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BRIEF COMMUNICATION

Changes in Cerebral Gray and White Matter in Patients with Pantothenate Kinase-Associated Neurodegeneration: A Long-Term Magnetic Resonance Imaging Follow-Up Study

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ABSTRACT

Objective To determine the volume changes in gray and white matter during a long-term follow-up in patients suffering from pantothenate kinase-associated neurodegeneration (PKAN).

Methods Magnetic resonance imaging was repeated in 13 patients and 14 age-matched controls after a mean interval of more than 7 years. T1-weighted sequences were evaluated by fully automated atlas-based volumetry, compared between groups and correlated with disease progression.

Results The patients did not show generalized cerebral atrophy but did show a significantly faster volume reduction in the globus pallidus during follow-up (between -0.96% and -1.02% per year, $p < 0.05$ adjusted for false discovery rate) than controls, which was significantly related to the progression in their dystonia scores ($p = 0.032$).

Conclusion The volume loss in the globus pallidus over time—together with the accumulation of iron known as the "tiger's eye"—supports the pathophysiologic concept of this nucleus as a center of inhibition and its severe malfunction in PKAN.

Key Words Cerebral gray and white matter volume; Long-term follow-up; Pantothenate kinase-associated neurodegeneration.

Pantothenate kinase-associated neurodegeneration (PKAN) is a rare autosomal-recessive disease and belongs to the group of neurodegenerative conditions involving brain iron accumulation.[1] Genetically, the large cohort of PKAN patients living in the Dominican Republic had been previously characterized; with very few exceptions, they suffer from the missense homogeneous mutation c.680 A>G, p.Y227C.[2] Most patients presented with the typical "tiger's eye," but in some of them, the bright spot in the anterior part of the globus pallidus was obscured by the heavy accumulation of iron deposits.[3,4]

In addition, some gray and white matter (GM and WM) anomalies have been reported in these patients as an increase in GM density in the basal ganglia and anterior cingulate cortex associated with age, as well as reductions in fractional anisotropy of frontal and periventricular WM tracts.[5,6] However, subsequent volume changes in these patients have not yet been investigated. Follow-up (FU) studies in PKAN are rare and have mostly described the clinical course and/or iron deposition in the globus pallidus.[7-10]

Here, we present a retrospective longitudinal magnetic reso-

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nance imaging (MRI) FU study over a mean of seven years looking for signs of neurodegeneration of the GM and WM.

MATERIALS & METHODS

This study was approved by the Local Ethics Committee (CEI-368/2019), and informed consent was received from all participants or their parents.

Patients and controls

The study included 13 patients and 14 volunteers whose GM and WM imaging findings at the first examination were previously reported.[5] All patients suffered from genetically confirmed PKAN dystonia and showed the "tiger's eye" on T2-weighted MRI (Supplementary Figure 1 in the online-only Data Supplement). The mean age at the first MR examination for both groups was comparable (17.0 ± 9.5 years vs. 18.0 ± 8.7 years), as was the interval to FU examination (7.1 years vs. 7.7 years). All patients were examined by an experienced neurologist (Dr. Pedro Roa-Sanchez) on both occasions. The examination included a scoring of dystonia (mean: 23.8 ± 24.4 points; progression per year: 1.0 ± 1.8 points) and of disability (mean: 8.4 ± 7.3 points; progression per year: 1.3 ± 0.9 points) on the Burke-Fahn-Marsden (BFM) scales.

MR examinations

All scans were measured on the same Philips 3T Achieva scanner:

1) MPRAGE:3-dimensional turbo field echo; TR/TE = 6.73/3.11 ms; voxel size = 1 mm^3.

2) T2W SE: TR/TE = 2,050/80 ms, voxel size = 5.0 × 0.5 × 0.5 mm.

3) In 7 patients, T2 times of the globus pallidus were measured: 3D gradient spin echo (GRASE) sequence: TR/TE = 1,000/6.7 ms; flip angle 90°; 32 echoes with 6.7 ms spacing; voxel size = 3.0 × 0.5 × 0.5 mm.

Scans showing obvious movement artifacts were excluded from further evaluation.

