Widespread neuronal damage and cognitive dysfunction in spinocerebellar ataxia type 3

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Received: 25 February 2013 / Revised: 2 May 2013 / Accepted: 5 June 2013 / Published online: 18 June 2013
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Abstract Previous studies demonstrated cognitive impairments in spinocerebellar ataxia type 3 (SCA3/MJD); however, there is no consensus about the cognitive domains affected and the correlation with structural brain abnormalities. We investigated the neuropsychological profile and 3T-MRI findings, including high-resolution T1-images, diffusion tensor imaging and magnetic resonance spectroscopy of 32 patients with SCA3/MJD and 32 age-, gender- and educational level–matched healthy controls. We reviewed patients’ clinical history and CAG repeat length, and performed assessment and rating of ataxia (SARA)-Brazilian version and the neuropsychiatric inventory. Patients presented worse performance in episodic and working memory and Beck inventories (depression and anxiety). SCA3/MJD patients had a reduction of gray matter volume (GM) in the cerebellum, putamen, cingulum, precentral and parietal lobe. A positive correlation was identified between the cognitive findings and GM of temporal, frontal, parietal, culmen and insula. We observed positive correlation between the brainstem's fractional anisotropy and digit span-forward. The following cerebellar metabolite groups (measured relative to creatine) were reduced in patients: N-acetyl-aspartate (NAA), NAA + N-acetyl-aspartate-glutamate and glutamate + glutamine (Glx). We found a positive correlation between Corsi’s block-tapping task forward with Glx; semantic verbal fluency with phosphorylcholine and glycerophosphorylcholine; digits span-forward with NAA. The cognitive impairments in SCA3/MJD are associated not only with cerebellar and brainstem abnormalities, but also with neuroimaging evidence of diffuse neuronal and axonal dysfunction, particularly in temporal, frontal, parietal and insular areas.

Keywords Spinocerebellar ataxia type 3 · Cognitive deficits · Voxel-based morphometry · Diffusion tensor imaging · Magnetic resonance spectroscopy

Introduction

Spinocerebellar ataxia type 3 (SCA3), or Machado–Joseph disease (MJD), is a neurodegenerative disorder. Although rare, it is the most common type of SCA worldwide and is caused by an abnormal CAG repeat expansion at the ATXN3 gene on chromosome 14q32.1 [1]. Cerebellar damage is responsible for the main clinical manifestation: ataxia. Traditionally, the cerebellum is
considered to play a major role in motor function, however cognitive and emotional dysfunctions have also been reported in patients with cerebellar lesions [2]. This emphasizes the importance of studies that consider non-motor aspects in SCA3/MJD.

Furthermore, the disease is characterized by wide morphological degeneration of the central and peripheral nervous systems [3] causing diffuse functional alterations. Neuropathological and neuroimaging studies have indicated a widespread involvement of the central nervous system including the cerebellum, brainstem, basal ganglia, spinal cord [3, 4], as well as the frontal, temporal, parietal and occipital lobes [4–6]; in addition, there is evidence of axonal dysfunction of the deep white-matter [7].

Cognitive function is impaired in SCA3/MJD, in domains such as attention, executive function, nonverbal abstract reasoning, visual ability and memory. However, most of the prior studies evaluated small samples of subjects and applied neuropsychological tools which usually neglected the motor difficulties presented by these patients, resulting in inappropriate tests [8–16]. Most importantly, there was no control group matched for age, gender and educational level in some. Only one study evaluated the correlation between neuroimaging and cognitive dysfunction [15]. Psychiatric dysfunction, especially depression, has been also described in SCA3/MJD. Nevertheless, little is known about its etiology [10] and there are no studies that evaluated the influence of the psychiatric disorders on the cognition of SCA3/MJD patients.

Hence, the profile of cognitive impairment in patients with SCA3/MJD is not entirely clear, and it has not yet been established whether or not the cognitive dysfunction can be exclusively attributed to the cerebellar abnormalities, although the disease has already been described as cerebellar cognitive affective syndrome [16]. Moreover, the impact of depression and anxiety in the cognitive dysfunction observed in SCA3/MJD has not been well characterized.

