Silent Saliva Aspiration in Parkinson's Disease

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ABSTRACT

Background: Silent laryngeal penetration and silent aspiration (SLP/SA) are common manifestations in Parkinson's disease (PD) patients and are frequently associated with dysphagia. However, little is known about saliva aspiration in this population. Objective: We investigated the frequency and characteristics of saliva SLP/SA in PD patients with daily drooling (Group A) and in individuals without PD or daily drooling (Group B).

Method: Both groups were evaluated by fiberoptic endoscopic evaluation of swallowing (FEES) after dyeing the oral cavity with blue dye. The oropharynx was assessed for the presence of the stasis of saliva, and sensitivity was tested by direct tactile stimuli.

Results: PD patients (n = 28) and controls (n = 18) were evaluated. We observed silent aspiration of saliva in 10.7% and silent laryngeal penetration of saliva near the vocal folds in 28.6% of Group A; however, none of these events was observed in Group B. Sensitivity in the epiglottis and posterior wall of the hypopharynx was decreased in 89.2% of Group A and in 33.3% of Group B, whereas in the aryepiglottic folds and interarytenoid area, a decrease in sensitivity was observed in 92.8% and in 44.4% of Groups A and B, respectively.

Conclusion: Silent aspiration and laryngeal penetration of saliva are common features in PD patients with daily drooling. The presence of hypoesthesia of the laryngeal structures and the lack of protective reflexes in such patients may play a major role in the mechanisms of SLP/SA.

Key Words: Parkinson's disease; swallowing disorders; dysphagia; sialorrhea; laryngeal penetration; silent aspiration; saliva aspiration

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 27 April 2009; **Revised** 29 December 2009; **Accepted** 17 May 2010
Published online 16 November 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23301

Parkinson's disease (PD) was first described in 1817 as a movement disorder characterized by bradykinesia, resting tremors, and rigidity. However, nonmotor symptoms such as depression, dementia, and olfactory deficits have also been described as components of PD.[1] The early onset of autonomic dysfunction presenting as vesical and erectile dysfunction, a decreased sympathetic skin response (SSR), cardiovascular disorders, and swallowing dysfunction are common features in PD patients.[2]

Sialorrhea, which is the visible manifestation of a swallowing dysfunction, has been correlated with dysphagia and predisposes patients to laryngeal penetration and aspiration, adding to the risk of respiratory tract infection and death in PD patients.[3,4] In these patients, laryngeal penetration and aspiration are more hazardous because the patients have a compromised coughing efficacy.[5] Another issue that is currently not well understood is the role of oropharyngeal bradykinesia in this group of patients.

Although videofluoroscopy is considered the gold standard for swallowing analysis, fiberoptic endoscopy examination of swallowing (FEES) can detect laryngeal penetration and aspiration.[6] FEES is a patient-friendly method that produces recorded and repeatable data without exposing the patient to radiation.[6]

Given that producing and swallowing saliva are major components of the swallowing process and are performed continuously, all swallowing studies have been conducted with food. Thus, our objective was to determine the frequency of laryngeal penetration and aspiration of saliva in PD patients with sialorrhea using FEES.

Patients and Methods

This study was approved by the Federal University of Bahia's Ethics Committee and was conducted according to the Helsinki Declaration (1964). Consecutive patients with PD who exhibited daily drooling (Group A) and age-matched controls without PD or daily drooling (Group B) participated in the study. All participants signed an informed consent form to participate in the study before any procedures and were submitted to the drooling score scale and FEES procedure.

The PD diagnosis was made by a certified neurologist and fulfilled the United Kingdom brain bank criteria (UKPDBB).[7] The disease stage was determined by the Hoehn and Yahr (H&Y) scale.[8] PD patients with daily drooling were assessed during the "on" stage. The drooling score scale (2: no sialorrhea; 9: worst

Table 1. Characteristics of PD patients with daily drooling and the presence of saliva stasis, decreased sensitivity, and SLP/SA

PT	Age	DD	HY	DS	SEN	STA	SLP	SLP*	SA
1	70	9	2	6	√	√	√	√	
2	79	6	1	5	√	√			
3	83	8	2	8	√				
4	62	3	2	6	√	√	√		
5	74	13	3	9	√	√	√	√	√
6	51	8	3	9	√	√	√	√	
7	55	4	2	5	√	√	√	√	
8	59	4	2	8	√				
9	49	4	2	5	√	√			
10	74	6	2	7	√		√		
11	75	11	2.5	7	√	√			
12	57	9	2	6					
13	60	18	2.5	6	√				
14	49	8	2.5	5	√				
15	79	5	4	7	√				
16	80	13	5	7	√	√	√	√	√
17	64	5	1.5	6	√				
18	67	10	4	5	√	√			
19	60	2	1	6	√	√			
20	77	3	1	5	√	√	√	√	√
21	77	5	4	8	√				
22	71	28	2.5	5	√	√	√	√	
23	71	7	2.5	8	√	√			
24	77	6	5	4		√	√		
25	51	7	1.5	6	√				
26	59	4	2.5	8	√	√	√		
27	71	8	2.5	5	√	√	√	√	
28	66	3	3	9	√				
Mean	66.67 ± 10.35	7.75 ± 5.37	2.51 ± 1.06	6.46 ± 1.45					

DD, disease duration; DS, drooling score scale; HY, Hoehn and Yahr scale; SA, silent aspiration; SEN, sensitivity deficit; SLP, silent laryngeal penetration; SLP*, silent laryngeal penetration near the vocal cords; STA, stasis. √ = Event present in the assessment.

drooling) sums the score for severity (1: dry, no sialorrhea; 5: profuse, moist hands, and clothes) and frequency (1: no sialorrhea; 4: constant drooling).[9] After proper coloration of the oral cavity with a nontoxic and edible blue dye, participants were submitted to the FEES procedure.[10] The participants were placed in an upright position and were asked to continue swallowing saliva normally. Direct tactile stimuli with the laryngoscope were applied to the epiglottis, vallecula, piriform sinus, and aryepiglottic folds to trigger and assess the sensorimotor response. The procedure was performed by an otorhinolaryngologist and was recorded on individual digital video disks (DVDs). Laryngeal penetration was defined as the presence of saliva up to the vocal folds, whereas aspiration was the presence of saliva beyond the folds. Exclusion criteria were the use of anticholinergic or antipsychotic drugs, previous treatment of sialorrhea with botulinum toxin, head and neck surgery, severe depression, stroke, and other neurodegenerative disorders.

Statistical Analysis

The data were analyzed using the statistical package SPSS 13.0, and descriptive results are presented as frequencies, means ± standard deviations and percentages. The Mann-Whitney test was performed to compare means between groups.

Results

In Group A, 28 PD patients (19 men, 9 women) were consecutively evaluated. The mean age was 66.67 ± 10.35 years with a mean disease duration of 7.75 ± 5.37 years, and the Hoehn and Yahr stages ranged from 1 to 5 (2.51 ± 1.06). Table 1 presents the characteristics of these patients and their drooling scores. In Group B, 18 individuals were evaluated (4 men, 14 women). The mean age was 65.44 ± 4.7 years.

Table 2 shows the findings from the FEES in both groups. In Group A, we observed silent aspiration of saliva in three patients (10.7%), laryngeal penetration of saliva in the superior and middle portion of the epiglottis in 12 patients (42.8%), and laryngeal penetration of saliva near the vocal folds without inducing a cough reflex in eight patients (28.6%) (Fig. 1). Some of these patients had multiple laryngeal penetration sites that accounted for one SLP event. There were no differences between the groups with and without silent

Table 2. Characteristics of the findings in groups A and B

	Age	DS						
	Mean	Mean	SEN (%)	STA (%)	SLP (%)	SLP* (%)	SA (%)	N
Group A	66.67 ± 10.35	6.46 ± 1.45	93	60.7	42.8	28.6	10.7	28
Group B	65.44 ± 4.7	2.0 ± 0.0	44.4	22.2	38.9	0	0	18

Group A, PD patients; Group B, controls; DS, drooling score scale; SA, silent aspiration; SEN, sensitivity deficit; SLP, silent laryngeal penetration in the superior and middle portions of the epiglottis; SLP*, silent laryngeal penetration near the vocal folds; STA, stasis.

laryngeal penetration and silent aspiration (SLP/SA) regarding disease duration ($P = 0.11$), H&Y scale ($P = 0.58$), drooling score ($P = 0.65$), or age ($P = 0.57$). In Group B, no silent aspiration events or silent laryngeal penetration of saliva near the vocal folds were observed. Conversely, the presence of laryngeal penetration of saliva in the superior and middle portions of the epiglottis was detected in seven participants (38.9%).

In Group A, the sensitivity of the epiglottis and posterior wall of the hypopharynx was decreased in 25 patients (89.28%), whereas sensitivity in the aryepiglottic folds and interarytenoid area was decreased in 26 patients (92.85%). In Group B, the sensitivity of the epiglottis and posterior wall of the hypopharynx was decreased in 6 participants (33.3%), and sensitivity in the aryepiglottic folds and interarytenoid area was decreased in 8 patients (44.4%).

Discussion

The findings of this study support the hypothesis that PD patients with daily sialorrhea have saliva aspiration or laryngeal penetration compared with the control group. Saliva is the major component in swallowing and the medium for the carriage of oral bacterial to the lower respiratory tract.[11] However, the frequency of saliva SLP/SA and its consequences and correlation with food aspiration in this population remain unclear.

In a previous study, we found episodes of silent aspiration or laryngeal penetration of food in 21% of PD patients who had daily drooling.[12] The 1-year follow-up of those patients showed a 9.7-fold higher risk of respiratory infection compared with controls without SLP/SA.[4]

A recent study showed SLP/SA of food in healthy older individuals.[13,14] Episodes of laryngeal

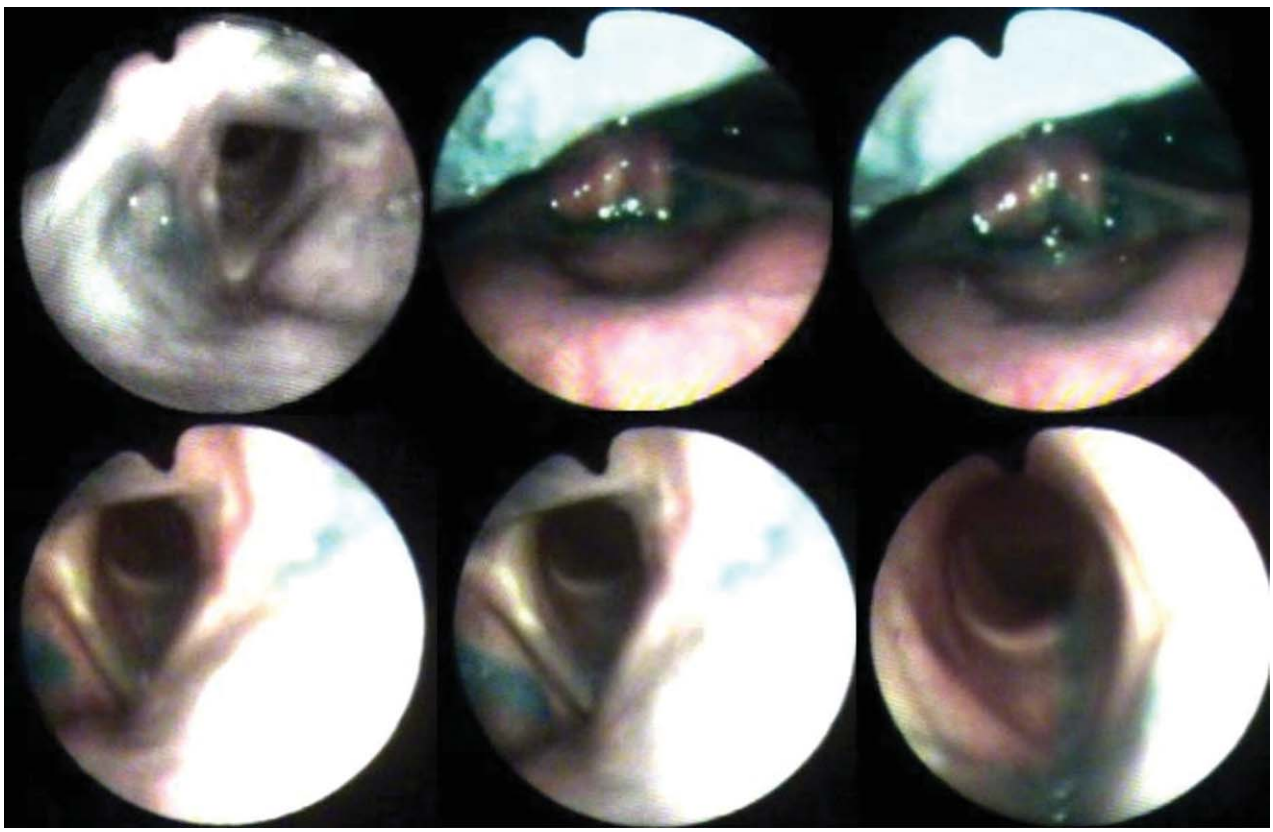

FIG. 1. Sequence of images showing silent penetration and aspiration of blue-dyed saliva in a PD patient with daily sialorrhea.

penetration above the vocal cords (high penetration) were described as common events in this population, with food being ejected from the airway before swallowing was complete.[13] In our study, we found the same findings with saliva, and the depth of the laryngeal penetration was shallow, being cleared before or after swallowing. Although food aspiration events have been described in healthy older individuals,[14] we did not find saliva aspiration in our control subjects. This difference may be due to methodological differences, because other authors have used topical nasal anesthetics in their subjects, whereas in our control and patient groups the the FEES procedure was performed without the use of anesthetics.

In this study, decreased sensitivity in the oropharynx was observed in most of the PD patients, a proportion of whom had oropharyngeal stasis of saliva and some of whom had SLP/SA. This chain of events may be a continuum associated with the compromise of airway protection mechanisms and oropharynx muscle coordination. As stated previously, PD patients have impaired motor and sensory components of coughing,[5] which is likely due to degeneration of the bulbar structures such as the ambiguous and solitary nuclei, emphasizing that PD is much more than a purely dopaminergic dysfunction.