Evaluation of data

We applied atlas-based volumetry, a fully automated and objective method for volumetric analysis in individual patients, using algorithms of Statistical Parametric Mapping (SPM) 12 software (www.fil.ion.ucl.ac.uk/spm). Individual brains were segmented into GM, WM, and CSF compartments, and the resulting tissue component images were mapped into a template space by means of high-dimensional elastic registration.[11] Predefined regions of interest (ROIs) derived from probabilistic brain atlases were used to extract regional brain volumes. Here, the Neuromorphometrics (NMO) maximum probability atlas (www.neuromorphometrics.com) and the "Hammersmith Atlas" (http://brain-development.org/brain-atlases/adult-brain-atlases/adult-brain-maximum-probability-map-hammers-mith-atlas-n30r83-in-mni-space/) were used to define most subcortical ROIs, including the caudate nucleus, putamen, globus pallidus and thalamus. The LPBA40 probabilistic brain atlas of the Los Angeles Neuroimaging Laboratory[12] was used to assess frontal and parietal lobes and the cingulated gyrus, which had shown volume differences between patients and controls in a previous study.[3] Altogether, 444 ROIs were analyzed, including the cerebellum and the hippocampi.

GM and WM volumes and ROI-defined segmented subvolumes and their changes over time were compared between both groups by 2 sample *t*-tests and by the nonparametric Mann-Whitney U-test. For comparison of volumes at the first examination, values were normalized to an intracranial volume of 1,400 cm^3, whereas volume changes over time were calculated from the original values. Significance was accepted on a 95% level, adjusted for the false discovery rate (FDR; www.sdmproject.com/utilities/?show=FDR).[13] This adjustment was performed for all volumes and subvolumes, as indicated in Table 1.

In patients, volume changes were correlated with changes in BFM dystonia and disability scores and with age of onset and duration of the disease at the time of the first examination, corrected for age at the first examination, by means of partial correlation analysis included in the statistical package SPSS 15.0 (SPSS Inc., Chicago, IL, USA). The "influence" of volume changes in GM and WM and in the globus pallidus, putamen and caudate nucleus on the variance in changes in dystonia and disability scores was estimated by linear regression analysis. To confirm the volumetric measurements of the putamen and globus pallidus, data from MPRAGE and T2-weighted sequences were independently evaluated using the Multimodal Image Segmentation Tool (MIST)[14] included in FSL (fsl.fmrib.ox.ac.uk/) (Supplementary Figure 2 and Supplementary Table 1 in the online-only Data Supplement).

To check for an influence of increasing iron deposition in the globus pallidus during the FU, we correlated the changes in T2 times to volume changes in the globus pallidus during the FU using Pearson's correlation analysis.

RESULTS

Global GM and WM Volumes

Normalized GM and WM volumes at the time of the 1st examination did not significantly differ between patients and controls (Table 1).

During the FU, GM volumes decreased in patients and con-

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Table 1. Changes in global and segmented gray and white matter volumes

	Volumes at 1st examination (cm³)		Change during FU (%)		CC of volume changes to clinical data in patients	
	Patients	Controls	Patients	Controls	Dystonia	Disability
GM brain	759.42 ± 60.23	716.85 ± 48.79	-0.79 ± 0.95	-0.74 ± 0.49	0.039	0.345
WM brain	429.75 ± 37.74	445.55 ± 26.59	0.29 ± 1.15	0.51 ± 0.72	0.375	-0.173
Globus pallidus[1]	3.06 ± 0.31	2.94 ± 0.31	-0.96 ± 1.19*	-0.16 ± 0.62*	-0.322	0.116
Globus pallidus R[1]	1.54 ± 0.16	1.46 ± 0.15	-1.20 ± 1.30*	-0.10 ± 0.60*	-0.380	0.209
Globus pallidus L[1]	1.52 ± 0.16	1.48 ± 0.16	-0.72 ± 1.14	-0.22 ± 0.69	0.492†	0.001
Nucleus caudatus[1]	6.09 ± 0.75	5.77 ± 0.59	-0.71 ± 1.19	-0.46 ± 0.49	0.408	-0.088
Putamen[1]	8.13 ± 1.43	7.64 ± 1.13	-0.30 ± 3.23	-0.40 ± 0.96	0.557	0.107
Thalamus[1]	12.92 ± 0.99	13.87 ± 0.92	-0.53 ± 0.61	-0.15 ± 0.34	-0.093	-0.205
Globus pallidus[2]	2.69 ± 0.30	2.58 ± 0.39	-1.02 ± 1.21*	0.19 ± 0.66*	-0.314	0.113
Globus pallidus R[2]	1.36 ± 0.15	1.29 ± 0.14	-1.26 ± 1.30*	0.13 ± 0.66*	-0.381	0.207
Globus pallidus L[2]	1.33 ± 0.15	1.29 ± 0.15	-0.77 ± 1.20	0.26 ± 0.72	0.472	-0.023
Nucleus caudatus[2]	7.18 ± 0.83	6.80 ± 0.67	-0.77 ± 1.14	-0.49± 0.46	0.384	-0.104
Putamen[2]	8.40 ± 1.46	7.89 ± 1.16	-0.33 ± 3.15	-0.41 ± 0.92	0.539	0.071
Thalamus[2]	11.76 ± 0.74	11.79 ± 0.79	-0.38 ± 0.38	-0.17± 0.32	-0.353	-0.488
Frontal GM[3]	218.04 ± 21.37	202.17 ± 15.92	-1.02 ± 1.07	-0.90 ± 0.63	-0.013	0.367
Frontal WM[3]	129.60 ± 14.27	134.50 ± 11.35	0.27 ± 1.17	0.51 ± 0.68	0.394	-0.134
Parietal GM[3]	124.89 ± 13.34	116.51 ± 9.62	-0.66 ± 1.76	-0.91 ± 0.75	0.022	0.347
Parietal WM[3]	70.60 ± 7.25	73.19 ± 5.12	0.33 ± 1.24	0.41 ± 0.66	0.333	-0.207
Cingulate gyrus R[3]	11.45 ± 1.18	10.76 ± 0.92	-0.33 ± 0.76	-0.88 ± 0.53	0.084	0.421
Cingulate gyrus L[3]	11.85 ± 1.13	11.02 ± 0.95	-1.01 ± 0.83	-0.93 ± 0.57	-0.094	0.290
Hippocampi + amygdala[3]	11.37 ± 1.17	11.90 ± 0.75	-0.40 ± 0.76	-0.12 ± 0.23	-0.170	-0.258
Cerebellum[3]	112.30 ± 10.13	126.20 ± 12.18	-0. 13 ± 0.76	-0.14 ± 0.32	-0.235	-0.293