Therefore, the aims of this study were to investigate the neuropsychological profile in patients with SCA3/MJD and assess the correlation between the cognitive findings and gray matter (GM) volume, white matter (WM) integrity, cerebellar metabolites concentration, clinical and genetic data. We hypothesized that the cognitive dysfunction in SCA3/MJD is not merely a manifestation of the cerebellar cognitive affective syndrome, but in fact a manifestation of a more widespread cerebral involvement.

Methodology

This study was approved by our local research ethics committee and all participants provided written informed consent for the clinical investigation. Thirty-two genetically confirmed patients with SCA3/MJD and 32 healthy controls were enrolled in this study. All patients were matched with controls for age, gender and educational level (in years) and underwent the same neuropsychological protocol and neuroimaging acquisition. We acquired T1 weighted 3D-MRI for all 64 participants in order to perform voxel-based morphometry (VBM) analyses; the diffusion tensor imaging (DTI) protocol was performed for 29 patients and 29 controls and the magnetic resonance spectroscopy-cerebellum (MRS-c) protocol for 26 per group. The discrepant number of patients in each imaging acquisitions was due to patients who could not finish the whole protocol due to dizziness or nausea, or some image acquisitions that were excluded due to movement artifacts.

A neurologist performed the scale for the assessment and rating of ataxia (SARA)-Brazilian version [17] at the same day of the image acquisition and the neuropsychological assessment or within 3 months after the cognitive assessments. For each subject we recorded the abnormal CAG repetition length; the age at onset of the disease and the neuropsychiatric inventory (NPI) [18].

We compared the differences between groups related to age and educational level using the Mann–Whitney test considering $p < 0.05$. We also considered the gender for our analysis using the same proportion of female and male (17 women and 15 men in each group). We also determined the mean and standard deviation of the age of onset, CAG lengths, duration and disease severity and score in the NPI of the patients with SCA3/MJD. The statistical analysis was performed using SYSTAT9.

Neuropsychological tests

We selected a comprehensive neuropsychological battery that assessed several cognitive domains, depression and anxiety indicators. The tests required minimum motor activity to be performed, so that the influence of motor deficits on the cognitive evaluation was dramatically reduced. These tests were: Rey auditory verbal learning test-RAVLT (coding, delayed recall and recognition) [19]; Raven’s progressive matrices (RPM) [20]; Corsi blocking task (forward and backward) [21]; digits span-WAIS III subtest (forward and backward) [22]; semantic verbal fluency (animal category) [21]; figural memory, logical memory I and II and visual paired associated I and II subtests (WMS-R) [23]; pseudo repetition word test [24]; similarities and picture completion (WAIS III subtest) [22]; Boston naming test (BNT) [25]; Hooper visual organization [26]; Brazilian version of the Wisconsin card test-categories achieved (ten consecutive correct responses is considered one category) including perseverative responses, total errors, perseverative errors, non-perseverative...
errors and failure to maintain set [27]; Beck inventories for depression (BDI) and anxiety (BAI) [28] and neuropsychiatric inventory (NPI) [18].

Imaging acquisitions

The images were acquired in a 3-Tesla magnetic resonance (MR) scanner (Achieva, Philips, The Netherlands).

Structural imaging

Volumetric (3D) T1-weighted images were acquired with 1 mm isotropic voxel, repetition time (TR) = 7 ms, echo time (TE) = 3.2 ms and field-of-view (FOV) = 240 × 240 × 180 mm³. This sequence was used for voxel-based morphometry (VBM) analysis.

Diffusion tensor imaging—fractional anisotropy

These images were acquired in the axial plane with 32 directions, echoplanar nonlinear sequence; FOV = 256 × 256 × 140 mm³; voxel resolution 1 × 1 × 2 mm³; TR = 8.5 ms; TE = 61 ms; b value = 1,000.