Hospitalized elderly individuals with accumulated secretions in the laryngeal vestibule have a higher risk of food aspiration. This accumulation of secretions is associated with a decreased number of swallows.[15] In PD patients with sialorrhea, there is stasis of food in the larynx,[3,12] which, together with, our findings for saliva stasis, can be assumed to be an additional risk factor for aspiration. Moreover, PD patients swallow during inspiration,[16] and the combination of these situations acts to favor laryngeal penetration and aspiration. Indeed, the silent laryngeal penetration of saliva near the vocal folds seems to be more related to the aspiration events observed in the PD group, because laryngeal penetration in the superior and middle portions of the epiglottis is an expected event in a portion of healthy individuals.[13]

As described in food aspiration,[17] the severity of PD was not correlated with saliva aspiration, reflecting that autonomic phenomena in PD dysphagia are likely unrelated to motor progression. Given that the severity of drooling is correlated with dysphagia, it seems apparent that SLP/SA of saliva should be more prevalent in patients with daily drooling; however, further studies are required to clarify this issue.

This is an initial observation regarding SLP/SA in individuals with PD. An important point to be clarified is the relationship between saliva and food SLP/SA because they may share pathophysiological mechanisms or may even be a spectrum of swallowing disorders observed in PD patients. Therefore, further investigations regarding the true significance of saliva aspiration as a risk factor for respiratory infection need to be performed.

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Regional Cortical Grey Matter Loss in Parkinson's Disease Without Dementia is Independent from Visual Hallucinations

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ABSTRACT

In our previous functional magnetic resonance imaging study, Parkinson's disease (PD) patients with visual hallucinations (VH) showed reduced activations in ventral/lateral visual association cortices preceding image recognition, compared with both PD patients without VH and healthy controls. The primary aim of the current study was to investigate whether functional deficits are associated with grey matter volume changes. In addition, possible grey matter differences between all PD patients and healthy controls were assessed. By using 3-Tesla magnetic resonance imaging (MRI) and voxel-based morphometry (VBM), we found no differences between PD patients with (n = 11) and without VH (n = 13). However, grey matter decreases of the bilateral prefrontal and parietal cortex, left anterior superior temporal, and left middle occipital gyrus were found in the total group of PD patients, compared with controls (n = 14). This indicates that previously demonstrated functional deficits in PD patients with VH are not associated with grey matter loss. The strong left parietal reduction in both nondemented patient groups was hemisphere specific and independent of the side of PD symptoms. © 2010 *Movement* Disorder Society

Key Words: MRI; VBM; Parkinson's disease; visual hallucinations

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 23 April 2009; **Revised** 15 May 2010; **Accepted** 6 July 2010
Published online 16 November 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23375

Parkinson's disease (PD) primarily affects the substantia nigra, including its striatum projections.[1] Classically, cortical pathology has received little attention in PD.[2] More recently, structural abnormalities have been described, although inconsistent and mainly focussed on cognitive impairment.[3,4] However, functional imaging in nondemented PD patients has revealed more consistent cortical impairment, both in motor and visual domains.[4,5] Using functional magnetic resonance imaging (fMRI), we recently specified a relation between visual cortex function and visual hallucinations (VH) in nondemented PD patients by demonstrating reduced extrastriate visual activations preceding image recognition.[6]

Now, we aimed to gain further insight in possible occipitotemporal pathology associated with such VH using 3-Tesla MRI and voxel-based morphometry (VBM). VBM allows determination of cortex density and/or volume changes without a regional bias. It has been used before by one other group to address this topic,[7,8] but using 1.5-Tesla MRI. We also compared all PD patients with healthy controls to see whether regional cortex atrophy would match previously described reduced regional metabolism in PD.

Methods

Subjects

The 38 included subjects were previously studied with fMRI[6] and divided in three groups: PD patients with VH, experienced at least weekly during the last month (n = 11), patients without VH (n = 13), and healthy controls (n = 14). Patients met the criteria of the UK PD Society Brain Bank. Cognition was assessed with the Mini-Mental State Examination (MMSE)[9] and the SCOPA-cog (Scales of Outcomes in Parkinson's disease—cognition).[10] Severity of motor symptoms was rated with the Unified Parkinson's Disease Rating Scale (UPDRS), Part III. Severity of VH and executive functioning were assessed with the Neuropsychiatric Inventory (B: "Hallucinations") and the Frontal Assessment Battery (FAB),[11] respectively. Exclusion criteria were dementia (MMSE < 24), neurological disorders other than PD, psychiatric disorders, visual acuity below 50%, and visual field defects. The local Medical Ethical Committee approved the study. Participants signed an informed consent.

Voxel-Based Morphometry

MRI was performed with a 3-Tesla scanner (Philips, Best, NL) using a standard 6-channel SENSE head coil. T1-weighted 3D anatomical images were defined by isotropic voxels 1 × 1 × 1 mm, matrix 256 × 256, and axial orientation.

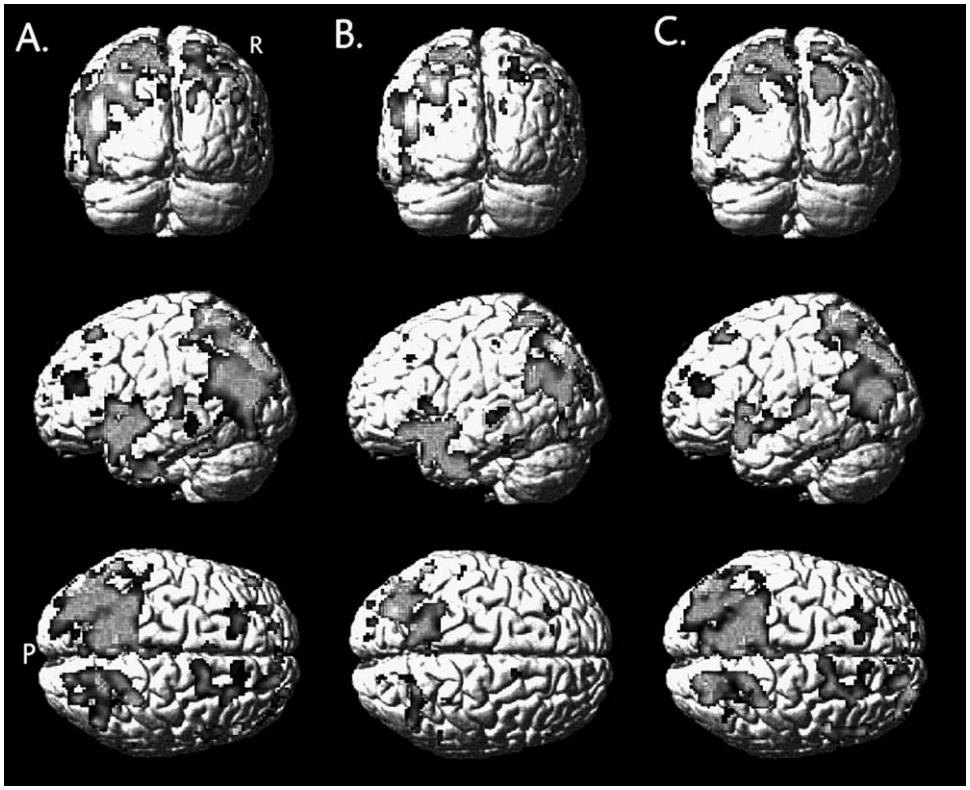

FIG. 1. Regional cortical grey matter changes in PD patients. Grey matter reductions in PD_{total}, compared with healthy controls (**A**), PD without VH, compared with healthy controls (**B**), and PD with VH, compared with healthy controls (**C**) at a threshold of $p < 0.001$ (uncorrected), $k = 20$. Regional grey matter reductions are rendered on a standard MNI brain. R, right; P, posterior.

Image processing and statistical analysis were conducted with Statistical Parametric Mapping [SPM,[12] version 5 (Wellcome Dept.Cogn.Neurol. London, UK; www.fil.ion.ucl.ac.uk/spm)].

Images were spatially normalized [T1 template Montreal Neurological Institute (MNI)] and segmented into grey matter, white matter, and cerebrospinal fluid. Grey matter images were modulated and smoothed (10 FWHM). We used modulated grey matter images, because modulation takes into account the deformation field generated during spatial normalization. In this way, the total amount of grey matter remains the same as it would be in the original images and grey matter volume changes rather than concentration differences can be assessed.

Statistical Analyses

MMSE, FAB and UPDRS-III scores for PD patients were not normally distributed and, therefore, compared using the Mann-Whitney test. Education levels, total SCOPA-cog, and SCOPA-cog subscores were compared with the Kruskal-Wallis test. Normally distributed age differences were tested with ANOVA. Grey matter volume changes were assessed with ANOVA (flexible factorial, main effect factor "group"). Total grey matter was calculated per subject and used as covariate to remove variance due to differences in head size. We compared PD with and without VH with each other and with healthy controls, and all PD versus healthy controls. Initial threshold was $p < 0.001$, voxel-level, uncorrected. Clusters were considered statistically significant at brain-volume corrected cluster-level $p < 0.05$. In addition, Regions Of Interest (ROI's) were defined with Marsbar in SPM5, based on previously reported activation decreases in PD with VH in the left fusiform gyrus.

Results

No differences existed between the three groups regarding age ($F = 0.62$, $p = 0.54$), gender ($\chi^2 = 2.55$, $p = 0.28$), and education level ($\chi^2 = 0.35$, $p = 0.84$). Mean (SD) PD disease duration was 8.0 (4.7) years in PD with VH and 7.9 (2.4) years in PD without VH. PD groups were similar regarding MMSE scores ($z = -1.45$, $p = 0.15$).

Total SCOPA-cog scores differed between groups ($\chi^2 = 9.0$, $p = 0.01$), with verbal memory being the only significant subscore ($\chi^2 = 8.37$, $p = 0.02$; attention: $\chi^2 = 0.81$, $p = 0.67$; executive functioning: $\chi^2 = 2.63$, $p = 0.27$; visuospatial: $\chi^2 = 0.18$, $p = 0.18$). Mann-Whitney test revealed significant differences on

Table 1. Location and MNI coordinates of significant clusters of grey matter loss of all PD patients compared with healthy controls (PD_{total}-HC), PD patients without VH compared with healthy controls (PDnonVH-HC,) and PD patients with VH compared with healthy controls (PDwithVH-HC)

		MNI coordinates				
$P_{corrected}$ (cluster level)	Cluster (*k*)	*x*	*y*	*z*	T (voxel level)	Location
PD_{total}-HC						
0.000	6480	−18	−60	64	6.19	Parietal superior L
		−48	−62	30	5.55	Angular gyrus L
		−4	−54	50	4.86	Precuneus L
		−40	−56	46	4.59	Parietal inferior L
		−30	−74	40	6.36	Occipital middle L
0.000	2764	−46	18	−14	5.72	Temporal superior pole L
		−50	16	4	4.81	Frontal inferior L
0.000	1472	26	−62	48	5.27	Angular gyrus R
		18	−50	68	5.06	Parietal superior R
		16	−70	50	4.80	Precuneus R
0.000	2338	20	32	−18	5.11	Frontal orbicularis sup R
		36	36	−18	4.68	Frontal orbicularis inf R
0.001	556	12	6	64	4.58	(Pre-) SMA
0.034	284	−40	−36	−24	4.70	Fusiform gyrus L
		−46	−46	−22	4.23	Temporal inferior L
0.032	288	52	28	26	4.59	Frontal inferior R
PDnonVH-HC						
0.000	2672	−30	−74	42	5.99	Occipital middle L
		−20	−60	64	5.17	Parietal superior L
		−48	−62	30	5.01	Angular gyrus L
		−8	−66	58	4.00	Precuneus L
		−40	−56	48	3.76	Parietal inferior L
0.000	1968	−40	16	−24	5.35	Temporal superior pole L
		−54	4	−34	5.30	Temporal inferior L
		−48	30	12	4.70	Front orbicularis inf L
		−38	−16	−42	3.69	Temporal inferior L
0.000	1727	20	32	−18	5.14	Frontal orbicularis sup R
		20	42	−16	4.78	Frontal orbicularis mid R
0.04	275	28	−60	46	5.12	Angular gyrus R
		24	−60	58	4.00	Parietal superior R
PDwithVH-HC						
0.000	5231	−8	−66	58	6.19	Precuneus L
		−46	−68	14	6.06	Temporal/occipital mid L
		−18	−60	64	5.69	Parietal superior L
		−48	−64	30	4.74	Angular gyrus L
		−40	−56	46	4.33	Parietal inferior L
0.000	1368	18	−50	68	5.87	Parietal superior R
		16	−72	50	4.82	Precuneus R
		28	−72	42	4.69	Occipital superior R
0.000	1014	−48	18	−14	5.04	Temporal superior pole L
		−60	−22	2	5.02	Temporal middle L
		−50	16	4	4.76	Frontal inferior L
0.003	493	18	56	28	5.08	Frontal superior R
		32	56	20	4.35	Frontal middle R
0.000	752	26	4	68	4.80	Frontal superior R
		8	4	62	4.33	(Pre-)SMA R
		8	30	54	4.07	Frontal sup med R
0.02	331	52	28	26	4.47	Frontal inferior R

Initial threshold in SPM5 was 0.001 (voxel level, uncorrected). Significant clusters ($p < 0.05$, brain-volume corrected) are reported (expected false discovery rate $p < 0.02$).

verbal memory between PD with VH versus controls ($z = -2.66$, $p = 0.008$). Differences were not significant between PD without VH versus either PD with VH ($z = -1.66$, $p = 0.10$) or controls ($z = -1.67$, $p = 0.09$). FAB-scores were lower in PD patients with VH, compared with patients without VH ($z = -2.29$, $p = 0.02$). UPDRS-III scores did not differ ($z = -0.70$, $p = 0.48$).

Voxel-based comparison between grey matter images of PD patients with VH and without VH did not show any differences between the two groups, ROI analysis of the left fusiform gyrus showed no differences either (data not shown). In comparison with healthy controls, however, each of the two PD patient groups, i.e., with or without VH, showed grey matter decreases in prefrontal, parietal, and temporal cortices (Fig. 1B,C).

The combined PD group (=PD_{total}) showed grey matter reductions in prefrontal and parietal cortices (bilaterally), the left temporal lobe, left middle occipital gyrus and right (pre-) supplementary motor area (SMA; Fig. 1A). Table 1 reports significant regions of grey matter decrease ($p < 0.05$, cluster-level, brain-volume corrected).

Parietal grey matter reductions were most apparent in the left hemisphere (Fig. 1, Table 1). To explore a possible relation with contralateral symptom dominance, a PD symptom lateralization index was calculated defined by negative values for left-sided dominance, positive values for right dominance, and zero for absent lateralization. Adding this index as a covariate in the analysis had no effect on the results, indicating that the observed lateralization was hemisphere-specific, independent from the side of symptom dominance. Because PD patients with and without VH differed on the FAB-scores, these were also added as a covariate, without effect on SPM results either.