Segmented volumes of GM and WM at first examination (normalized to an intracranial volume of 1,400 cm³), reduction (-)/increase (+) per year during the FU relative to the volumes at the first examination, and CCs to changes in BFM scale scores for dystonia and disability, based on NMO atlas segmentation (1), Hammersmith atlas segmentation (2) and LPB atlas segmentation (3). *significance at $p < 0.05$, corrected for FDR, of difference between patients and controls, †significant relationship between volume loss and progression of dystonia score in linear regression analysis ($p < 0.05$). FU: follow-up, CC: correlation coefficient, GM: gray matter, WM: white matter, R: right, L: left, BFM: Burke-Fahn-Marsden, NMO: Neuromorphometrics, LPB: LONI Probabilistic Brain Atlas, FDR: false discovery rate.

trols by -0.79% and -0.74% per year, whereas WM volumes increased (0.29% and 0.51% per year), and there were no significant differences. In patients, these GM and WM volume changes did not significantly correlate with the clinical course as increases in dystonia and disability scores or age at onset or duration of symptoms.

Segmented Subvolumes of GM and WM

At the first examination, none of the subvolumes that were segmented based on the NMO and Hammersmith atlases, significantly differed between patients and controls, and there were no significant correlations between the clinical data and these subvolumes.

During the FU, all segmented GM areas lost some volume in the patients. Compared to the controls, the patients showed a difference in volume loss only in the globus pallidus that reached significance in the *t*-test and Mann-Whitney U-test (-0.96% vs. -0.16% per year according to NMO atlas and -1.02% vs. a slight increase of 0.19% per year according to Hammersmith atlas; FDR-adjusted $p < 0.05$) (Fig. 1). Linear regression analysis showed a significant ($p = 0.032$) relation between an increase in dystonia scores (set as the dependent variable) and volume reduction in the left globus pallidus with an adjusted $R^2 = 0.295$, which suggested that the linear regression could "explain" approximately 30% of the data variance. Independent evaluation of patient data using the segmentation program MIST confirmed a significant volume reduction in the globus pallidus of 1.35 ± 2.79% per year of the FU ($p = 0.046$), in contrast to a nonsignificant volume change in the putamina.

In the 7 patients with the T2 times of the globus pallidus measured, we observed a mean reduction in the T2 time of 0.92 (± 0.94) ms per year. The correlation coefficients of this reduction to volume loss in the globus pallidus were far beyond any level of significance (Supplementary Table 2 in the online-only Data Supplement).