Magnetic resonance spectroscopy—cerebellum (MRS-c)

1H-MR spectra were acquired with a PRESS sequence (TR/TE = 2,000/144 ms, 128 scans, 2 kHz bandwidth, 1,024 data points), using a single voxel of 1.5 × 1.5 × 1.5 cm³ placed over the white matter in the left cerebellum. Localized B0 shimming was automatically performed in the preparation phase of the MRS sequence.

Imaging analysis

Voxel-based morphometry (VBM)

This technique involved an automatic imaging analysis to investigate differences between whole brain anatomy by a voxel-to-voxel analysis using Statistical Parametric Mapping SPM8® (http://www.fil.ion.ucl.ac.uk/spm/). First, images were converted from DICOM to Niftii format using the dcm2niigui routine from the MRIcron program (http://www.bioimagesuite.org/Mni2Tal/index.html).

Diffusion tensor imaging—fractional anisotropy (DTI- FA)

We transformed the reconstructed DTI images from DICOM to Niftii format (www.mricron.com). The analysis involved the automatic preprocessing of the imaging data using the FSL DTI diffusion toolbox 4.1 (http://www.fmrib.ox.ac.uk/fsl/) following the steps: (1) correction of the spatial distortion by the eddy current tool; (2) mask implementation using the brain extraction (BET) tool to differentiate brain and skull; (3) creation of the diffusion image directions, calculation of the diffusion tensor and fractional anisotropy (FA) maps with DTIfit algorithm and (4) the Tract-Based Spatial Statistics-v1.2 (TBSS) package was used to compare the differences between groups related to WM-FA integrity.

In a second step of analysis we extracted individual FA values from areas with significant differences in order to investigate possible correlations with cognitive findings applying the Spearman’s correlation test by SYSTAT9.

Magnetic resonance spectroscopy—cerebellum (MRS-c)

The quantification of the spectra was performed using the software LCModel [29]. This method uses a basis of measured or simulated spectra from individual metabolites and combines them linearly to fit the measured data. We employed a basis set, specific for the 3T field and TE = 144 ms, provided by LCModel’s author Stephen Provencher, which was simulated using guidance and routines using the GAMMA simulation library (http://www.gamma.ethz.ch) and data from Govindaraju et al. [30]. The metabolite groups evaluated were: N-acetyl-aspartate (NAA), NAA + N-acetyl-aspartyl-glutamate (NAAG + NAA), total creatine (Cr + PCr), glutamate (Glu), Glu + glutamine (Glx), phosphorylcholine (PCh), PCh + glycerophosphorylcholine (GPC + PCh), and myo-inositol (Ins).

Data mean signal-to-noise (SNR), as reported by LCModel, was (7.58 ± 2.04), ranging from 4 to 12, for
patients; and (6.89 ± 1.28), ranging from 4 to 9, for controls. Data mean full width at half maximum (FWHM), also as reported by LCModel, was 0.064 ± 0.016, ranging from 0.038 to 0.099, for patients; and 0.060 ± 0.014, ranging from 0.038 to 0.084, for controls. There were no significant differences of SNR (t test, \( p = 0.15 \)) or FWHM (t test, \( p = 0.33 \)) among the groups.

Only metabolites quantified with Cramer–Rao lower bounds [31] smaller than 30 % were used in the statistical analysis. We first performed a comparison of Cr + PCr levels between patients and controls using a Mann–Whitney test. As we found no statistically significant differences regarding gender was performed using the Mann–Whitney test considering age and educational level using the Mann–Whitney test.

For demographic and clinical analysis

We compared the difference between groups related to age and educational level using the Mann–Whitney test considering \( p < 0.05 \). The difference between groups regarding gender was performed using \( \chi^2 \) with Yates’s correction. We also determined the mean and standard deviation of the age of onset, CAG length, duration and disease severity and score in the NPI of the patients with SCA3/MJD.