Discussion

Equal Grey Matter in PD with and without VH

Reduced cortical grey matter volume was a general PD characteristic, without differences between patients with or without VH. This indicates that the functional differences we previously found between these two patient groups, i.e., reduced activation of ventral/lateral extrastriate visual cortices in PD with VH,[6] were not associated with local cortical atrophy. This seeming discrepancy with Ramirez-Ruiz et al.,[7] who associated VH in PD with grey matter reductions in the lingual gyrus and superior parietal lobe, is likely explained by the advanced disease stage in their study. Although they included Hoehn and Yahr stage as a covariate in their analysis, this corrects only for differences between their two PD groups and not between their patients and ours. VH-related functional impairment without anatomy changes in our patients suggests specific neurochemical deficits preceding structural changes. Cholinergic deficit might be considered in this respect possibly causing impaired selection of subcortical information streams, subsequently predisposing to hallucinations.[13] Higher density of Lewy bodies in the temporal lobe might also play a role.[14]

In nondemented PD patients, VH have been associated with cognitive impairment[15–19] and may predict dementia.[20,21] In pathologically proven PD, VH were an initial milestone of advanced disease[22] independent from disease duration. The association between VH and cognitive decline in PD is consistent with enhanced brain atrophy in PD patients with VH,[8] particularly when dementia follows.[8] In this respect, our PD patients with VH might show cognitive impairment and atrophy in follow-up assessments.

Grey Matter Reductions in PD

Although we saw no grey matter differences between the two nondemented PD patient groups, PD_{total} showed grey matter reductions in specific parietal, temporal, occipital, and frontal regions, compared with healthy controls. These reductions were more extensive than previously described, possibly explained by higher sensitivity of 3-Tesla imaging. Frontal and temporal cortex atrophy in nondemented PD patients has been described before with 1.5-Tesla MRI,[7,23–27] but not consistently[28,29] and depending on cognitive impairment[28] or depression.[29] Medial frontal atrophy in our study particularly concerned the rostral (or pre-) SMA, which is consistent with functional cortical impairment in PD due to loss of basal ganglia–thalamus output.[30,31] Associated frontal and parietal grey matter reductions may further reflect impaired neuronal circuitry implicated in both motor and cognitive functions.[5,32,33]

To explain cortical volume reduction, a first consideration is disease-inflicted cell loss. This might be, e.g., a consequence of α-synuclein pathology,[34] although subsequent cortical Lewy body deposition in nondemented PD remains an issue of debate.[35–39] However, tissue pathology, does not explain the left-sided predominance of parietal atrophy we found, because it was not contralateral to the side of dominant symptoms. Volume reduction might alternatively be a dynamic consequence of reduced neuronal activity, leading to reduced dendritic spine volume or astroglial volume reduction.[40] The opposite effect, i.e., action-induced volume increase, has been demonstrated.[40]

Bilateral parietal atrophy in PD has been described before, also with left-sided dominance.[24] To provide a functional explanation for left-sided parietal atrophy, possible associations between parietal motor functions[41] and PD symptoms need to be considered. Left parietal processing of body scheme- or self-referenced (motor) information subserves prehension[41,42] and contributes to the initiation of new motor programs,[42,43] while deficit may result in

ideomotor apraxia.[44] Although apraxia is not a key symptom of PD, reduced internally-driven performance would fit the hypothesis of "de-learning" skilled movements in PD. Thus, atrophy would be secondary to reduced purposeful action, in which a general intentional drive is impaired due to basal ganglia disease. Such dynamic volume change has been demonstrated by the opposite effect: grey matter of visual motion area MT/V5 and the left parietal cortex thickens after learning a new skill such as juggling[45]; left frontoparietal cortex volume enlarges in skilled golfers.[46] Finally, although PD patients, especially those with VH, scored lower at the verbal memory subtask of the SCOPA-cog, we regard it unlikely that the left lateralized parietal atrophy reflects impairment of language-related function.

Conclusions

Compared with healthy controls, gray matter was equally reduced in PD patients with and without VH, indicating that previously found VH-related functional deficits in these patients were not associated with detectable anatomical changes. Hemisphere-specific left parietal atrophy in PD_{total} might reflect a secondary effect of basal ganglia disease, leading to impaired recruitment of internally guided motor programs. ■

Acknowledgments: We would like to thank professor K.L. Leenders for critically reading previous versions of this manuscript.

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Tardive Dyskinesia and Other Movement Disorders Secondary to Aripiprazole

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ABSTRACT

The objective of this report is to draw attention to tardive dyskinesia (TD) caused by aripiprazole, a third generation antipsychotic. TD has been traditionally attributed to typical (first-generation) antipsychotics, but other dopamine receptor blocking drugs and atypical (second- and third-generation) neuroleptics are emerging as an important cause of TD. We reviewed the medical records of patients with TD seen at the Baylor College of Medicine Movement Disorders Clinic between 2002 and 2010 to identify patients with TD associated with aripiprazole. Among 236 patients with TD seen over the specified period, 8 (3.4%) were found to have aripiprazole-associated TD. In 5 patients, TD occurred after exclusive exposure to aripiprazole. The mean age at onset was 55.8 ± 14.8 years with a female predominance. The average duration of treatment with aripiprazole was 18.4 ± 26.4 months. Oro-bucco-lingual stereotypy was seen in all patients. In most patients, TD did not spontaneously improve after stopping aripiprazole. Of the 5 patients treated with tetrabenazine, 4 improved during follow-up. Although aripiprazole, a third generation antipsychotic, has been promoted to have a low risk of TD, the drug accounts for about 3.5% of patients with TD evaluated in a movement disorders clinic. This largest reported series draws attention to the growing incidence of TD and other drug-induced movement disorders associated with "atypical antipsychotics."

Key Words: antipsychotics; aripiprazole; neuroleptics; tardive dyskinesia; tetrabenazine

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Additional Supporting Information may be found in the online version of this article.

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 24 March 2010; **Revised** 17 May 2010; **Accepted** 13 July 2010
Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23329

Tardive dyskinesia (TD), first described in 1957,[1] is a relatively common iatrogenic and potentially irreversible movement disorder that has been traditionally attributed to D2 receptor antagonist activity of ''typical'' or first-generation antipsychotics (FGA), such as haloperidol, chlorpromazine, fluphenazine, thioridizine, and pimozide. Second-generation antipsychotics (SGA) have largely replaced these older medications, partly because of presumably improved pharmacologic profile, but also because of more aggressive marketing. The lower risk of TD secondary to the SGAs was believed to be due to their 5HT2A receptor antagonist activity with less D2 receptor antagonism.[2]

Aripiprazole (Abilify) is a third-generation antipsychotic (TGA), which acts as an agonist, partial agonist, and an antagonist at both dopamine and serotonin receptors.[3] It is currently approved by the US Food and Drug Administration (FDA) for the treatment of schizophrenia and bipolar I disorder.[4] It is also approved as adjunctive treatment for major depressive disorder.[5] The risk of TD associated with aripiprazole is not known, and there is evidence that it may be higher than initially suspected.[2] We report a series of patients with TD and other movement disorders associated with aripiprazole treatment referred to our movement disorders clinic since the drug was approved by the FDA.

Methods

We reviewed the database of all cases of TD evaluated at the Baylor College of Medicine Movement Disorders Clinic seen between January 2002 and January 2010. All patients underwent a detailed neurological examination and were evaluated by a movement disorders specialist. TD was defined as an involuntary movement, usually stereotypic or choreic, resulting from exposure to at least one dopamine receptor blocking agent (DRBA) and persisting for at least 1 month after stopping the offending drug.[6,7] Suspected cases of TD secondary to aripiprazole were identified and their medical records were reviewed in detail.

We categorized our patients according to a level of certainty whether the movement disorder was caused by aripiprazole into two groups: definite and probable. Patients were classified as definite aripiprazole-associated TD if aripiprazole was the only neuroleptic used prior to the onset of the movement disorder and probable if the patient was exposed to multiple neuroleptics, but aripiprazole was the last one before the movement disorder emerged. Demographic data, clinical characteristics, treatment indication, and medication history were entered into a database and analyzed. We also searched Pub Med using the keywords: ''tardive,'' ''dyskinesia,'' and ''aripiprazole'' to systematically review previously published cases.

Results

Of the 236 patients with TD seen at the Movement Disorders Clinic at Baylor College of Medicine between January 2002 and January 2010, 8 (3.4%) patients, 5 women, were identified with TD associated with aripiprazole. Five patients were classified definite aripiprazole-associated TD as the movement disorder occurred after exclusive exposure to aripiprazole, and 3 patients were classified probable (Table 1). Six additional patients with possible aripiprazole-associated TD were not included in our analysis, as the temporal correlation was not known or aripiprazole was not the last neuroleptic added.

The duration of treatment with aripiprazole and the mean age at onset of dyskinesias was known in 7/8 patients and averaged 18.4 $\pm$ 26.4 months and 55.8 $\pm$ 14.8 years, respectively. All patients had oro-buccolingual stereotypies. Of these patients, oro-buccal-lingual stereotypy alone was observed in 4 patients and 4 other patients had a mixed phenomenology. Stereotypy was defined as an involuntary, patterned, repetitive, continuous, coordinated, purposeless, or ritualistic movement, posture, or utterance.[7] Akathisia was reported in one patient 2 weeks after discontinuation of aripiprazole and tardive dystonia was identified in another patient. Only one patient had parkinsonism in addition to TD. Three patients were on additional medications, most frequently risperidone and quetiapine, which had the potential to cause TD. Aripiprazole was used to treat bipolar disorder in most patients and none of our patients had schizophrenia. The majority of patients failed to show meaningful spontaneous improvement in their symptoms after stopping aripiprazole over an average period of 18.4 $\pm$ 22.4 months (range: 4 to 72 months) of follow-up. One patient reported worsening of symptoms after stopping aripiprazole. Five patients were treated with tetrabenazine (TBZ) and of the 4 patients with follow-up data available, all had a moderate improvement in their dyskinesias.

Discussion

We identified 8 patients manifesting various movement disorders associated with the use of aripiprazole. This represents 3.4% of all our cases (N = 236) of TD that had presented to the Baylor College of Medicine Movement Disorder Clinic during the 8 years since the FDA approved aripiprazole. Oro-bucco-lingual stereotypies were the most common type of dyskinesia observed followed by a mixed phenomenology. Our data is consistent with previously published cases of aripirazole-associated TD[2,8–19] including risk factors of advanced age, female gender, presence of mood disorder, diabetes, and prolonged exposure to neuroleptics[15,20–22] (Table 2). The relatively high

Table 1. Tardive dyskinesia definitively and probably attributed to aripiprazole

Patient	Age at onset/ gender (age at first visit)	Underlying disorder	Type of dyskinesia	Duration of treatment with aripiprazole	Dose of Abilify received per day	Other drugs which could cause TD	Spontaneous improvement after discontinuing aripiprazole	Treatment	Improvement after treatment
1[a]	60F (60)	Stress-induced anxiety	Oral stereotypy, rapid dystonic movements, L > R arm, and akathisia 2 weeks after discontinuation	4 months	5 mg	None	Worsening symptoms after discontinuing	Amantadine TBZ	Yes (TBZ)
2	72F (73)	Depression	Oro-bucco-lingual stereotypy	36 months	Unknown	None	Yes	None	–
3	Not stated/F (69)	Bipolar disorder	Oro-bucco-lingual stereotypy	72 months	Unknown	None	No	None	–
4	33F (34)	Bipolar disorder	Dystonic posturing in lower extremities, mild lingual stereotypy	4 months	Unknown	None before the dyskinesias. Quetiapine and ziprasidone (after aripiprazole induced-TD)	No	Trihexyphenidyl TBZ	Yes (TBZ)
5	52M (53)	Bipolar disorder	Oro-bucco-lingual, fingers, lower extremities stereotypy, myoclonic jerks	Unknown	Unknown	None before the dyskinesias. Quetiapine (after stopping aripiprazole)	Yes	None	–
6	34F (34)	Bipolar disorder	Oro-bucco-lingual and lower extremity dyskinesias	5 months	Unknown	Quetiapine, ziprasidone (aripiprazole was the last neuroleptic added)	No	TBZ	Yes
7	60M (67)	Bipolar disorder	Oro-bucco-lingual	4 months	20 mg	Haloperidol, fluphenazide, risperidone, mesoridazine, olanzapine (aripiprazole was the last neuroleptic added)	No follow-up	TBZ	No follow-up
8	63M (63)	Anger/irritability	Oro-bucco-lingual	4 months	Unknown	Metoclopramide, quetiapine (aripiprazole started 4 months before dyskinesia onset)	No	TBZ	Yes

[a]See accompanying video.