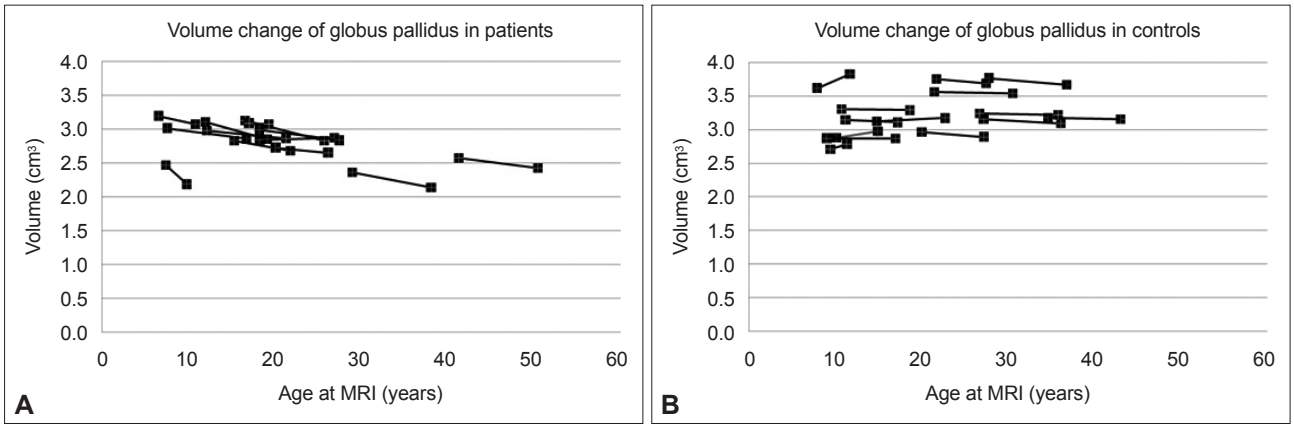

Figure 1. Change in the volume of the globus pallidus over time in patients (A) and controls (B). Mean volumes based on the NMO atlas. MRI: magnetic resonance imaging, NMO: Neuromorphometrics.

DISCUSSION

For the first time, this study presents a long-term observation of GM and WM volume changes in patients suffering from PKAN over a FU time of more than 7 years. The reduction in global GM and increase in global WM were not significantly different in patients and controls. Thus, generalized cerebral atrophy during the FU, as one might expect based on early publications, in which patients have been reported to suffer from severe cognitive impairment,[15,16] can be excluded.

In contrast to our previous study,[5] we did not see significant GM differences at the first examination. This might be due to the main limitation of the study, the rather small sample size, as well as the fact that the subgroup of patients included here had a lower disability score than the total group (8.4 points vs. 21.2 points); thus, these individuals had obviously been in a less advanced stage of the disease at the time of their first examination.

During the FU, the only area where shrinkage was significantly faster in patients than in controls was the globus pallidus. As shown by linear regression analysis, this volume loss was significantly related to the progression of dystonia. This finding has not been reported before and is in line with the suspected malfunction of the globus pallidus in dystonia.[17,18] The relation is especially evident in PKAN, where this nucleus is the site of the primary lesion.[19]

Although we cannot completely exclude some influence of susceptibility alterations in the segmentation process due to the accumulation of iron within the globus pallidus,[20] true volume loss appears to be a more accurate hypothesis because 1) volumes of the globus pallidus at the first examination, when severe iron accumulation had already been present in patients, were (non-significantly) larger in patients than in controls, 2) measurements were confirmed by a second independent segmentation program using T2-weighted images, and 3) we did not see a significant correlation between reductions in T2 times and volume loss in the globus pallidus in the 7 patients with the relevant measurements.

Conclusion

In contrast to cortical GM volume reductions, which were similar in both groups, the present study showed a faster shrinkage of the globus pallidus in PKAN patients than in controls. This finding supports the traditional pathophysiologic concept of this nucleus as a center of inhibition and its severe malfunction in PKAN.

Supplementary Materials

The online-only Data Supplement is available with this article at https://doi.org/10.14802/jmd.20102.

Conflicts of Interest

The authors have no financial conflicts of interest.

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Author Contributions

Conceptualization: all authors. Data curation: Pedro Roa-Sanchez, Jairo Oviedo, Hans-Jürgen Huppertz, Peter Stoeter. Formal analysis: Hans-Jürgen Huppertz, Peter Stoeter. Investigation: Pedro Roa-Sanchez, Pamela Bido, Jairo Oviedo, Herwin Speckter, Peter Stoeter. Methodology: Jairo Oviedo, Hans-Jürgen Huppertz, Peter Stoeter. Project administration: Herwin Speckter, Peter Stoeter. Resources: all authors. Software: Dr. Hans-Jürgen Huppertz, Peter Stoeter. Supervision: Peter Stoeter. Validation: Hans-Jürgen Huppertz, Peter Stoeter. Visualization: Jairo Oviedo, Hans-Jürgen Huppertz, Herwin Speckter, Peter Stoeter. Writing—original draft: Peter Stoeter. Writing—review & editing: all authors.