For MRS-c analysis

We correlated the cognitive findings with the significant metabolites. These values were compared between patients and controls groups using the Mann–Whitney test running on SYSTAT9 and we correlated the cognitive findings with the significant metabolite findings in patients with SCA3/MJD.

For VBM analysis

We used the two-sample t test from SPM8 to evaluate GM differences between patients and controls. We selected clusters with 50 voxels and \( p \) value <0.05 (FWE corrected). Then, to investigate the association between cognitive findings and GM maps we performed the multiple regression tests, including clusters with at least 50 voxels and \( p \) value <0.001 (non-corrected).

For DTI analysis

First, we evaluated the difference between groups related to the WM-FA integrity using Student’s t-test by the Tract-Based Spatial Statistics-v1.2 (TBSS) package considering \( p < 0.05 \) corrected for FWE. We then extracted the FA values from the significant areas and used these values to correlate with cognitive findings that were different from controls as mentioned above. For this analysis we used Spearman’s correlation test with significance at \( p < 0.05 \).

For MRS-c analysis

First, we compared the difference between groups regarding metabolites concentration using the Mann–Whitney test, considering the level of significance at \( p < 0.05 \) with Bonferroni correction for multiple comparisons. In addition, we performed Spearman’s correlation between SCA3’s performance in the cognitive tests and the MRS-c metabolites.

Results

Demographic and clinical data

No differences were observed between controls and patients regarding age (46.97 ± 12.06 vs 46.78 ± 11.47 years, \( p = 0.846 \)), educational level (10.40 ± 4.27 vs 10.19 ± 3.78, \( p = 0.590 \)) or gender (17 female SCA3 and controls; 15 male SCA3 and controls). The mean age of disease onset was 36.72 ± 10.91 years (ranging from 15 to 58 years) and the mean disease duration was 10.09 ± 5.78 years (ranging from 2 to 30 years). The mean CAG expansion length was 69.0 ± 5.0 (ranging from 62 to 81). Mean SARA and NPI scores were 13.6 ± 4.27 (ranging from 4.5 to 30) and 11.47 ± 12.26 (ranging from 0 to 51), respectively. Thirteen patients were related to each other with the following degree of relationship: brothers, mother/daughter and aunt/nephew.

Five patients (16 %) were grouped into the subtype I of the disease (large expansions of CAG, early onset and prominent dystonia), 22 patients (69 %) into the subtype II.
(onset in the 30–50 years, cerebellar ataxia and pyramidal signs), four (12%) into the subtype III (short CAG expansions, late onset and prominent neuropathy) and one (3%) into the subtype IV (characterized by parkinsonism) [32].

Neuropsychological evaluation

SCA3/MJD patients had worse cognitive performance compared to controls in the following tests: RAVLT-coding, delayed recall; Corsi block tapping task-forward; Corsi block tapping task-backward; as well as in the depression and anxiety Beck’s inventory (Table 1).

As we identified significant differences between groups regarding depression and anxiety scores as measured by both Beck inventories, we performed a secondary analysis excluding patients with depression and anxiety symptoms (four patients who scored more than 19 for depression symptoms and four patients who scored more than 19 for depression and anxiety) in order to exclude the mood's influence in the cognitive performance. This new analysis comprised 24 SCA3/MJD patients and 24 matched controls.

We observed differences in this secondary analysis in the same tests as in the first analysis (with Bonferroni correction for multiple comparisons): RAVLT-coding: 43.46 ± 11.30 vs 53.83 ± 9.11, p = 0.008; RAVLT-delayed recall: 8.58 ± 3.19 vs. 11.37 ± 2.73, p = 0.004; Corsi block tapping task-backward: 4.79 ± 1.53 vs 6.12 ± 1.19, p = 0.008.

We also classified the performance of SCA3/MJD patients in the cognitive tests since we had a control group well matched and we found mild damage in the RAVLT-coding and inferior average damage in the RAVLT-delayed recall and recognition, Raven’s progressive matrices, Corsi block tapping task-forward and backward, digits span-forward and semantic verbal fluency.