Table 2. Cases of aripiprazole-associated tardive dyskinesia reported in the literature

Author	Year	Number of patients	Age (years)	Dose/day (mg)	Underlying disorder	Type of dyskinesia	Treatment duration with aripiprazole	Other drugs	Improvement alter stopping aripiprazole	Treatment
Hall et al.[14]	2009	6 (5F, 1M)	52.2 (range 22–67)	10–15 mg	Motor tics (3), bipolar disorder (1) schizophrenia (1), PD (1)	Akathisia (3), chorea (1), orofacial dyskinesia (3), torticollis (1)	2 weeks to 2 years	Risperidone, quetiapine, olanzapine	Yes—2, Partial—1, Not stated—3	Quetiapine—1, None—2, Not stated—3
Lungu et al.[17]	2009	2 (1F, 1M)	40.5 (19–62)	7.5–15 mg	Anxiety and violent outbursts	Cervical dystonia and upper limbs (1) involuntary movements of tongue, mouth, neck and trunk (1)	18 months to 3 years	None	Yes—1, No—1	DBS in the patient who did not improve
Wang et al.[21]	2009	2 (2F)	46.5 (41–52)	10–15 mg	Schizophrenia	Oro-buccal dyskinesia (2), finger athetosis (1)	2–15 months	Amisulpiride, sulpiride, risperidone (before)	2N (better after treatment)	Vit E (1), Diphenhydramine (1)

frequency of aripiprazole-related TD highlights the importance of obtaining detailed medication history in patients presenting with a movement disorder, specifically focusing on DRBAs, including the TGAs, previously thought to have a low risk of TD.[23–26]

The pathophysiology of TD is not well understood, but many studies support the involvement of dopaminergic system as suggested by increased dopamine receptor sensitivity (possibly associated with D2 receptor upregulation) following chronic blockade of the receptors, particularly in the frontal-subcortical motor circuit.[27–32] With the introduction of the SGAs, which include olanzapine, risperidone, quetiapine, and ziprasidone, it was initially thought that the incidence of TD would decline due to improved tolerability and pharmacologic properties and, indeed, these drugs have almost completely replaced the FGAs in the treatment of schizophrenia.[33] The SGAs differ from the older antipsychotics in that they block 5HT2A receptors and by relatively weak binding to and rapidly dissociating from dopamine receptors.[34,35] Despite the modifications in the pharmacologic profile, the SGAs still have many adverse effects, including hyperprolactinemia, weight gain, diabetes mellitus, metabolic syndrome, and prolongation of the QT interval.[36] Early studies indicated that the risk of TD with SGAs was lower compared with FGAs,[22,37] but long-term studies are lacking.[38,39] The incidence of TD associated with FGAs has been reported to be ~5% per year in adults and 25–30% in the elderly,[40–42] while the reported incidence of TD due to SGAs has been reported to range from 0% in children and 6.8% per year in the combined adult and elderly population.[40,41,43] One study suggested the following rank regarding the potential of atypical neuroleptics to cause TD: clozapine<quetiapine<aripiprazole <olanzapine=ziprasidone <risperidone.[44]

Although TD is usually attributed to the traditional antipsychotic drugs, our study draws attention to the increasing incidence of TD caused by SGAs and TGAs. Our findings are supported by a recent study demonstrating that the incidence of TD associated with atypical antipsychotics is similar to conventional antipsychotics.[45] Other medications causing tardive movement disorders include metoclopramide, the calcium channel blockers flunnarizine and cinnarizine, and certain antidepressants.[46,47] Indeed, metoclopramide has replaced haloperidol as the most common cause of TD in our clinic.[41]

Because of lack of convincing epidemiological studies, the true prevalence and incidence of TD and other drug-induced movement disorders associated with TGAs, such as aripiprazole, are not known. Aripiprazole has been demonstrated to be a 5-HT2A antagonist with partial 5-HT1A agonist activity.[25] In addition, it has unique D2, D3, D4 partial agonist activity (mainly at the presynaptic dopamine

autoreceptors and postsynaptic D2 receptors) thus considered a ''dopaminergic stabilizer'' acting as a dopamine agonist in hypodopaminergic states and as a dopamine antagonist in hyperdopaminergic states.[24,26,48]

One limitation of our study, other than its retrospective design, is that patients categorized as probably were also taking other antipsychotic medications prior to the addition to aripiprazole. Also in most cases, the dosage of aripiprazole was not known. Although we classified as ''definite'' only those patients taking aripiprazole alone before the development of TD, we cannot exclude the possibility that they were also taking other neuroleptics as medication history was obtained only from the patient interview. Most patients did not experience a spontaneous improvement after stopping aripiprazole, supporting the notion that TD may be an irreversible condition, but long-term follow-up is required to establish the natural history of movement disorders associated with aripiprazole. TBZ treatment was associated with improvement in TD in our patients and this monoamine-depleting drug, although currently approved only for the treatment of chorea associated with Huntington disease, is now considered by many as the treatment of choice for patients with troublesome TD.[7,49,50] In contrast to other neuroleptics, TBZ has not been documented to cause TD.[51]

Although there are case reports (but no long-term prospective studies) of improvement of TD after treatment with aripiprazole,[9,24,26,52–59] this may not be a prudent treatment strategy. Spontaneous, coincidental remission of TD rather than a true therapeutic effect of aripiprazole could explain this apparent therapeutic effect.[14] Clinicians should be cautioned against the abrupt withdrawal of neuroleptics which can result in persistent akathisia or other tardive syndromes in adults.[6] Withdrawal emergent syndrome has been described in children with DRDAs including haloperidol, fluphenazine, thiothixene, thiodazine, and trifluoperazine.[51] An early withdrawal effect may occur even when tapering off an antipsychotic medication and can transiently worsen TD.[60]

In conclusion, our series comprises the largest number of patients reported with aripiprazole associated TD and draws attention to the emerging link of TD and other drug-induced movement disorders associated with ''atypical antipsychotics.'' Prospective long-term studies are needed to better understand the underlying pathophysiology, risk factors, and natural history of aripiprazole-induced movement disorders.

Legends to the Video

Table 1, Patient #1.

Segment 1. The video shows a 60-year-old woman with aripiprazole-induced TD manifested by orofacial-lingual stereotypy, rhythmic dystonic pronation of left arm, postural tremor of right arm, akathisia with repetitive, restless touching of her head, and exaggerated arm swing when walking.

Segment 2. Marked improvement of TD while on TBZ, 75 mg/day. The patient is now able to perform repetitive movements in both hands and write without any difficulty.

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Apomorphine Effect on Pain Threshold in Parkinson's Disease: A Clinical and Positron Emission Tomography Study

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ABSTRACT

Patients with Parkinson's disease (PD) frequently experience pain that could be in part due to central modification of nociception. In this randomized controlled double blind study, we compared the effect of apomorphine versus placebo on pain thresholds and pain-induced cerebral activity in 25 patients with PD. Subjective pain threshold (using thermal stimulation, thermotest), objective pain threshold (nociceptive flexion reflex), and cerebral activity ($H_2^{15}O$ PET) during noxious and innocuous stimulations were performed. Neither subjective nor objective pain thresholds nor pain activation profile were modified by apomorphine compared with placebo in 25 PD patients. Apomorphine has no effect on pain processing in PD. We suggest that other monoamine systems than dopaminergic system could be involved. © 2010 *Movement* Disorder Society

Key Words: Parkinson's disease; pain threshold; $H_2^{15}O$ positron emission tomography; apomorphine

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Funding agency: This work was supported by PHRC grant 2006 led by Toulouse Hospital.
Relevant conflict of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 9 June 2010; **Revised** 22 July 2010; **Accepted** 25 July 2010
Published online 19 October 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23406

Introduction

Several recent epidemiological studies have shown pain's prevalence in Parkinson's disease (PD) was higher than in general population.[1,2] In PD, pain can be classified in two physiopathological types: nociceptive pain directly relating to motor symptoms and neuropathic pain resulting from an abnormal nociceptive information process. Previous clinical and neuroimaging studies[3–5] have reported lowered pain thresholds and abnormal activations of nociceptive areas in PD patients. In addition, levodopa (L-dopa) administration reduced pain sensitivity by raising subjective and objective pain thresholds and decreasing nociceptive brain areas hyperactivations.[3,4,6]

However, in the central nervous system, L-dopa is not only converted into dopamine but also in other monoamines like norepinephrine.[7] As monoamine systems were shown to play a major role in nociception,[8] we wondered whether L-dopa antinociceptive effect observed in previous studies could directly result from a dopaminergic effect or from another monoaminergic effect.

Therefore, we assessed the effect of a dopamine agonist, versus placebo, on subjective and objective pain thresholds and on cerebral activity (PET $H_2^{15}O$) during experimental nociceptive stimulations in PD patients.

Methods

Twenty five patients with clinical diagnosis of PD according to UKPDSBB criteria were included. Thirteen were pain free, and 12 experienced neuropathic pain defined as a score ≥ 4 using DN4 questionnaire (Table 1).[9] All were treated by dopaminergic drugs (L-dopa and/or dopamine agonists). Ethic committee approval and written informed consent were obtained.

This randomized, double-blind, apomorphine versus placebo controlled, cross over trial consisted in two periods of 2 days. In each PD patient, apomorphine dosage was chosen as those inducing a motor improvement (at least 30%) on UPDRS motor scale. During the 1st day, after 12 hours of dopamine treatment withdrawal, 2 subcutaneous injections (apomorphine or placebo) were performed 30 minutes before subjective pain threshold determination and PET scans. During the 2nd day, after 12 hours of dopamine treatment withdrawal, 1 subcutaneous injection (apomorphine or placebo) was performed 30 minutes before objective pain threshold determination. Two days after, the second period was realized. The primary efficacy parameter was subjective pain threshold assessed using a Peltier-based contact temperature stimulation with a 12 mm $\times$ 25 mm contact thermode (MSA Thermotest, Somedic AB, Sweden).[10] Heat pain threshold was measured on the thenar of the most affected hemibody using the methods of levels[11] which did not take into account reaction time (often increased in PD patients in OFF condition). Initial

Table 1. Clinical characteristics of neuropathic pain experienced by the painful PD patients group (n=12)

		Pain related to PD					
Patients	Location of pain	Occurs at the beginning of PD or is dependent on motor fluctuations	Is located in the most affected hemibody	Is influenced by dopaminergic drugs	Without other etiology evidence	Is perceived by patient as related to PD	Clinical characterictics of pain
1	Bilateral lower limbs, trunk	yes	yes	no	yes	yes	Burning
2	Left upper limb, trunk	yes	yes	yes	yes	yes	Pins and needles
3	Left upper limb, trunk	yes	yes	no	yes	yes	Tingling, pressure, squeezing
4	Lower limbs	yes	yes	no	yes	yes	Burning
5	Lower limbs	yes	yes	no	yes	yes	Pins and needles
6	Upper and lower limbs	yes	yes	no	yes	yes	Tingling
7	Upper and lower limbs, trunk	yes	yes	yes	yes	yes	Squeezing, pins and needles, tingling
8	Left lower limb, trunk	yes	yes	yes	yes	yes	Burning
9	Upper and lower limbs, trunk	yes	yes	yes	yes	yes	Burning
10	Left lower limb, trunk	yes	yes	yes	yes	yes	Squeezing, electric shocks, itching
11	Left upper limb	yes	yes	no	yes	yes	Burning, numbness
12	Right upper and lower limb, trunk	yes	yes	no	yes	yes	Burning, pins and needles

This table defines the location, the relation between pain and PD and clinical characteristics of neuropathic pain experienced by painful PD patients group. The relation between pain and PD was established using a clinical questionnaire developed in the department of neurology of Toulouse Hospital. A relation was considered between pain and PD if at least 3 of these 5 items were positive. Pain (1) occurs at the beginning of PD or is dependent on motor fluctuations; (2) is located in the most affected hemibody; (3) is influenced by dopaminergic medication; (4) is without other etiology evidence; (5) is perceived by patient as related to PD.

temperature of the thermode of 30°C was increased by steps of 3°C. At the end of the 30 seconds stimulation, patients were asked whether they felt pain or not. Secondary criteria were objective pain threshold and cerebral activity (PET scans). We recorded nociceptive reflex with an OXFORD SYNERGY data acquisition electromyography device.[12] The sural nerve stimulation in the retro-malleolar patch and the recording of electromyographic responses in ipsilateral Biceps Femoris were realized by a pair of surface electrodes in patients into complete muscular relaxation. The electrical stimulation consisted of a train of 5 rectangular pulses delivered over 21 ms from a constant current stimulator (stimulation rate: 0.2 Hz). EMG responses were amplified, digitized, full-wave rectified. Twenty two random intensities were applied. Objective pain threshold (RIII threshold) was defined, as the mean of minimal intensity inducing a RIII reflex response.

The scanner used in this study was an EXACT HR + (CTI/Siemens, Knoxville, TN). After reconstruction, axial and in-plane resolution was 4.1 to 4.5 mm.[13] For each PET scan, patients received six injections of 300 MBq of oxygen-15 radiolabeled water ($H_2^{15}O$) to measure regional cerebral blood flow (rCBF) during two alternated conditions of heat stimulations: painful (P) experimental stimulations (subjective pain threshold plus 1°C) and nonpainful (NP) experimental stimulations (subjective pain threshold less 5°C). The order of painful and nonpainful stimulations was randomized. Each thermal stimulation lasted 80 seconds (20 seconds before and 60 seconds throughout data acquisition).

Data Analysis

Based on a previous study,[6] we needed at least 16 patients to show a 3°C difference between apomorphine and placebo with a standard deviation of 3.1, 80% power and 5% level of significance. A possible order effect and treatment interaction was tested by the model of grizzle (multivaried analysis). Subjective and objective pain thresholds of the 25 patients in apomorphine and placebo conditions were compared using a paired test (Student *t* test). We compared pain thresholds between painful and nonpainful patients (unpaired test). Statistical analyses were done using SAS 9.1. Results were considered to be significant at $P < 0.05$. Clinical values were expressed as means ± standard deviation.

Data analysis of PET scanning was performed using Statistical Parametric Mapping (SPM2), developed by the Functional Imaging Laboratory (Wellcome Trust Centre for Neuroimaging, London, UK). Comparisons between conditions (placebo—NP stimulation;

Table 2. Baseline characteristics and pain thresholds after apomorphine and placebo in PD patients

		All PD patients n = 25 (18 males and 7 females)	Nonpainful patients n = 13 (11 males and 2 females)	Painful patients n = 12 (7 males and 5 females)
Mean age (years)		63.0 ± 6.2	62.2 ± 6.1	63.9 ± 6.5
Mean duration of PD (years)		8.4 ± 2.7	8.2 ± 0.7	8.7 ± 0.9
Mean duration of chronic pain (years)		/	/	5.1 ± 0.6
Mean dose of apomorphine (mg)		4.1 ± 0.8	4.2 ± 0.8	4 ± 0.8
Dopaminergic treatment (mg/d) (Levodopa Equivalent Dosage)		907.8 ± 355.4	876.6 ± 338.2	944.6 ± 387.8
DN4 (mean score)		/	/	4.75
Subjective pain threshold (° C)	Apomorphine	45.5 ± 2.7	46.4 ± 2.4	44.5 ± 2.8
	Placebo	45.6 ± 2.8	46.5 ± 2.70	44.6 ± 2.9
Objective pain threshold (mA)	Apomorphine	10.7 ± 3.6	10.6 ± 3.0	10.8 ± 4.2
	Placebo	9.4 ± 3.7	9.6 ± 3.3	9.1 ± 4.2

There were no significant differences for all the parameters between the two groups of PD patients ($p>0.05$).
No significant differences were found in subjective and objective pain thresholds with placebo or apomorphine neither in all PD patients nor in each PD patients group.

placebo—P stimulation; apomorphine—NP stimulation and apomorphine—P stimulation) were made using *t* statistics with appropriate linear contrasts and then converted to Z-scores. Only clusters exceeding 50 voxels and a threshold of $P_{uncorrected} \leq 0.01$ (Z-score ≥ 3.0) were considered as statistically significant.

Results

There was no order effect and treatment interaction.

Apomorphine significantly improved motor scale (UPDRS III) by 58% ($P < 0.0001$), whereas placebo did not ($P = 0.1$).

Pain Thresholds

Apomorphine did not significantly modify subjective (45.5 ± 2.7°C) and objective pain thresholds (10.7 ± 3.6 mA) compared with placebo (45.6 ± 2.8°C, $P =$ 0.2 and 9.4 ± 3.7 mA, respectively, $P = 0.8$) in the 25 PD patients. Moreover, in each patients group, subjective and objective pain thresholds were not significantly different after apomorphine and placebo. Whatever the treatment (placebo or apomorphine), subjective pain threshold were lower, but did not reach the significant level, in painful patients compared with nonpainful patients (Table 2).

