Coordinates represent the peak value of the cluster in accordance with the Neurological Institute of Montreal (MNI) template. Approximate Brodmann areas were obtained through the Talairach Daemon System [44].

* Indicates no Brodmann’s area reported.
episodic memory to perform adequate social and spatial mobilization [5,61]. These mnemonic processes implicate a network comprising high cortical functions regulating visuo-constructive skills and emotions [9] that are impaired in SCA2 patients [25]. Additionally, SCA2 patients also manifest memory complaints when remembering recent events or incorporating new information [49]. Since damage in the cerebellum influences deficits in verbal working memory, the loss in the parahippocampal region along with the cerebellar, frontal, and temporal regions observed could contribute to the low scores in the MMSE and the MoCA test involving articulatory rehearsal [82].

Reduced volume in the vermis has been previously reported in SCA2 using VBM [20,93]. This reduction may be linked to impairments on executive and coordinative functions in SCA2 patients [17] that could be explained by the fact that the vermis acts on a network comprising other cerebellar regions, such as the floculonodular lobe, the fastigial nucleus, the globose nucleus, and the limbic regions involved in the regulation of executive decisions [34,87]. The vermis is fundamental to regulating emotional expressions [71] and its damage could be associated with the cerebellar cognitive affective syndrome and with emotional fragility and alterations in facial recognition in several SCA types [17,72,81,82].
Our results also support previous SCA2 findings reporting the loss of cortical gray matter in the premotor cortex and the inferior frontal gyrus [21], the temporal and parietal cortices [17,30], as well as, the insula that has been associated to dystonia, myoclonus, tremor and pyramidal signs in patients with multiple system atrophy [7].

Fig 2. Sagittal, axial and coronal slices showing subcortical and cerebellar regions with significant gray matter volume reductions in SCA2 patients. Green crosshairs indicate the local maxima differences. A. Right insula (BA 13), B. left insula (BA 13), C. right parahippocampal gyrus (BA 28), D. left parahippocampal gyrus (BA 28), E. left brainstem (pons), F. left posterior cerebellum (uvula of vermis). BA = Brodmann’s area; S = superior face; I = inferior face, A = anterior face; P = posterior face; R = right face; L = left face.
It has been reported that decreased activity in the left inferior frontal gyrus in Brodmann area 45 is associated with reduced body movements [67], which may occur in SCA2 progression. The inferior frontal gyrus is also necessary for language production and higher-order motor sequences [10,40,53]. The loss of gray matter in this frontal region observed in SCA2 patients may affect speech production and language processes [13,69] and could be related to the low scores in the MMSE and the MoCA test which include verbal production and repetition tasks.

The insular cortex is anatomically and functionally linked with the inferior frontal and temporal cortices, and its function is required for goal-directed behaviors, sound to speech transformations [11] and interoceptive information allowing for bodily maps and social skills [27, 50,54]. The gray matter reduction in the insula may be related with speech impairments and deficits in social and perceptual guides to motor decisions and emotional attributions observed in SCA patients [17,23,28,80].

It has been proposed that the temporal pole (Brodmann area 38) plays a role in social cognition, emotional experiences and learning of linguistic concepts [57,94] when working in conjunction with the frontal cortex [Brodmann area 45], parietal cortex (Brodmann area 40) and insula [51] observed as atrophied in our study. Taking into account the role of the cerebellar vermis in emotional regulation [81], morphological alterations in the temporal pole could be related to eventual social cognitive alterations in SCA2 [28,80] affecting emotional face recognition [17].

As we hypothesized, some clinical features in SCA2 patients correlated with low performance of behavioral and cognitive tasks and with reduced brain volume, as it was the case for the SARA ratings. Nevertheless, other features such as CAG repetitions and disease evolution did not show any correlation. The use of VBM is an important tool to clarify cerebellar and non-cerebellar atrophies in SCA2 patients but findings may not always be strictly related with unique clinical manifestations, as has been proposed before [93]. Nevertheless, to assess more accurate correlations, VBM analysis could be complemented with other volumetric techniques, such as cortical regional thickness, or functional analysis using resting state to identify disrupted connectivity between reduced brain regions [35].

The pattern of cerebellar and non-cerebellar morphological alterations associated with SCA2 has to be considered to understand the deficient performances of these patients in psychiatric and neurological tests evaluating visual motor coordination, and non-motor functions such as verbal and visual memory, attention, learning abilities, language comprehension, and emotion recognition [9,17,45,85]. Besides, the influence of factors such as gender and education involving social cognition and emotion in SCA2 needs the integration of interdisciplinary phenomenological and neurobiological methodologies in order to describe in a more accurate manner the social consequences of this disease, as it has been recently made for other neuropsychiatric disorders [14,52].

In comparison with healthy participants SCA2 patients made more incorrect responses and required more time to respond in the CANTAB tests. Besides, latency was correlated with the SARA ratings and with the years of disease progression. These results confirm that SCA2 patients exhibit low performance in non-verbal task abilities such as working memory, retrieval, attention, and visuomotor learning [9,64, 85] and that these cognitive abilities get worse as the motor difficulties aggravate and disease progression moves forward [41,45].

SCA2 patients exhibited lower scores than control participants in the MMSE and in the MoCA indicating cognitive impairments. In this regard, it has been reported that SCA2 patients do not show severe impairments evaluated by the MMSE but show evident deficits in other cognitive tests such as the Wechsler Adult Intelligence Scale-R and the Wisconsin Card Sorting Test [75]. In addition, scores for the MMSE and the MoCA were negatively correlated with the years of disease evolution but not with the scores for the CANTAB neither for the SARA ratings. These results may suggest that, similar to SCA1 [91] and SCA6 patients [12], the spectrum of cognitive impairing in SCA2 is diverse. So, although eventually this disease results in high cognitive process alterations [17,45,66], these alterations may not be directly related with the motor difficulties classically observed in these patients.

On the other hand, it is reported that age and education are two demographic qualities influencing the cognitive performance in SCA patients and that education may represent a protective factor [58]. In our study, age was correlated with a better performance for the Spatial Span task of the CANTAB and years of education was correlated with higher scores for the MMSE and the MoCA and with better performance of the CANTAB test for both patients and control participants. These results are important for several reasons. CANTAB tests are non-verbal tasks suitable for testing individuals with low educational levels and the MMSE is validated to be applied in samples with 5 years of education in Mexico [59]. This is relevant since most of the SCA2 participants show low socioeconomic levels probably linked to limited educational access in their places of residency, and social discrimination dynamics that we have detected in field observations during this research, similar to other medical anthropological observations made in other countries [6,33]. Therefore, although education may influence the cognitive performance in our SCA2 patients, a clear relation between both variables requires more research.

In reference to the clinical features, it has been proposed that CAG repetitions can be considered as a genotypic indicator of the evolution and behavioral and cognitive impairments in SCA1, 2, 3 and 6 [25,36, 63]. Nevertheless, in our study CAG repetitions did not correlate either with the scores for the MMSE, the MoCA and the SARA test, or with the calculated gray, white and total volume of the patients’ brain. These results agree with previous reports [30] and may be attributable to the small sample and the variable clinical characteristics of the patients in our study. Conversely, we found a clear correlation between the SARA ratings and the gray matter, white matter and total brain volumes. In fact, SARA scores predicted the calculated patients’ brain volumes which confirm that SCA2 is a fundamental motor affection and suggest that the SARA test is a viable clinical evaluation.

**Conflict of interests**

The authors declare no conflict of interests.

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