Cognitive performance and VBM

The two-sample t-test analysis showed differences between groups in the GM volume at right and left cerebellum, right putamen, left posterior cingulum, left superior parietal lobe (Brodmann area = 7) and left precentral gyrus (Brodmann area = 4).

Table 1 Comparison of neuropsychological tests between SCA3 patients and controls

<table>
<thead>
<tr>
<th>Neuropsychological evaluation</th>
<th>SCA3/MJD (n = 32) Mean (DP)</th>
<th>Controls (n = 32) Mean (DP)</th>
<th>Non-corrected p value</th>
<th>Corrected p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAVLT-coding</td>
<td>41.97 (11.02)</td>
<td>51.69 (9.55)</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>RAVLT-delayed recall</td>
<td>8.38 (3.16)</td>
<td>11.00 (2.81)</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>RAVLT-recognition</td>
<td>9.81 (7.15)</td>
<td>12.38 (3.73)</td>
<td>0.027</td>
<td>NS</td>
</tr>
<tr>
<td>Raven’s progressive matrices</td>
<td>29.19 (11.47)</td>
<td>35.88 (13.39)</td>
<td>0.003</td>
<td>NS</td>
</tr>
<tr>
<td>CBT task-forward</td>
<td>4.72 (0.89)</td>
<td>5.50 (0.98)</td>
<td>0.002</td>
<td>0.05</td>
</tr>
<tr>
<td>CBT task-backward</td>
<td>4.81 (1.36)</td>
<td>5.94 (1.22)</td>
<td>0.001</td>
<td>0.03</td>
</tr>
<tr>
<td>Digit span-forward</td>
<td>4.56 (1.19)</td>
<td>5.06 (1.11)</td>
<td>0.024</td>
<td>NS</td>
</tr>
<tr>
<td>Semantic verbal fluency</td>
<td>15.66 (5.60)</td>
<td>19.09 (6.36)</td>
<td>0.029</td>
<td>NS</td>
</tr>
<tr>
<td>Beck depression inventory</td>
<td>13.63 (12.25)</td>
<td>3.53 (2.84)</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Beck anxiety inventory</td>
<td>9.44 (9.27)</td>
<td>2.44 (2.36)</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Digits span-backward</td>
<td>3.50 (0.84)</td>
<td>3.84 (1.14)</td>
<td>0.175</td>
<td>NS</td>
</tr>
<tr>
<td>Logical memory I</td>
<td>17.56 (9.36)</td>
<td>21.50 (8.06)</td>
<td>0.061</td>
<td>NS</td>
</tr>
<tr>
<td>Logical memory II</td>
<td>14.69 (8.90)</td>
<td>17.72 (8.29)</td>
<td>0.160</td>
<td>NS</td>
</tr>
<tr>
<td>Pseudorepetition test</td>
<td>37.20 (3.06)</td>
<td>38.41 (2.00)</td>
<td>0.069</td>
<td>NS</td>
</tr>
<tr>
<td>Similarities</td>
<td>12.50 (8.25)</td>
<td>16.06 (9.96)</td>
<td>0.119</td>
<td>NS</td>
</tr>
<tr>
<td>Boston naming test-total</td>
<td>50.34 (7.57)</td>
<td>51.72 (8.54)</td>
<td>0.296</td>
<td>NS</td>
</tr>
<tr>
<td>Figural memory</td>
<td>5.94 (2.08)</td>
<td>6.69 (2.06)</td>
<td>0.302</td>
<td>NS</td>
</tr>
<tr>
<td>Picture completion</td>
<td>12.88 (5.16)</td>
<td>14.56 (6.68)</td>
<td>0.223</td>
<td>NS</td>
</tr>
<tr>
<td>Visual paired association I</td>
<td>8.09 (4.69)</td>
<td>9.88 (5.39)</td>
<td>0.163</td>
<td>NS</td>
</tr>
<tr>
<td>Visual paired association II</td>
<td>5.16 (2.07)</td>
<td>5.06 (2.26)</td>
<td>0.863</td>
<td>NS</td>
</tr>
<tr>
<td>Hooper visual organization</td>
<td>19.00 (5.46)</td>
<td>20.58 (6.63)</td>
<td>0.302</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-categories achieved</td>
<td>3.25 (1.11)</td>
<td>3.34 (1.68)</td>
<td>0.793</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-total errors</td>
<td>26.13 (9.60)</td>
<td>24.81 (13.24)</td>
<td>0.375</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-perseverative responses</td>
<td>15.59 (11.60)</td>
<td>17.69 (15.62)</td>
<td>0.545</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-perseverative errors</td>
<td>13.31 (8.52)</td>
<td>14.72 (11.31)</td>
<td>0.576</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-non-perseverative errors</td>
<td>13.75 (8.58)</td>
<td>11.06 (7.72)</td>
<td>0.192</td>
<td>NS</td>
</tr>
<tr>
<td>WCT-failure to maintain set</td>
<td>1.63 (0.83)</td>
<td>1.41 (0.84)</td>
<td>0.299</td>
<td>NS</td>
</tr>
</tbody>
</table>
There was a significant correlation between patients’ cognitive performance in the RAVLT-coding and right angular gyrus, right superior temporal gyrus, right superior frontal gyrus, left medial frontal gyrus, left insula, left medial temporal gyrus, right parahippocampal gyrus and left culmen; RAVLT-delayed recall and right precentral gyrus, right culmen, left insula, left inferior temporal gyrus, left superior temporal gyrus, left inferior parietal lobe and left culmen; RAVLT-recognition and right inferior frontal gyrus; Raven’s progressive matrices and right precuneus, left inferior parietal lobe and left insula. However, there was no correlation between GM volume and Corsi block tapping task-forward (Table 2; Fig. 1). For more details about the correlation analyses and results see Supplemental Figs. 1, 2).

