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Cognitive and olfactory deficits in Machado–Joseph disease: A dopamine transporter study

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ABSTRACT

Cognitive and olfactory impairments have been demonstrated in patients with Machado–Joseph disease (MJD), and a possible relationship with dopaminergic dysfunction is implicated. However, there is still controversy regarding the pattern of striatal dopaminergic dysfunction in patients with MJD. In this study, we investigated whether these patients had different Dopamine Transporter (DAT) densities as compared to healthy subjects, and correlated these data with cognitive performance and sense of smell. Twenty-two MJD patients and 20 control subjects were enrolled. The neuropsychological assessment comprised the Spatial Span, Symbol Search, Picture Completion, Stroop Color Word Test, Trail Making Test and Phonemic Verbal Fluency test. The 16-item Sniffin' Sticks was used to evaluate odor identification. DAT imaging was performed using the SPECT radioligand [^{99m}Tc]-TRODAT-1, alongside with Magnetic Resonance imaging. Patients with MJD showed significantly lower DAT density in the caudate (1.34 ± 0.27 versus 2.02 ± 0.50, $p < 0.001$), posterior putamen (0.81 ± 0.32 versus 1.32 ± 0.34, $p < 0.001$) and anterior putamen (1.10 ± 0.31 versus 1.85 ± 0.45, $p < 0.001$) compared with healthy controls. The putamen/caudate ratio was also significantly lower in patients compared with controls (0.73 ± 0.038 versus 0.85 ± 0.032, $p = 0.027$). Even though we had only two patients with parkinsonism, we detected striatal dopaminergic deficits in those patients. No significant correlations were detected between DAT density and cognitive performance or Sniffin' Sticks scores. The data suggests that striatal dopamine deficit is not involved in cognitive or sense of smell deficits. This finding raises the possibility of extra-striatal dopamine and other neurotransmitter system involvement or of cerebellum neurodegeneration exerting a direct influence on cognitive and sensorial information processing in MJD.

1. Introduction

Spinocerebellar ataxia type 3 (SCA3), also called Machado–Joseph disease (MJD) – SCA3/MJD, has been recognized as a diffuse neurodegenerative disorder that comprises not only cerebellar, but also diverse neurological manifestations such as cognitive and olfactory deficits [1–5]. The main cognitive impairments found in patients with SCA3/MJD in previous studies were fronto-executive dysfunction, visuospatial, verbal and visual memory deficits [3–5]. As seen in Parkinson's disease (PD), these cognitive deficits could also have an underlying fronto-striatal dopaminergic dysfunction [6].

Furthermore, smell sense loss is a common and early sign in neurodegenerative disorders including PD [7], and has been more recently described in hereditary ataxias [2,8]. Recently, our group

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demonstrated olfactory dysfunction in a large group of patients with SCA3/MJD [2], but to date studies have been unable to explain the pathophysiological mechanism of hyposmia in patients with SCA3/MJD.

The development of TRODAT-1, a technetium-99m labeled Dopamine Transporter (DAT) tracer, provided an efficient and less expensive alternative for molecular neuroimaging studies [9]. DAT imaging using [^{99m}Tc]-TRODAT-1 and has been reported to be significantly decreased in the *striatum* of most SCA3/MJD patients, including asymptomatic carriers and those with no clinical parkinsonian signs [10,11]. Another study using the PET ligand [^{18}F]-DOPA also showed striatal uptake reduction [12]. However, there is still a controversy regarding the pattern of striatal dopaminergic dysfunction. To illustrate this, a recent [^{123}I]-FP-CIT SPECT study showed greater asymmetrical involvement in the *striatum* (putamen > caudate) in the parkinsonian rather than the cerebellar SCA3/MJD phenotype [13] while another TRODAT-1 study detected the same asymmetrical uptake decrease of DAT densities in two sibs with parkinsonian phenotype and also one with ataxic phenotype [14].

Taking into account a possible relationship of cognitive and olfactory deficits with striatal dopaminergic dysfunction and the controversial DAT imaging findings in SCA3/MJD patients we aimed to investigate whether these patients had different DAT densities as compared with age- and gender-matched control subjects, and correlate these results with our data regarding cognitive and olfactory measurements.

2. Material and methods

2.1. Subjects and clinical protocol

Twenty-two genetically-confirmed SCA3/MJD patients and 20 control subjects were enrolled in this study. All study subjects were from the same pool of 38 patients and 31 controls examined in the previous study by our group [5]. The assessment of ataxia was conducted in all subjects using the International Cooperative Ataxia Rating Scale (ICARS) and the scale for the assessment and rating of ataxia (SARA). Psychiatric symptoms were evaluated by the Hamilton Anxiety Scale (HAMA) and Beck Depression Inventory (BDI). Information on the presence of parkinsonism and dystonia was also recorded. All patients and controls met the study inclusion criteria, namely: younger than 60 years of age (to preclude any age-related cognitive phenomena), Mini-Mental State Examination (MMSE) score greater than 24, and at least eight years of education.

The local research ethics committee approved this study. All participants provided written informed consent for the clinical investigation and subsequent analysis.

2.2. Evaluation of sense of smell

Subjects were submitted to odor identification evaluation using the 16-item, culturally adapted, Portuguese translation of the Sniffin' Sticks (SS-16), described in our previous report [2]. Briefly, in this test the patient is asked to sniff the tip of a pen, and identify the correct odor from four possible choices. Subjects' scores range from 0 to 16.