PET Scanning

During the placebo condition, pain induced activation of the left insula, the medial supplementary motor area (SMA), the left prefrontal cortex (superior and inferior frontal gyri; BA 10 and 47, respectively), the right cerebellum, and the left thalamus in the 25 patients (Table 3).

Table 3. Sites of pain-induced activations during placebo and apomorphine conditions in the 25 PD patients

			Placebo condition			Apomorphine condition		
Cerebral areas localization	Brodmann areas	Laterality	x, y, z	z-score	k	x, y, z	z-score	k
SII	40	R	-	-	-	48; −66; 48	3.94	159
Insula	13	L	−42; 8; 0	3.39	272	−38; 22; 4	3.89	617
SMA	6	Medial	0; 8; 62	3.70	476	4; 20; 50	3.47	1491
Prefrontal cortex	9	L	-	-	-	−28; 26; 42	3.56	760
Prefrontal cortex	10	R	-	-	-	34; 56; 20	3.51	256
Prefrontal cortex	10	L	−24; 52; 22	3.07	202	-	-	-
Prefrontal cortex	11	L	-	-	-	−28; 50; −14	3.47	372
Prefrontal cortex	11	R	-	-	-	26; 64; −16	3.34	105
Prefrontal cortex	47	L	−32; 22; −4	3.08	335	-	-	-
Cerebellum	-	R	44; −66; −34	3.2	282	52; −68; −32	3.28	436
Cerebellum	-	Medial	-	-	-	0; −62; −10	3.37	186
Thalamus	-	L	−18; −12; 2	3.12	118	-	-	-
Thalamus	-	R	-	-	-	6; −18; 10	3.34	1072

x, y, z correspond to mediolateral, rostrocaudal and dorsoventral MNI coordinates; k: cluster size (number of voxels).

During the apomorphine condition, pain induced activation of the right secondary somatosensory cortex and thalamus, the left insula, the medial SMA, the bilateral prefrontal cortex (BA 9, 10, 11, and 47), the right and medial cerebellum in those patients.

In all PD patients and in each group, neither the comparison placebo versus apomorphine condition nor the opposite comparison (apomorphine versus placebo condition) revealed any differences in pain activation profiles.

Intergroup comparisons did not reveal any difference in pain-induced activations between painful and nonpainful patients.

Discussion

Our study showed that, compared to placebo, apomorphine had no specific effect on pain threshold and on pain-induced cerebral activity in PD patients.

We could address two comments about values of these present pain thresholds. First, both subjective and objective pain thresholds values are higher than those reported in our previous studies.[6,14] This might be related to placebo analgesic effect because pain thresholds were not determined under baseline condition (i.e., before any injection) but always following subcutaneous injections (saline or apomorphine) which probably induced a placebo effect. Studies of placebo analgesia have shown that pain relief expectation or desire was to reduce significantly pain ratings.[15] Second, no significant differences in subjective pain thresholds in painful and pain-free PD patients was found, whereas Djaldetti et al. study[14] reported that painful PD patients had a lower pain threshold than pain-free ones. From our study, it may be considered that because of the small patients sample size, subjective pain thresholds only tended to be lowered in painful PD patients with no significant level.

Our imaging results revealed cerebral activations of areas classically involved in the nociceptive network such as the thalamic nuclei, insula, somatosensory, and prefrontal cortices[16,17] and showed that apomorphine did not influence pain cerebral activation pattern confirming our clinical findings.

Considering our clinical and neuroimaging results reporting a lack of apomorphine effect, we can hypothesize the role of other monoamine systems in the antinociceptive effect of L-dopa. L-dopa is not only converted into dopamine but also in norepinephrine[7] and could act as a "false transmitter" in serotoninergic terminals too.[18] Therefore, L-dopa effectiveness in pain threshold rising might result from noradrenergic and/or serotoninergic interaction. To argue our hypothesis, these two monoamines systems are involved in nociceptive process[8] and undergo some alteration in Parkinson's disease.[19–21] Actually, severe neuronal loss was found in locus coeruleus (80%) and in raphe magnus (56%). There are also evidence of abnormal low levels of Norepinephrine levels in the striatum and of 5-HT1A (reflective of serotonin) in the cerebrospinal fluid of PD patients. Lesions of the locus coeruleus and raphe nuclei would even occur during presymptomatic stage of PD and earlier than degeneration of the substancia nigra pars compacta.[22] Taken into consideration that pain may precede motor symptoms, locus coeruleus and raphe magnus lesions could be more closely related to pain than those of the substancia nigra. Recently, a clinical trial supported the noradrenergic and/or serotoninergic hypothesis in pain perception in PD demonstrating a decrease in clinical pain scores after duloxetine treatment, a selective serotonine and norepinephrine reuptake inhibitor.[23]

In conclusion, our clinical and neuroimaging results suggest that the dopaminergic system would probably not be directly involved in pain in PD patients and that L-dopa could exert its antinociceptive effect acting on the noradrenergic and/or the serotoninergic systems. ■

Acknowledgments: We address special thanks to the PET team, G Viallard and H Gros-Dagnac, and to the staff of the Clinical Investigation Center for expert help in patient's management.

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Clinical and Biochemical Characterization of Patients with Early Infantile Onset of Autosomal Recessive GTP Cyclohydrolase I Deficiency without Hyperphenylalaninemia

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ABSTRACT

Autosomal recessive guanosine triphosphate cyclohydrolase (GTPCH) type I deficiency is characterized by complex neurological dysfunction. Patients are usually diagnosed with hyperphenylalaninemia in newborn screening. We describe two unrelated patients without hyperphenylalaninemia who presented during early infancy with severe motor retardation, hypokinesia, and truncal hypotonia. CSF homovanillic acid and 5-hydroxyindoleacetic acid as well as tetrahydrobiopterin and neopterin were decreased. Diagnosis of recessive GTPCH deficiency was confirmed biochemically, and a novel homozygous mutation was identified in one patient and a compound-heterozygous mutation of GCH1 in the other. Treatment with Levodopa/Carbidopa resulted in striking clinical improvement, with age-appropriate development at follow-up at 6 years. Autosomal recessive GTPCH deficiency should be considered in infants with severe truncal hypotonia even if hyperphenylalaninemia or classical extrapyramidal symptoms are missing. Neurotransmitter analysis followed by enzyme or mutation analysis can confirm the diagnosis, and Levodopa treatment should be started at high-doses.

Key Words: autosomal recessive GTP cyclohydrolase I; dopa-responsive dystonia; extrapyramidal movements; truncal hyptonia; tetrahydrobiopterin; hyperphenylalaninemia

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Additional Supporting Information may be found in the online version of this article.

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 10 December 2009; **Revised** 18 February 2010; **Accepted** 2 June 2010
Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23329

Guanosine-triphophate cyclohydrolase Type I (GTPCH, EC 3.5.4.16), encoded by the *GCH1* gene, is the rate-limiting enzyme in the synthesis of tetrahydrobiopterin (BH_4), the cofactor of phenylalanine, tyrosine, and tryptophan hydroxylases.[1] Homozygous or compound-heterozygous recessive GTPCH deficiency (OMIM #233910) results in insufficient biosynthesis of dopamine and serotonin. Usually plasma hyperphenylalaninemia is detected in newborn screening.[2] Here we describe the clinical and biochemical spectrum of 2 patients with an early onset GTPCH deficiency without hyperphenylalaninemia and suggest a stepwise diagnostic approach.

Patients and Methods

Patient I

Patient 1 was born in the 41st week of pregnancy by secondary caesarean section because of malposition (BW 3840 g, BL 60 cm, HC 38 cm). Apgar scores were normal. Both parents are first-degree cousins of Pakistani origin. The mother is healthy; the father describes a slightly delayed motor development during infancy that completely resolved. There are no limitations of daily life, but a standardized neuropsychological or motor assessment of both parents was not performed. The index patient is their first son. His younger sister is healthy.

Newborn screening was normal. His early psychomotor development was already retarded: First social contact was observed at the age of 3 months, reaching for objects at the age of 4 months. Further developmental milestones were not reached. At this time, further diagnostic investigations including MRI and basal metabolic investigations were normal.

Because of suspected myopathy, the patient was referred at the age of 17 months. At presentation, the boy showed severe hypotonia. Head control was possible only shortly whereas arms and legs appeared rigid; passive flexion was barely possible. Hands were fisted. Spontaneous movements were scarce. At the same time, the patient appeared surprisingly alert. It was easy to establish eye contact, and he followed objects with clear interest (see Supporting Information Video Segment 1). Apart from miosis, the remaining neurological examination was normal.

Investigations

Laboratory tests including lactic acid and creatine kinase as well as plasma amino acids including phenylalanine were normal (Table 1). Further investigations including electromyography and cerebral MRI were normal.

Neurotransmitter Analysis

Analysis of biogenic amines and pterins in CSF exhibited a clearly reduced concentration of homovanilic acid (HVA). 5-hydroxyindoleacetic acid (5-HIAA) was initially only marginally low. More pronounced abnormalities were found during follow-up. Neopterin was at the lower normal range, whilst tetrahydrobiopterin was significantly reduced. DHPR activity was normal. The data for biogenic amines are summarized in Table 1.

Phenylalanine Loading

A phenylalanine loading test with 100 mg/kg phenylalanine was performed. Phenylalanine/tyrosine ratio was elevated during the first 4 hour of the test, with a striking reduction of plasma biopterin.

GTPCH Enzyme Activity

GTPCH activity analyzed in fibroblasts as previously published,[3] revealed an activity reduced down to 35%, when compared with healthy controls.

GCH1 Analysis

Molecular genetic analysis of the *GCH1* gene revealed a novel homozygous missense mutation in exon 1 (c. 218C > A; p. A73D), which was present in heterozygous state in both parents and in the younger sister.

Treatment and Follow-Up

At the age of 18 months, a treatment trial with Levodopa (L-dopa) with 25% carbidopa (decarboxylase inhibitor) was started with an initial dose of 2 mg/kg BW/d. Within 3 weeks, a significant improvement was observed (Supporting Information Video Segment 2). The L-dopa dosage was slowly increased, without significant side effects. The patient showed still a slight truncal hypotonia, which made sitting impossible, 3 months after start of therapy (Supporting Information Video Segment 3) with 8 mg/kg BW/d L-Dopa/carbidopa. He started to move forward from a supine position. He also became able to reach for things and started imitating syllables. After 1 year of treatment, he was able to sit without support. Shortly there after, he started walking. Although he showed still some instability mainly during fast movements, he was able to climb stairs alternately at the age of 28 months (Supporting Information Video Segment 4). Similar to the motor development also mental development was very satisfactory: Hannover-Wechsler intelligence test III (HAWIVA III) performed at the age of 6 years showed average age-related results in all subtests (Supporting Information Video Segment 5).

Table 1. Biochemical findings of patients 1 and 2 in CSF, plasma and urine

		Patient 1						Patient 2	
Age		16 mo	20 mo	2 5/12 yr	3 8/12 yr	4 8/12 yr	5 8/12 yr	12 mo	18 mo
Biogenic amines [nmol/l]	HVA	145	316	586	459	222	247	142	218
	5HIAA	151	146	171	130	97	103	145	206
Pterins [nmol/l]	BH4	6	10	13	8	3	9	<2	<2
	NEO	7	3	3	4	12	3	<2	<2
	Norm [nmol/l]	HVA 364–870; 5HIAA 155–359; BH4 20–61; Neo 5–53			HVA 313–824; 5HIAA 130–362; BH4 20–61; Neo 5–53			HVA 403–919; 5HIAA 170–412; BH4 24–59; Neo 7–31	HVA 364–870; 5HIAA 155–359; BH4 20–61; Neo 5–53
Urine Pterins [nmol/l]	BIO (Norm 0.5–3.0 nmol/mol Crea)	1.2	1.0						0.1
	NEO (Norm 1.1–4.0 nmol/mol Crea)	0.6	0.7						0.1
Amino acids	PHE (Norm 23–75 µmol/l)	55	49					91 (pp*)	59
	TYR (Norm 20–120 µmol/l)	46	49					72	63
GTPCH enzyme activity		35%						n.d.	
GCH1 analysis				exon 1 (c. 218C > A; p. A73D)				exon 5 (c.640G > A; V204I) exon 6 (c.656_663delAGAAAATG)	
L-Dopa Treatment [mg/kg BW]		–	8/d	7/d	7/d	8/d	6.4/d	8.5 mg/kg BW	8.5 mg/kg BW

Follow-up under treatment is shown.
HVA: homovanillic acid; 5HIAA: 5-hydroxyindoleacetic acid; BIO: biopterin; BH4: tetrahydrobiopterin, NEO: Neopterin; PHE: phenylalanine; TYR: tyrosine; pp: postprandial; n.d.: not done.

Patient II

Patient 2 is the first child of healthy, nonconsanguineous German parents. Family history is unremarkable. She was born after uneventful pregnancy at term (BW 3850 g, BL 52 cm, HC 39 cm). Apart from a fracture of the left clavicula, perinatal adaptation and neonatal period were without complications. Psychomotor development stagnated at the third month of life. From the sixth months of life, progressive hypotonia, mainly in the shoulder girdle was observed. In traction test, no head control was achieved. At the same time, arms and legs were hypertonic. Symptoms worsened during the day and after longer periods awake, while resting led to improvement.

Investigations

Newborn screening and basic laboratory tests were normal. Plasma amino acids including phenylalanine were normal (Table 1). Cerebral MRI and further diagnostic investigations were normal.

Neurotransmitter Analysis

The concentrations of HVA and 5-HIAA, as well as of tetrahydrobiopterin and neopterin were all reduced (see Table 1).

Mutation Analysis

A compound-heterozygous mutation in the *GCH1* gene (exon 5 (c. 640G > A; V204I) and exon 6 (c.656_663delAGAAAATG) were found. The mother was heterozygous for the mutation in exon 5, the father for the eight base pair deletion in exon 6.

GTPCH Enzyme Activity

Measurement of GTPCH activity in fibroblasts was not performed in this child, since her parents declined skin biopsy.