Cognitive performance and DTI

There were differences between groups regarding the WM-FA in brainstem indicating worse white matter integrity in SCA3/MJD patients. WM-FA in brainstem correlated with digits span-forward \((r = 0.485; p < 0.05)\).

### Table 2 Positive correlation areas between gray matter volume and cognitive findings in patients with SCA3/MJD

<table>
<thead>
<tr>
<th>Cognitive tests</th>
<th>Talarach coordinate</th>
<th>Brodmann area</th>
<th>Anatomic area</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x (mm)</td>
<td>y (mm)</td>
<td>z (mm)</td>
<td></td>
</tr>
<tr>
<td>RAVLT coding</td>
<td>41</td>
<td>−69</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>−19</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>−5</td>
<td>53</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>−43</td>
<td>31</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>−42</td>
<td>16</td>
<td>−1</td>
<td>13</td>
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<td>−31</td>
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<td>8</td>
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<td>17</td>
<td>−19</td>
<td>−10</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>−11</td>
<td>−45</td>
<td>−10</td>
<td>–</td>
</tr>
<tr>
<td>RAVLT delayed recall</td>
<td>29</td>
<td>−14</td>
<td>65</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>−34</td>
<td>−15</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>−42</td>
<td>16</td>
<td>−1</td>
<td>13</td>
</tr>
<tr>
<td></td>
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<td>−29</td>
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<td>20</td>
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<td>40</td>
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<td></td>
<td>−11</td>
<td>−43</td>
<td>−10</td>
<td>–</td>
</tr>
<tr>
<td>RAVLT recognition</td>
<td>54</td>
<td>37</td>
<td>9</td>
<td>46</td>
</tr>
<tr>
<td>Raven’s progressive matrices</td>
<td>−18</td>
<td>−48</td>
<td>50</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>−60</td>
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<td>−11</td>
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<td></td>
<td>22</td>
<td>−52</td>
<td>−47</td>
<td>–</td>
</tr>
<tr>
<td>Corsi block tapping test-forward</td>
<td>−43</td>
<td>−33</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>Digit span-forward</td>
<td>−46</td>
<td>−10</td>
<td>−15</td>
<td>20</td>
</tr>
<tr>
<td>Semantic verbal fluency</td>
<td>−28</td>
<td>−7</td>
<td>47</td>
<td>6</td>
</tr>
</tbody>
</table>