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Supplementary Table 1. Volume changes of globus pallidus and putamen as calculated by MIST program included in FSL during FU

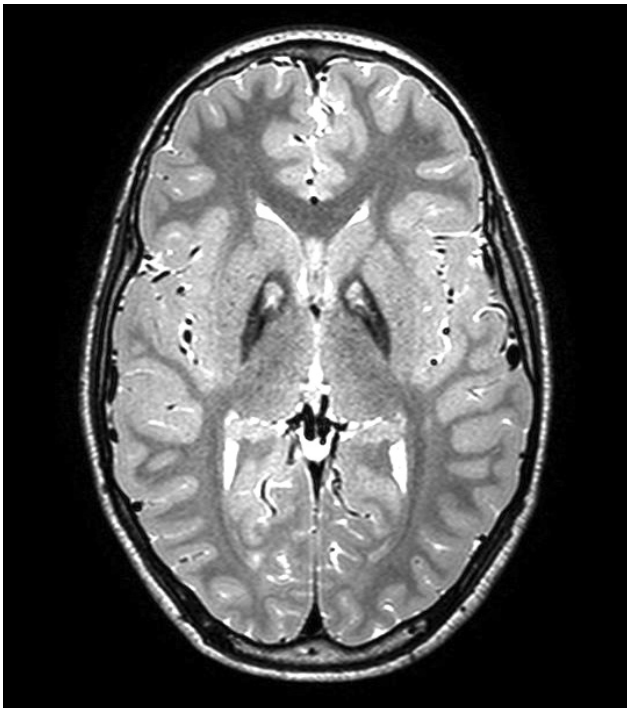
	Mean volume at 1st exam	Mean volume at 2nd exam
Globus pallidus	1.925 ± 0.495 cm^3	1.848 ± 0.358 cm^{3*}
Putamen	5.724 ± 0.569 cm^3	5.481 ± 0.425 cm^3

*significant difference at $p < 0.05$, paired *t*-test. MIST: multimodal image segmentation tool.

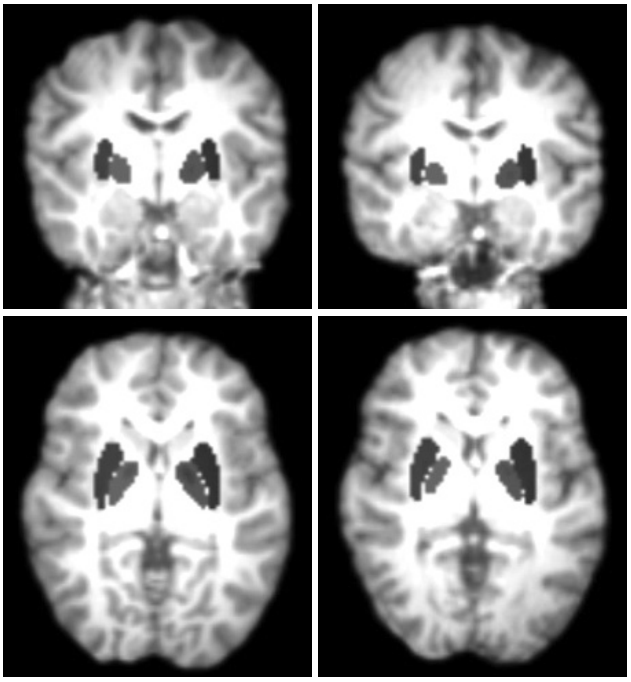
Supplementary Table 2. Correlation of Change of T2 time and volume change of globus pallidus according to segmentation from NMO and Hammersmith atlas

	Difference of T2-time per year	Volume change per year according to NMO atlas	Volume change per year according to Hammersmith atlas
Mean of 7 patients	0.924 ± 0.944 ms	-0.603 ± 0.270%	-0.657 ± 0.273%
CC of T2 time difference to volume change		0.084	0.153

CCs did not reach significance. NMO: Neuromorphometrics, CC: correlation coefficient.



Supplementary Figure 1. The “tiger’s eye” consists of a bright area of gliosis surrounded by a dark area of accumulation of iron in the globus pallidus in T2-weighted MR images. Images are from a 10.8-year-old male PKAN patient with dystonic movement for 4 years, a BFM dystonia score of 10, and a disability score of 8.



Supplementary Figure 2. Volume changesin the globus pallidus and putamen during the follow-up. Normalized MRI of the same female PKAN patient in coronal (upper images) and transversal (lower images) projections showing a normal-sized basal ganglia at the age of 12 years (left images) and volume reductions of 0.26 cm^3 in the globus pallidus and 1.72 cm^3 in the putamen at the age of 19 years (right images).