Treatment and Follow-Up

L-dopa with 25% Carbidopa supplementation was started at the age of twelve months at 5.3 mg/kg/d, which improved muscle tone within days. At 13 months of life, the girl reached a developmental age of nine months. She was then able to turn from the dorsal to the ventral position and started to crawl. Truncal hypotonia was still detectable, but the hypertonic posture of her arms and legs as well as diurnal fluctuations disappeared. Due to truncal hypotonia, L-dopa was increased in the following two months stepwise to 8.5 mg/kg/d. From the age of 18 months she was able to sit. CSF analysis at this time revealed an increased but not yet normalized concentration of HVA (Table

Table 2. Clinical symptoms and follow-up under L-Dopa treatment in patients 1 and 2

	Patient 1					Patient 2			
Age	17 mo	18 mo	20 mo	28 mo	5 yr	6 mo	12 mo	18 mo	22 mo
Musulcar hypotonia	+++	++	+	+	+	+++	+	−	−
Hypertonic arms and legs	+++	++	−	−	−	++	−	−	−
Fisted hands	+	−	−	−	−	+	+	−	−
Eye contact	+	+	+	+	+	+	+	+	+
Oculogyric crises	−	−	−	−	−	+	−	−	−
Reaching for objects	−	Unsighted	Targeted	Targeted	Targeted	−	+	+	+
Turning ventral to dorsal position	−	−	+	++	n.d.	−	−	+	+
Sitting	−	−	−	+	+++	−	−	+	+
Crawling			+	+	+++	−	−	−	+
Walking	−	−	−	+	+++	−	−	−	+
L-Dopa dose	−	2 mg/kg BW/d	8 mg/kg BW/d	8 mg/kg/BW	6.8 mg/kg/d	−	5.3 mg/kg/BW	8.5 mg/kg/BW	8.5 mg/kg/BW
Video	1	2	3	4	5			n.a.	

n.a.: not available; n.d.: not done.

1). From the age of 22 months, she became able to crawl and started walking. Unfortunately video documentation of this patient is not available (Table 2).

Discussion

The phenotypic spectrum of GTPCH type I deficiency can be regarded as a continuum between the milder dominant and the severe recessive forms.[4] The clinical spectrum reaches from the classical dopa-responsive dystonia, type Segawa, at the mildest, to neonatal onset of progressive spasticity, rigidity, tremor and dystonia in autosomal recessive DRD at the other end of the continuum.[4–7] Intermediate phenotypes with different clinical symptoms have been described. The presence or absence of hyperphenylalaninemia seems to be one of the key features to distinguish between the different forms. In this report, we describe two patients with a recessive form of GTPCH deficiency without hyperphenylalaninemia, characterized by early infantile onset and severe motor symptoms.

Striking in our patients was the onset of clinical symptoms before the age of 6 months whilst phenylalanine levels were repeatedly normal. The dominating clinical feature in both patients was their severe axial hypotonia in combination with extrapyramidal limb hypertonia. Both children appeared alert leading to the hypothesis of a neuromuscular disease. As creatine kinase and neurophysiological tests were normal, analysis of biogenic amines and pterins in CSF was performed. The abnormal neurotransmitter and pterine constellation in CSF was the first hint pointing to a defect in BH_4 synthesis, specifically GTPCH deficiency.

As the clinical picture of GTPH deficiency is heterogeneous, reliable diagnostic work-up is necessary. As the concentration of 5-HIAA in CSF was only slightly reduced compared to HVA, tyrosine hydroxylase deficiency was initially suspected, but could be excluded by mutation analysis. For further differentiation an oral phenylalanine-loading test was performed. Here mainly the missing increase of biopterin in plasma pointed towards a defect in pterin metabolism. Diagnosis in patient 1 was confirmed by determination of GTPCH activity in fibroblasts. Surprisingly, the residual GTPCH activity was relatively high. It is still controversial whether GTPCH enzyme activity correlates with the clinical picture.[8]

In both children, treatment with L-dopa and carbidopa had a dramatic immediate effect and allowed a more or less normal long term development. The dosage of L-dopa used corresponds to those published in other forms of recessive GTPCH deficiency and differs from the low doses (2–3 mg/kg/d) sufficient in the dominant form of L-dopa responsive dystonia. This stresses the importance of adequate elucidation of the underlying biochemical defect before using a treatment trial with L-dopa as a diagnostic criterion.

Conclusion

Early infantile onset autosomal recessive GTP cyclohydrolase I deficiency without hyperphenylalaninemia is a treatable disorder, which requires high doses of L-dopa. Therefore, in patients presenting in infancy or early childhood with delayed motor development, severe hyptonia and hypokinesia, determination of biogenic amines, and pterins in CSF should be performed. In case of ambiguous results, an oral phenylalanine loading test[8] can be helpful. Confirmation of the diagnosis can be achieved by enzyme analysis in cultured skin fibroblasts[8] or by mutation analysis of *GCH1*. Treatment with L-dopa leads to a very

favorable long-term outcome regarding motor as well as mental development.

Legends to the Video

Video segment 1. Patient 1 at the age of 16 months without L-dopa treatment.

Video segment 2. Patient 1 at the age of 17 months. 4 weeks under L-dopa treatment (2 mg/kg/d).

Video segment 3. Patient 1 at the age of 19 months. 3 months under L-dopa treatment (8 mg/kg/d).

Video segment 4. Patient 1 follow-up at the age of 28 months; 11 months under L-dopa treatment (8 mg/kg/d).

Video segment 5. Patient 1 follow-up at the age of 5 years; L-dopa treatment (6.8 mg/kg/d).

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How Well Do Caregivers Detect Mild Cognitive Change in Parkinson's Disease?

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ABSTRACT

Using the Cambridge Behavior Inventory-Revised, this study evaluated the relationship between caregiver ratings of cognitive change and neuropsychological performance. In sixty-one nondemented patients with Parkinson's Disease (PD; mean age = 64.5 years, MMSE = 28.7), 62% met criteria for mild cognitive impairment. This group were rated as having more overall change as well as memory and behavior change. Caregiver ratings were related to poorer psychomotor speed, learning/memory, language, and executive functioning. The capacity for caregivers to rate mild cognitive change in PD may be useful to assist in early screening and intervention approaches.

Key Words: Parkinson's Disease; cognition; caregiver; CBI-R; mild cognitive impairment

In comparison with the normal population, there is a six-fold increase in the prevalence of dementia associated with Parkinson's Disease (PD).[1] At disease onset, around 25 to 30% of patients will have some degree of cognitive impairment whilst frank dementia (PDD) can eventually affect up to 80% of patients with advanced disease.[2,3] Of significance, the cognitive decline and dementia in PDD are associated with behavioral disturbance, reduced quality of life, caregiver burden, high health care utilization, and the need for institutional care.[3,4]

The ability to identify patients "at risk" of PDD may in the future lead to increased opportunities for

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Relevant conflicts of interest: Nothing to report
Full financial disclosures and author roles may be found in the online version of this article.

Received 11 May 2010; **Revised** 3 June 2010; **Accepted** 6 June 2010

Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23331

early intervention. In this regard, the term "mild cognitive impairment" has been used in neurodegenerative diseases including PD (PD-MCI) to identify individuals who exhibit objective evidence of cognitive impairment but do not meet criteria for dementia. Incidence studies suggest that in drug naïve patients, nearly 20% meet criteria for PD-MCI, mostly in nonmemory domains, double the rate observed within healthy controls. In patients with disease durations of several years, recent data suggests that around half meet criteria for PD-MCI[5] and longitudinal studies support the notion that this decline indeed represents a risk factor for PDD.[6]

Efforts directed toward improved detection of PD-MCI are therefore warranted but current tools designed to assess for PDD or cognition at a gross level are unsuitable for detecting this early change. Neuropsychological assessment may be available for this purpose in some clinical settings but may be inaccessible and/or costly. Because there is considerable heterogeneity in the nature and progression of cognitive impairment in PD,[7] neuropsychological assessment may also be unnecessary for all PD patients. An alternative approach that may assist early detection would be to utilize caregiver reports of cognitive change. In this study, we aimed to examine the relationship between caregiver ratings of cognitive and behavioral change against objective neuropsychological test performance and evaluated whether caregiver ratings differed between those patients with and without PD-MCI.

Methods

Participants

Sixty-one nondemented patients (38 men) were recruited from the Brain & Mind Research Institute PD Research Clinic, University of Sydney. All patients who satisfied UKPDS Brain Bank criteria[8] were required to have a Mini-Mental State Examination (MMSE) score[9] of $\geq$ 24 and were assessed on their regular medication. Demographic details are presented in Table 1. Permission for the study was obtained from the local research ethics committee, and all patients gave written informed consent.

Assessments

All patients underwent neurological examination conducted in their "on" state. They were rated on the Unified Parkinson's Disease Rating Scale sections I–IV[10] and as between Hoehn and Yahr stages I to IV. Disease duration was calculated from age at disease diagnosis. The Beck Depression Inventory-II (BDI-II)[11] was also given to determine depressive symptom severity.

A clinical neuropsychologist assessed cognition using standardized tests. *Working memory* was assessed using the Digit Span sub-test of the Wechsler adult intelligence scale (Digit Span total score).[12] *Psychomotor speed* was assessed using Part A of the trailmaking test (TMT-A, seconds).[13] *Verbal learning and memory* was assessed using the Wechsler memory scale-III logical memory subtest.[12] This prose passage task assesses for both learning (encoding; maximum = 75) and 25 to 35 minute delay. For the delay, percentage retention was calculated so that performance reflected memory storage (% retention). *Language* was assessed by phonetic fluency (letters F, A, S) and semantic fluency (animal names).[14] *Executive functioning* was assessed using Part B of the trailmaking test (TMT-B, seconds).[13] This component reflects set-shifting under timed conditions.[15] In addition, to obtain a measure of *predicted intelligence quotient* (IQ), the National Adult Reading Test[16] was administered.

Table 1. Demographic, neurological, neuropsychiatric and neuropsychological data (n = 61)

	Mean ± sd	Frequency (%)
Age (yr)	64.5 ± 7.9	–
UPDRS, total	36.3 ± 18.1	–
Hoehn & Yahr, stage	2.2 ± 0.6	–
Disease duration (yr)	6.2 ± 5.8	–
Treatment, untreated	–	11/61 (18)
Treatment, L-dopa	–	48/61 (79)
Treatment, L-dopa, plus other[a]	–	36/61 (59)
Treatment, DA agonist monotherapy	–	2/61 (3)
Beck Depression Inventory-II	10.4 ± 7.4	–
NART predicted IQ	112.0 ± 10.4	–
Mini Mental State Examination	28.7 ± 1.5	–
Digit Span, total score	17.4 ± 3.7	–
Logical Memory, encoding	34.0 ± 11.3	–
Logical Memory, % retention	72.5 ± 20.6	–
Verbal fluency, animals	18.9 ± 5.3	–
Verbal fluency, letters F,A,S	41.3 ± 12.7	–
Trailmaking Test Part A (s)	41.2 ± 18.5	–
Trailmaking Test Part B (s)	113.4 ± 78.7	–

Other includes dopamine agonists, COMT inhibitors, MAO inhibitors.

Mild Cognitive Impairment Classification

Diagnoses were conducted by consensus between a Neuropsychologist (SN) and a Neurologist (SJGL) with expertise in PD. Scores on neuropsychological tests were converted to *z*-scores, based on appropriate age-adjusted normative data.[12,17–19] An individual was classified as being either positive or negative for PD-MCI (MCI+, MCI−, respectively) if they demonstrated impairment (1.5 standard deviation decrement, relative to predicted IQ) on at least one domain of neuropsychological testing.[20] All *z*-scores on the TMT-B were considered in relation to TMT-A (i.e., to ensure that scores on the set-shifting component of TMT-B were not simply due to slowed psychomotor speed).

Caregiver Ratings

The Cambridge Behavioral Inventory-Revised (CBI-R)[21] was used to obtain caregiver ratings of cognitive and behavior change. The CBI-R is a 45-item questionnaire that is divided into sections measuring memory/orientation, everyday skills, self-care, abnormal behavior, mood, beliefs, eating habits, sleep, stereotypic behaviors, and motivation. Individual items are rated on a frequency scale from 0 (''never'') to 4 (''constant'') problems.

Statistical Analysis

Data analysis was performed using *SPSS version 16*. Between groups comparisons used analysis of variance or Mann-Whitney U tests depending on homogeneity of variance. Continuous data used Pearson correlation coefficients. All analyses were two-tailed with an alpha level of 0.05.

Results

Table 1 displays demographic data for the sample. Sixty-two percent (n = 38) of the sample demonstrated PD-MCI on at least one domain of functioning. This comprised 23 individuals (37.7%) showing single-domain MCI and 15 patients who had at least two areas of impairment (24.6%).

As shown in Table 2, there was no difference in age, sex, predicted IQ, disease duration, Hoehn and Yahr Stage or depressive symptoms between those patients who did and did not meet criteria for MCI. However, those with PD-MCI did have higher total UPDRS scores (reflecting nonmotor, motor, and activities of daily living features).

Caregiver Ratings

Table 2 shows that for those patients with PD-MCI, caregivers reported significantly more change in CBI-R total scores, as well as memory and behavior. For those domains in which caregivers rated impairments for PD-MCI patients, higher ratings on CBI-R total and memory scores were associated with poorer performance on the MMSE (total, $r = -0.31$, $P < 0.05$; memory, $r = -0.30$, $P < 0.05$, respectively), TMT-A (total, $r = -0.26$, $P<0.05$; memory, $r = 0.28$, $P < 0.05$, respectively), Logical Memory encoding (total, $r = -0.31$, $P < 0.05$; memory, $r = -0.35$, $P < 0.01$, respectively), phonetic fluency (total, $r = -0.31$, $P < 0.05$; memory, $r = -0.27$, $P < 0.05$, respectively), and TMT-B (total, $r = 0.32$, $P < 0.05$; memory, $r = 0.37$, $P < 0.05$, respectively). Caregiver ratings of behavior change were not related to neuropsychological test performance. Caregiver rated mood disturbance was also correlated with BDI-II scores ($r = 0.52$, $P < 0.001$).

Table 2. Demographic, clinical, and CBI-R scores for patients with (MCI+) and without (MCI−) mild cognitive impairment (mean ± sd)

	MCI+ (N = 38)	MCI− (N = 23)	*F*
% male	60.5	65.2	0.1[a]
Age (yr)	65.4 ± 6.5	63.0 ± 9.8	1.3
Predicted IQ	111.3 ± 10.4	113.1 ± 10.4	0.4
BDI-II, total	10.9 ± 8.4	9.9 ± 8.5	0.2
Hoehn & Yahr, stage	2.3 ± 0.7	2.1 ± 0.4	0.6
UPDRS, total	40.6 ± 18.7	29.2 ± 15.0	6.1*
Disease duration (yr)	6.6 ± 6.7	5.6 ± 3.9	429.5[b]
Mini Mental State Examination[c]	28.5 ± 1.6	29.0 ± 1.1	1.8
CBI-R scores			
Total score	27.9 ± 24.2	16.2 ± 14.9	590.5[b,*]
Memory	6.6 ± 6.3	2.5 ± 2.9	633.0[b,**]
Everyday skills	3.1 ± 3.7	1.2 ± 1.5	555.5[b]
Self-care	2.5 ± 3.2	1.3 ± 2.0	513.0[b]
Behavior	2.2 ± 3.2	0.9 ± 1.9	580.0[b,*]
Mood	3.2 ± 2.7	1.9 ± 2.5	3.4
Beliefs	0.5 ± 1.1	0.0 ± 0.2	512.0[b]
Eating	1.7 ± 2.9	1.1 ± 1.8	0.8
Sleep	3.2 ± 2.2	3.3 ± 2.2	0.0
Stereotypies	2.3 ± 2.8	1.3 ± 2.2	1.8
Motivation	2.9 ± 3.6	2.6 ± 3.7	0.1

[a]Chi-square analysis.
[b]Mann-Whitney U test due to unequal variances.
[c]Median MMSE for both groups = 29.0.
*$P < 0.05$.
**$P < 0.01$.