RAVLT Rey auditory verbal learning test, Sup superior, Med medial, Inf inferior, R right, L left

\(p < 0.001\)

We observed reduced relative metabolites concentrations in SCA3/MJD patients compared to controls (with Bonferroni correction for multiple comparisons) (See Supplemental Fig. 3 for sample spectra): NAA/Cr + PCr (0.76 ± 0.15 vs 0.98 ± 0.19, \(p = 0.001\)), NAA + NAAG/Cr + PCr (0.88 ± 0.15 vs 1.28 ± 0.41, \(p = 0.001\)), Glx/Cr + PCr (0.37 ± 0.09 vs 0.47 ± 0.15, \(p = 0.035\)).

There was no significant difference in Glu/Cr + PCr (0.35 ± 0.10 vs 0.44 ± 0.17, \(p = 0.11\)), PCh/Cr + PCr (0.22 ± 0.06 vs 0.25 ± 0.08, \(p = 0.13\)), GPC + PCh/Cr + PCr (0.24 ± 0.04 vs 0.27 ± 0.05, \(p = 0.098\)), and Ins/Cr + PCr (0.83 ± 0.20 vs 0.81 ± 4.09, \(p = 1.00\)). The correlation between the cognitive findings and the relative metabolites concentration findings showed: Corsi block tapping task-forward with Glu (\(r = -0.419\)) and Glx (\(r = -0.405, p < 0.05\)); semantic verbal fluency with PCh (\(r = 0.661, p < 0.05\)) and GPC + PCh (\(r = 0.692, p < 0.05\)).
Cognitive performance and genetic and clinical features

We did not find significant correlation between the cognitive findings and CAG expansion length, duration and disease severity.

Discussion

We performed a detailed cognitive profiling in SCA3/MJD compared to matched control group and correlated cognitive performance with anatomical and functional neuroimaging findings. In addition, our sample is well representative of the population with SCA3/MJD, because we had a large number of patients allocated in the four...
main subtypes. Moreover, the subtype II was the most frequent as shown in other study [33].

We also corrected the analyses of cognitive findings for the influence of mood disorders in a secondary analysis, excluding eight patients with symptoms indicative of depression and anxiety and their respective controls. Therefore, we demonstrated that cognitive changes are present regardless of depressive symptoms.

Although previous studies reported cognitive deficits in SCA3/MJD, there was no consensus regarding the existence of such manifestations [9, 34]. Factors such as sample size, unmatched control group and inappropriate use of tests that required adequate motor control were confounding features in those studies.

In this study, we demonstrated that SCA3/MJD patients have mild impairment in the test that evaluate episodic memory-coding and inferior mild impairment in episodic memory-delayed recall; non-verbal abstract reasoning and working memory (phonological loop and visuospatial sketchpad) as showed by other researchers [8–16]. This performance classification is probably the reason SCA3/MJD patients do not present objective cognitive complains.

Nevertheless, the correlation with anatomic brain areas did not systematically show an association between the cognitive dysfunction with the neuroanatomic area expected. For instance, there were correlations between the coding and delayed recall tasks of episodic memory and anterior cerebellum, which has been essentially considered a sensory-motor nucleus [35]. This result suggests the co-occurrence of cognitive impairment and the atrophy in this area or an association between these aspects that have not been described. It is worth remembering that the association found needs to be validated further, as this was an exploratory study. In addition, we found the non-verbal abstract reasoning function to be correlated with precuneus, left inferior temporal gyrus and cerebellar tonsil. These are areas that may be implicated in visuospatial ability and a complex spatial processing [36, 37].