2.3. Neuropsychological tests

Based on our previous study, the neuropsychological tests on which SCA3/MJD patients had showed impairment were selected after controlling for multiple comparisons and, independently of demographic factors, for anxiety and depressive symptoms [5]. These tests were: the Spatial Span Backward and Forward subtest of the WAIS-R (visual attention and working memory); the Symbol Search subtest of the WAIS-III (processing speed and visuospatial function integrity); the Picture Completion subtest of the WAIS-III (visual perception and attention); the Stroop Color Word Test (SCWT) parts 1, 2 and 3 (selective attention, cognitive flexibility and inhibitory control); the Trail Making Test parts A and B (speed of visual search, mental flexibility, attention resources and motor abilities) and the Phonemic Verbal Fluency test (association fluency) [5].

2.4. SPECT imaging procedure

All subjects were submitted to brain [^{99m}Tc]-TRODAT-1 SPECT imaging, a radiotracer with high selectivity and specificity for the DAT. Images were acquired 4 h after injection of 740 ± 74 MBq (~20 mCi) of [^{99m}Tc]-TRODAT-1 using a Dual-head gamma camera "Hawk Eye" (GE Infinia, USA), equipped with high-resolution fan beam collimators. For each scan, a total of 128 projections (30 s per frame) were collected in a step-and shoot mode on a circular 360° orbit in a 128 × 128 matrix with a mean radius of rotation of 15.5 cm. The image data were then reconstructed by standard filtered back projection using a Butterworth filter (cut-off frequency 0.45) with attenuations by Chang's method.

2.5. Imaging analysis

For accurate determination of volumetric regions of interest (vROI), all subjects also underwent structural magnetic resonance imaging (1.5 T Philips Sigma scanner – General Electric, Milwaukee WI, USA) (T1-3D MPRAGE sequence, 248 slices, voxel size = 0.94 mm × 0.94 mm × 0.80 mm, TE = 4.20 ms, TR = 10.5 ms, flip angle = 15, acquisition matrix = 256 × 192; slice thickness = 1.6 mm). The MRI-based ROI analysis were performed by co-registering each subject's TRODAT-1 SPECT scans with his or her own MRI scan using the Statistic Parametric Mapping 5 program (SPM5) (Wellcome Department of Imaging Neuroscience, London, United Kingdom), executed in Matlab (Mathworks, Sherborn, Massachusetts) through a three-dimensional transformation. This process considered intersubject neuroanatomic variability and allowed for measurement of DAT density in each individual subject. Six spheres representing the vROI (right and left caudate, right and left anterior putamen, and right and left posterior putamen) were plotted on individually reoriented MRI scans by a researcher (P.B.N) blind to subject identity and diagnosis, using a SPM5 toolbox (http://marsbar.sourceforge.net/). The centroids of these spheres were defined visually based on anatomical knowledge extracted from individual MRIs. The occipital was used as a reference region. Bilaterally, a sphere with a 3-mm radius was chosen to represent the caudate and anterior putamen whereas a sphere with a 2-mm radius was taken to present the posterior putamen. A sphere with a 12-mm radius represented the occipital in each hemisphere. All individual vROI were smaller than the actual structures they represented to minimize undefined edges and effects of volume averaging. The ratio of the specific DAT density was calculated for each separate vROI according to the following formula: average radioactivity count per voxel per volume of interest minus average radioactivity count per voxel in occipital/average radioactivity count per voxel in occipital. For each region, the average of both right and left sides were calculated. The putamen to caudate ratio (P/C) was also determined by the following formula: average DAT density at anterior and posterior putamen/DAT density at caudate.

2.6. Statistical analysis

The chi-square test (X^2) (without Yates correction) was used for comparing categorical data. Differences in the means of continuous measurements were assessed by Student's *t*-test (*t*).

Pearson's (*r*) correlation coefficients were calculated to verify the strength of the relationship of DAT density with psychiatric assessment scales, neuropsychological tests and sniff tests.

All tests were two tailed and a p value <0.05 was considered to indicate statistical significance except for those involving correlation coefficients, where multiple comparisons were undertaken and the subsequent p value set at a lower value ($p < 0.0033$ for controls and less than 0.0027 for patients) according to Bonferroni's correction. Ninety-five percent confidence intervals (CI) were calculated for the difference between means. The entire analysis was calculated using the statistical software SPSS 19 for Windows.

3. Results

No differences were identified between control subjects and SCA3/MJD patients regarding age (41.7 ± 9.1 ys versus 43.8 ± 9.6 ys, $p = 0.479$), sex (48% females versus 52% of males, $p = 1.000$) or years of education (12.4 ± 2.7 versus 12.7 ± 2.9, $p = 0.730$).

Among the patients with SCA3/MJD, the mean age of the disease onset was 34.7 ± 9.7 years and mean disease duration was 7.2 ± 4.6 years. Mean CAG repetition length was 71.0 ± 3.9. Regarding patients' clinical features, mean score was 32.3 ± 16.6 on the ICARS and 10.9 ± 6.0 on the SARA. Five patients had dystonia (22.7%) and two parkinsonism (9.1%).

Table 1 shows a comparison of all neuropsychological tests performed, as well as the psychiatric assessment and SS-16. The test results were similar to those found in our previous report [16].

Regarding DAT density, patients with SCA3/MJD showed significantly DAT density reduction compared with normal controls in the caudate (1.34 ± 0.27 versus 2.02 ± 0.50, $p < 0.001$), posterior