Discussion

This study aimed to determine the extent to which caregivers rate cognitive change in patients with PD-MCI. Consistent with studies examining patients with longer disease durations[5] around 60% of this sample demonstrated at least single-domain MCI, and of these, 24% showed evidence of impairment on at least two neuropsychological domains. Most importantly, the results presented here suggest that caregivers are sensitive to subtle cognitive decline that would not ordinarily be detected on routine clinical assessment. That is, these effects were evident despite the sample having average MMSE scores of 28, suggesting the lack of sensitivity of this screening instrument for detecting cognitive impairment in PD.

The findings demonstrate that caregivers report greater changes in memory and behavior in those with PD-MCI. CBI-R rated changes in memory include poor day-to-day memory, losing or misplacing items, forgetting names, poor concentration, and orientation. Changes in behavior include temper outbursts, uncooperative behavior, inappropriate humour/social behavior or impulsive or tactless behavior. Clearly, these behaviors have major impacts on caregivers and are thus likely to be noticed. While caregiver ratings of behavior change were not related to neuropsychological test performance (possibly reflecting the nature

of the tests which did not specifically probe for social or inappropriate behavior, impulsivity or humour), memory ratings were associated with poorer performance across the domains of psychomotor speed, learning/memory, language, and executive functioning. Interestingly, these correlations were significant, despite the fact that patients were tested in a quiet structured environment using novel tests that potentially lack ecological validity. In the ''real-world'' environment, patients may also use compensatory mechanisms (e.g., external memory aids such as diaries), or may tend to predominantly perform tasks that are routine in nature. In addition, it is worth noting that caregivers' detected cognitive change despite the difference in time-scales (i.e., CBI-R ratings over the last month compared with an average disease duration of 6 years). Thus, although cognitive change in PD may emerge over the course of disease, caregiver ratings assessing recent change seem to be sufficient.

Improved detection of cognitive change early in PD may be useful to guide the use of cholinesterase inhibitors and future neuroprotective strategies. In addition, cognitive training techniques are showing promise at both primary and secondary levels of prevention across many neuropsychiatric and neurodegenerative diseases.[22–24] Prevention of cognitive decline may also be addressed by targeting other neuropsychiatric features that are linked to dementia.[25] In this regard, caregiver ratings of mood change were related to patient reported depressive symptoms. Future studies examining the cognitive benefits of depression treatments in those with PD and PD-MCI would thus be worthwhile. In addition, examination of PD-MCI with respect to disease heterogeneity[7] may reveal phenotypes that could be preferentially screened and targeted for early intervention. ■

Acknowledgments: We thank the patients who participated in this research.

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Estimation of Skeletal Muscle Energy Metabolism in Machado-Joseph Disease Using ^{31}P-MR Spectroscopy

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ABSTRACT

The aim of this study was to determine if muscle energy metabolism, as measured by ^{31}P-magnetic resonance spectroscopy (MRS), is a metabolic marker for the efficacy of treatment of Machado-Joseph disease (MJD). We obtained ^{31}P-MRS in the calf muscle of 8 male patients with MJD and 11 healthy men before, during, and after a 4 minute plantar flexion exercise in a supine position. The data showed that there was a significant difference between the groups in terms of the PCr/(Pi + PCr) ratio at rest ($P = 0.03$) and the maximum rate of mitochondrial ATP production (V_{max}) ($P < 0.01$). In addition, V_{max} was inversely correlated with the scale for the assessment and rating of ataxia score ($r = -0.34$, $P = 0.04$). The MJD group also showed a reduction in V_{max} over the course of 2 years ($P < 0.05$). These data suggest that this noninvasive measurement of muscle energy metabolism may represent a surrogate marker for MJD. © 2010 *Movement* Disorder Society

Key Words: Machado-Joseph disease; muscle energy metabolism; SARA; cerebellar ataxia; surrogate marker; ^{31}P-MRS

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 19 February 2010; **Revised** 12 April 2010; **Accepted** 9 June 2010
Published online 3 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23335

Abnormal in vivo skeletal muscle energy metabolism has been observed in polyglutamine disorders such as Huntington's disease and dentatorubropallidoluysian atrophy (DRPLA).[1] In patients with similar disorders, such as Friedreich's ataxia, antioxidant therapy has been shown to improve muscle energy metabolism, specifically the production of adenosine triphosphate (ATP) in skeletal muscle mitochondria.[2] These data suggest that muscle energy metabolism might be a suitable surrogate marker in some neurodegenerative disorders.

Machado-Joseph disease (MJD) is a common spinocerebellar ataxia[3–5] that is also characterized by polyglutamine dysfunction. Although there is no cure at present, various therapies have been developed to treat other polyglutamine disorders.[6,7] However, surrogate markers are necessary to evaluate the effectiveness of these new treatments because of the slowly progressive clinical course of the disease.[8] In this study, we sought to measure muscle energy metabolism by ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) and to evaluate this measurement as a metabolic marker for the efficacy of treatment of MJD.

Subjects and Methods

Subjects

This study was approved by the Hokkaido University ethics committee. Written informed consent was obtained from MJD patients and healthy controls. Subjects were 8 male patients with MJD and 11 healthy men. The mean age of the MJD group was 49.9 ± 10.8 (±SD) years (range: 30–65 years) and that of the healthy group was 40.9 ± 12.0 years (range: 30–72 years). There was no significant difference in the ages of the two groups ($P = 0.13$).

The mean disease duration of the MJD group was 12.4 ± 6.9 (±SD) years (range: 5–22 years). The diagnosis of MJD was based on genetic analysis.[3] The mean number of expanded CAG repeats was 70.8 ± 2.8 (±SD) (range: 67–75 repeats). To assess ataxia severity, the scale for the assessment and rating of ataxia (SARA) was evaluated before patients underwent ^{31}P-MRS.[9] Five patients and 5 healthy controls underwent the same sessions both after 1 year and 2 years. In the 5 patients with MJD, simultaneous SARA evaluations and ^{31}P-MRS analyses were done a total of 15 times. In addition, 2 of the other 3 MJD patients underwent simultaneous sessions after 1 year; the remaining patient underwent only one simultaneous session. Therefore, the total number of times that SARA evaluations and ^{31}P-MRS measurements were done simultaneously was 20 times in the 8 MJD patients.

^{31}P-MRS

As described in previous reports,[1,10] ^{31}P-MRS was carried out in the calf muscle to measure high-energy phosphates using a 1.5T MR imager (Magnetom Vision, Siemens Medical Solutions, Erlangen, Germany) before, during, and after a 4 minute plantar flexion exercise in a supine position. Sixty-four MRS scans were obtained at rest. The plantar flexion exercise was performed with a constant load of one-tenth of the body weight, as described previously.[1] Sixteen scans were recorded while the subject performed a plantar flexion exercise for 4 minutes, and another 8 scans were recorded immediately after the exercise. After the exercise, four sets of eight scans, four sets of 16 scans, three sets of 32 scans, and two sets of 64 scans were obtained at rest. The ratio of phosphocreatine to phosphocreatine plus inorganic phosphate {PCr/(Pi + PCr)}, the ratio Pi/PCr, and the maximum rate (V_{max}) of mitochondrial ATP production were calculated. Intramuscular pH was calculated by the chemical shift of Pi and PCr.[10] The time constant (1/k) was calculated using IGOR Pro software (Wave Metrics Inc, Lake Oswego, OR) using previously reported equation {$PCr(t) = PCr_0 + \Delta PCr\ (1 - e^{-kt})$}. The V_{max} of mitochondrial ATP production was then calculated using this k and the equation {$V_{max} = PCr_{end} * k$}.[10]

Statistical Analysis

We compared the MJD group and the healthy control group using the Student's t-test. Correlations between SARA scores and ^{31}P-MRS measurements were analyzed using the Spearman's rank-correlation coefficient. Change in intramuscular pH was analyzed using the Wilcoxon-Mann-Whitny test. Changes in V_{max} and PCr/(Pi + PCr) ratios at rest over time were analyzed using a repeated ANOVA. All results are expressed as means ± standard deviation (SD).

Results

In Figure 1, example ^{31}P-MRS spectra are shown. Figure 2 demonstrates that there were significant differences in resting PCr/(Pi + PCr) ratios (MJD: 0.864 ± 0.019, control: 0.889 ± 0.027, $P = 0.03$; Fig. 2a) and V_{max} of mitochondrial ATP production (MJD: 13.84 ± 6.38, control: 24.98 ± 5.82, $P < 0.01$; Fig. 2b) between the two groups.

However, there was no significant difference in the time constant (MJD: 28.29 ± 8.13, control: 29.68 ± 4.86, $P = 0.64$), in the resting Pi/PCr (MJD: 0.15 ± 0.03, control: 0.12 ± 0.03, $P = 0.07$), or in the end exercise PCr/(Pi + PCr) ratios (MJD: 0.609 ± 0.197, control: 0.708 ± 0.063, $P = 0.14$). Intramuscular pH change during exercise was mild: in the MJD group, the pH at rest was 7.03 ± 0.02 and the pH at the end of exercise was 7.00 ± 0.02 ($P = 0.396$). In the control group, the pH at rest was 7.03 ± 0.02 and at the end of exercise was 6.97 ± 0.11 ($P = 0.149$).

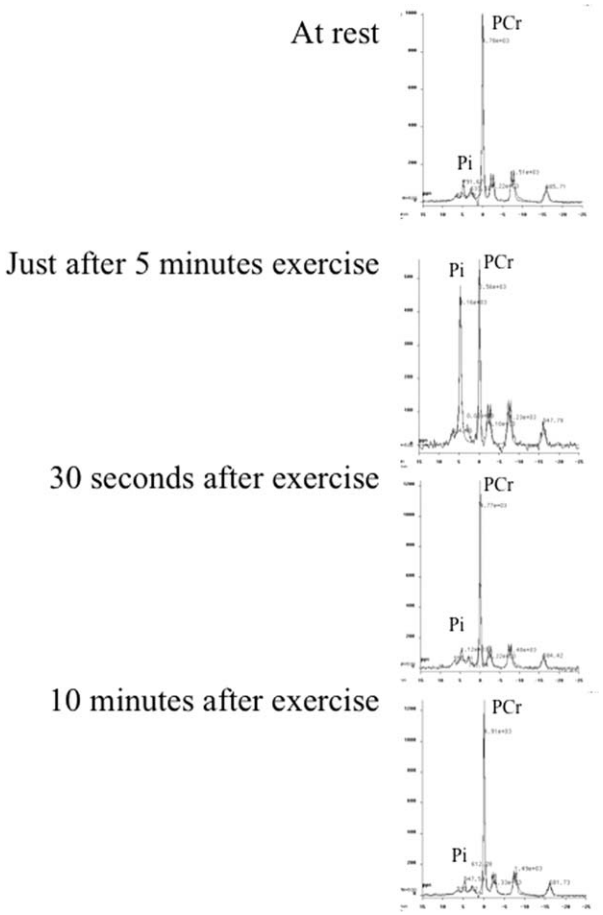

FIG. 1. An example of ^{31}P-MRS spectra. During exercise, inorganic phosphate (Pi) was elevated and phosphocreatine (PCr) was reduced. Both recovered after exercise.

Although the resting PCr/(Pi + PCr) ratios did not correlate with SARA scores ($P = 0.28$), V_{max} was significantly inversely correlated with the SARA total score ($P = 0.04$; Fig. 2c). There were no correlations between either V_{max} ($P = 0.80$) or resting PCr/(Pi + PCr) ($P = 0.16$) and the number of CAG repeats. In addition, there were no correlations between either V_{max} ($P = 0.85$) or resting PCr/ (Pi + PCr) ($P = 0.13$) and the disease duration. Although resting PCr/(Pi + PCr) ratios did not change over time ($P = 0.18$), V_{max} was significantly reduced in MJD patients, but not in controls, after 2 years ($P < 0.05$; Fig. 2d).