The same unexpected finding related to the semantic verbal fluency since this impairment was only associated with precentral gyrus morphometry, that is a region usually involved in motor functions. One study in healthy people interpreted this involvement as being secondary to the overt speech production (articulation) necessary to generate words. Then, the association between semantic verbal fluency and the precentral gyrus in our SCA3/MJD sample likely suggests a motor damage related to dysarthria and not to semantic verbal fluency [38].

The remaining cognitive alterations in SCA3/MJD patients—episodic memory and executive dysfunction—were associated with neuroanatomical areas consistently described in previous studies, namely the temporal, parietal and frontal lobes [6].

It is important to remember that the most of these areas that we showed significant correlation with the cognitive tests present functional connections with the cerebellum. Therefore, this structure can be implicated with the cognitive deficits in these patients, even if still indirectly.

The differences between areas with significant grey matter atrophy and areas of significant correlations with cognitive parameters are not so much discrepant as it may seem. Since this is a group analysis and due to the technical characteristics of VBM analysis, the areas of grey matter atrophy that survive the statistical threshold are those which are consistently present among one group (i.e., patients) and not in the other (i.e., controls). Other areas of atrophy that are more heterogeneously found among patients will be “washed out” in the group data analyses. This heterogeneity can be in terms of location and intensity of changes. In addition, when comparing two groups, the overall VBM analyses are set to avoid as much as possible false positives, but not so much false negatives. So, there may be many other areas of atrophy that will not appear as significant in the group comparisons even though these may affect cognitive functions. On the other hand, when performing a correlation analysis, instead of relying so much in a “more homogeneous” atrophy, the test is set to look at continuous variables that vary along the y and x axis (i.e., if the changes are similar across individuals there will be no correlation). In addition, we are not comparing the VBM changes directly with controls. Therefore, areas that vary among subjects in relation to the scores of cognitive tests may be statistically significant and yet not be the same as those shown in the comparisons with controls, even if the degrees of atrophy of these areas are much less pronounced.

We also found abnormal white matter integrity in the brainstem of our patients using DTI, which is in accordance with neuropathological [3] and neuroimaging studies [7, 39]. The correlation between the WM-FA and the cognitive impairments suggests that there is an attentional dysfunction associated with brainstem damage in our SCA3/MJD patients. This result supports that the brainstem is part of the brain network for cognitive functioning as shown in other studies [36, 37].

Concerning the metabolites’ quantification it is possible to conclude that, the decrease in NAA/Cr or PCr, NAA + NAAG/Cr or PCr, and Glx/Cr or PCr indicates that patients with SCA3/MJD present loss or dysfunction of the axonal projections of Purkinje cells [40]. The correlations between cognition and these metabolites’ concentrations suggest that cerebellar dysfunction is associated with executive function as shown in other related diseases [41] and in healthy elderly [42].

In SCA3/MJD, some aspects in the phenotype are largely dependent on the length of CAG expansion such as the
age at onset [41], whereas others essentially rely upon disease duration such as peripheral neuropathy. Regarding cognitive dysfunction, our results demonstrate it occurs independently of the CAG expansion and disease duration [42]. In addition, we failed to find an association between motor disability and cognition. Taken together, these findings suggest that cognitive dysfunction in SCA3 is probably multifactorial and may have multiple mechanisms.

Conclusions

The cognitive alterations in SCA3/MJD are associated with damage to the associative cortex, the brainstem and also the cerebellum. However the impairments are classified as mild regarding episodic verbal memory, working memory and visuospatial abilities. There are neuropsychiatric alterations in some SCA3/MJD patients, but not severe enough to influence their cognitive function. Moreover, cognitive performance in SCA3/MJD was not associated to the CAG expansion, disease duration or ataxia severity.

Acknowledgments This study was supported by the Fundação de Amparo à Pesquisa de São Paulo (FAPESP) and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Brazil. We would like to thank the patients and the healthier volunteers for participating in this study.

Conflicts of interest None.

Ethical standard This study was approved by the ethics committee of the Faculty of Medical Sciences of the State University of Campinas (FCM-UNICAMP).

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