Discussion

Our data show that V_{max} of mitochondrial ATP production provides an index of skeletal muscle energy metabolism that can be used to assess the

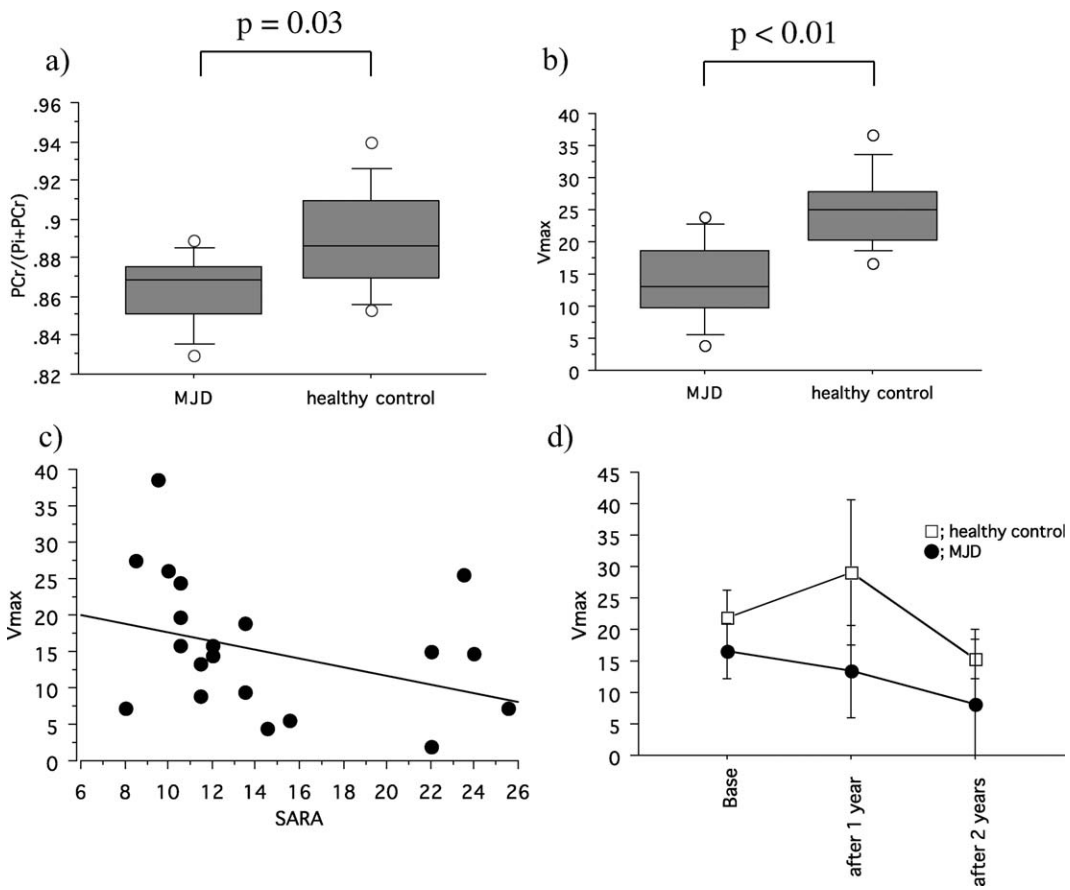

FIG. 2. (**a**) Comparison of rest PCr/(Pi + PCr) between the MJD group and healthy control groups. There were significant differences in resting PCr/(Pi + PCr) ratios (MJD: 0.864 ± 0.019, n = 8, control: 0.889 ± 0.027, n = 11, $P = 0.03$). The box indicates the spread of the central 50% of the data; the median is indicated by a horizontal line through the box. The upper whiskers denote the 90th percentile, and the lower whiskers denote the lower 10th percentile. The circles are data that are lower than the 10th percentile or higher than the 90th percentile. (**b**) Comparison of V_{max} between MJD group and healthy control group. There were significant differences in V_{max} (MJD: 13.84 ± 6.38, control: 24.98 ± 5.82, $P < 0.01$). (**c**) Inverse correlation between V_{max} and SARA (n = 20, r = −0.34, $P = 0.04$) (**d**) Reduction in V_{max} of the MJD group over a period of 2 years when compared with controls (5 subjects in each group, $P < 0.05$).

efficacy of treatment in MJD. In addition, significant differences between patients and the control group were detected in both resting PCr/(Pi + PCr) ratios and V_{max}. However, in our study, the resting PCr/(Pi + PCr) ratio showed no significant correlation with SARA scores and no significant change over 2 years. In addition, the time constant was not significantly different between the two groups. Thus, any interpretation of V_{max} needs to consider that the PCr time constants were not different. This means the true difference in metabolism might be in the resting metabolism, which somehow lowers PCr values.

These data may be due to the small variance of the PCr/(Pi + PCr) ratios and time constants, or the small number of subjects. In addition, while resting PCr/(Pi + PCr) values may be sensitive to muscle damage, PCr recovery may not be very sensitive to injury but very sensitive to metabolic changes. Indeed, Pi has been shown to be increased by ischemia and PCr recovery is associated with mitochondrial function.[1,11] For the results from patients at rest, the ratio of PCr/(Pi + PCr) will reflect changes in PCr. Rising Pi might indicate muscle damage, whereas low PCr might indicate mitochondria activity.[1,11] Our data showed that the change in the PCr/(Pi + PCr) ratio was more sensitive than the Pi/PCr ratio when comparing the MJD patient group and the healthy controls. In addition, intramuscular pH change during exercise was not significant in this study. These facts suggest that mitochondrial dysfunction may influence the pathological mechanism. A muscle pathological study of MJD will be needed to resolve these issues.

Because the resting PCr/(Pi + PCr) ratio can be detected easier than Vmax, it may also be a surrogate marker; however, additional studies with more subjects will be needed to verify this. In our study, the decline of the V_{max} was significant between the MJD group and normal controls; however, the V_{max} of the latter group was also reduced over 2 years (Fig. 2d). This phenomenon might be caused by a general decline of muscle metabolism during the aging process. However, we saw no correlation between V_{max}

and disease duration. Again, this result may be due to the small number of subjects or the age at onset, as MJD is a very slow, progressive disorder. Further study is also needed with a larger number of subjects, to confirm the progressive deterioration of V_{max} over a longer period of study.

In a previous study by Lodi et al., V_{max} in symptomatic patients with Huntington's disease did not correlate with clinical severity.[1] However, in our MJD patients, V_{max} was inversely correlated with clinical severity (SARA score). The reason for this disagreement is not clear. Lodi et al. argued that reduced V_{max} was caused by mitochondrial dysfunction in patients with Huntington's disease and DRPLA. It is possible that the V_{max} reduction in MJD is caused by a different mechanism. Little is known about the relationship between MJD and muscle energy metabolism. Mitochondrial function and muscle pathology in general have also not been carefully examined in MJD. Some patients do show a high rate of motor axon excitability, which may exert a secondary influence on muscle energy metabolism.[12,13] Although intramuscular pH change during exercise was mild in this study, this may be due to the mild exercise load. However, it would be difficult to exercise with a bigger load because the exercise capacity of MJD patients is low. Further study is needed to elucidate the exact mechanism of reduced V_{max} in MJD.

The development of a surrogate marker would be indispensable for the evaluation of new drugs targeting this neurodegenerative disease. Although our results should be confirmed by another MRI machine at another institution, the present data suggest that muscle energy metabolism is a possible marker in patients with MJD. ■

Acknowledgments: We thank all patients and control subjects for their active cooperation. In addition, we also thank Mrs. Mika Omatsu and Mr. Kinya Ishizaka for technical support of the ^{31}P-MRS studies.

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Cerebrospinal Fluid Tau and Phosphorylated Tau Protein are Elevated in Corticobasal Syndrome

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ABSTRACT

Differentiating corticobasal syndrome (CBS) from progressive supranuclear palsy (PSP) and idiopathic Parkinson's disease (PD) can be difficult. To investigate the additional value of cerebrospinal fluid (CSF) biomarkers in the diagnostic differentiation of parkinsonism, we analyzed the CSF concentrations of total protein, lactate and brain specific proteins amyloid-β_{42} protein, tau protein (t-tau), and tau protein phosphorylated at Thr181 (p-tau), in CSF samples from patients with PSP (n = 21), CBS (n = 12), and PD (n = 28). CBS patients demonstrated higher concentrations of t-tau and p-tau compared with PSP and PD patients. In discriminating CBS and PD, t-tau offered the best combination of sensitivity (75%) and specificity (90.9%), followed by p-tau (sensitivity 87.5% and specificity 75%). The p-tau/t-tau ratio resulted in sensitivity of 84.2% and specificity of 66.7% in discriminating PSP and CBS. In conclusion, our results suggest that CSF parameters are of additional value in the diagnostic differentiation of CBS and PD. © 2010 *Movement* Disorder Society

Key Words: Parkinson's disease; corticobasal syndrome; progressive supranuclear palsy; cerebrospinal fluid; sensitivity and specificity

Corticobasal syndrome (CBS) is an atypical parkinsonian disorder characterized by the combination of cortical dysfunction and extrapyramidal symptoms with profound appendicular dystonia and/or a poor response on levodopa therapy. The cortical dysfunction can comprise a variety of symptoms including the "alien-limb phenomenon."[1] Because Parkinson's disease (PD) and other atypical parkinsonian disorders, like progressive supranuclear palsy (PSP), can closely resemble CBD, the initial diagnosis can be challenging, as reflected by the relatively poor diagnostic accuracy of the clinical diagnosis on neuropathological examination.[1–5]

Corticobasal degeneration is the neuropathological substrate of CBS, and is characterized by the intraneuronal aggregation of tau protein like PSP and Alzheimer's disease (AD), commonly referred to as tauopathies.[6] In contrast, PD is characterized by α-synuclein inclusions in neurons of the substantia nigra and cortical areas and is therefore classified as an α-synucleinopathy.[7]

Currently, the distinction between CBS and other parkinsonian disorders is based mainly on clinical grounds, supported to a limited extent by ancillary investigations.[8] It would be helpful to identify new biomarkers that would facilitate the differential diagnosis of parkinsonism. Due to its proximity to the brain parenchyma, the composition of the cerebrospinal fluid (CSF) may reflect pathologic changes.

Herein, we analyzed the CSF concentrations of Amyloid-β_{42} ($A\beta_{42}$), lactate, total protein, tau protein (t-tau), and tau protein phosphorylated at Thr181 (p-tau), in patients with CBS, PSP, and PD, to investigate the diagnostic ability in differentiating between these parkinsonian syndromes.

Patients and Methods

In 2009, a single rater (MBA) retrospectively reassessed the clinical charts of 48 patients, suspected of either PSP or CBS, referred to the Department of Neurology (Radboud University Nijmegen Medical Centre) between 1998 and 2007, who underwent a lumbar puncture during their diagnostic work-up. Reassessment of the clinical diagnosis was performed after a minimum 5-years-follow-up period according to international consensus criteria (UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria for PD,[9] Litvan criteria for PSP,[10] Boeve criteria for CBS[1]). Diagnostic evaluation included detailed medical history, systematic neurological examination, routine laboratory testing, and a brain magnetic resonance imaging-scan. In addition, many patients underwent neuropsychological assessment, nuclear imaging of cerebral metabolism and/or dopaminergic pathways, electro-oculography, and electromyography of the anal sphincter. Only patients diagnosed with either PSP (n = 21) or CBS (n = 12) and available CSF results were included in this study.

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Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 22 April 2010; **Revised** 25 May 2010; **Accepted** 14 June 2010
Published online 10 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23341

Table 1. Demographic characteristics and results of CSF analysis of the diagnostic groups

Characteristic	PSP	CBS	PD	Controls	*P*-value overall
Number of patients	21	12	28	49	
Demographic characteristics					
Age (yr)[a]	65.5 (61.0–71.5)	69.0 (62.5–73.0)	62.5 (55.0–69.6)	55.0 (52.0–61.9)	<0.0001[b]
Number of men (%)	10 (48%)	6 (50%)	21 (75%)	26 (53.1%)	NS
Disease duration (yr)[a]	3.0 (1.0–4.0)	2.0 (1.5–4.0)	2.2 (1.7–4.4)	NA	NS
Disease severity, H&Y score[a]	3.0 (3.0–4.0)	2.5 (1.8–3.5)	2.0 (1.5–2.5)	NA	<0.0001[c]
Cognitive function, MMSE score[a]	26.2 (2.5)	21.3 (6.8)	NA	NA	NS
Duration of follow up (yr)	6.1 (3.2)	5.3 (3.1)	7.8 (4.9)	NA	NA
CSF parameters					
T-tau (ng/L)	234 (138)	402 (199)	151 (67.0)	161 (60,7)	<0.0001[d]
P-tau (ng/L)	46.0 (34.5–51.5)	48.0 (38.0–59.0)	46.0 (34.5–51.5)	44.0 (35.5–53.5)	<0.01[e]
$A\beta_{42}$ (ng/L)	704 (181)	730 (316)	744 (201)	838 (253)	NS
Lactate (μmol/L)	1927 (1645–2079)	1666 (1437–1808)	1734 (1478–1941)	1673 (1509–1828)	<0.05[f]
Total protein (mg/L)	584 (157)	488 (126)	511 (129)	475 (135)	<0.05[g]
$A\beta_{42}$/t-tau	3.69 (2.68–4.30)	2.28 (0.64–3.69)	5.79 (3.62–7.68)	5.14 (4.10–6.76)	<0.0001[h]
$A\beta_{42}$/p-tau[a]	15.5 (5.4)	12.9 (7.1)	17.8 (9.7)	18.8 (5.1)	<0.05[i]
P-tau/t-tau	0.21 (0.19–0.27)	0.18 (0.13–0.20)	0.24 (0.22–0.33)	0.26 (0.24–0.32)	<0.05[j]

Data represent median and interquartile range (non-Gaussian distributed data), mean and standard deviation (Gaussian distributed data) or number and percentage.
P value for differences using 1-way ANOVA. Bonferroni post-hoc test for multiple comparisons was used to identify between-group differences. In cases of non-Gaussian distribution the Kruskal-Wallis test was applied, using Dunn's post-hoc test for multiple comparisons. Gender distribution was analyzed using χ^2 test.
[a]At the time of lumbar puncture.
[b]Controls vs. PSP ($P < 0.001$), vs. CBS ($P < 0.01$) and vs. PD ($P < 0.05$).
[c]PSP vs. PD ($P < 0.001$).
[d]CBS vs. controls, PSP and PD ($P < 0.001$). PSP vs. PD and controls ($P < 0.05$).
[e]CBS vs. controls ($P < 0.01$), and vs. PD ($P < 0.05$).
[f]PSP vs. CBS ($P < 0.05$).
[g]PSP vs. controls ($P < 0.05$).
[h]CBS vs. PD and controls ($P < 0.001$). PSP vs. controls ($P < 0.05$).
[i]Between group difference not significant after correction for multiple comparisons.
[j]CBS vs. controls ($P < 0.05$).
PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PD, Parkinson's disease; H&Y score, Hoehn and Yahr score; MMSE, mini mental state examination; NS, not significant; NA, not assessed.

In addition, CSF data of 28 consecutive PD patients, aged older than 50 years and 49 age-matched patients referred to our Neurology Department in that period were analyzed for comparison (reasons for referral: headache (n = 15), memory complaints (n = 6), functional complaints (n = 10), sensory deficits (n = 9), dizziness (n = 4), and fatigue (n = 5)). All controls were diagnosed as not having a neurodegenerative disorder after extensive work-up and had normal routine CSF parameters (normal leukocyte and erythrocyte count, normal protein, glucose and lactate levels, and neither oligoclonal IgG bands nor blood pigments).

The protocols of CSF analysis of $A\beta_{42}$ t-tau, lactate, and total protein were described previously.[11,12] P-tau concentrations in CSF were analyzed using the Innotest Phospho-$Tau_{(181)}$-assay (Innogenetics, Ghent, Belgium; linearity up to 500 ng/L). The interassay variation coefficients over a period of 6 years were 8.8% for t-tau, 6.0% for $A\beta_{42}$, 4.5% for p-tau, and <3% for lactate.

Statistical analysis was performed using *GraphPad Prism version 4* (San Diego, CA, USA) and *SPSS software version 16.0* (Chicago, IL, USA). Between-groups-analysis was performed using a one-way analysis of variance test or Kruskal-Wallis test for non-normally distributed data. Correction for multiple comparisons was applied. Receiver operator characteristic analysis was used to evaluate the value of individual biochemical variables and their optimal cut-off values discriminating PSP, CBS, and PD. Multivariate logistic regression with backward selection procedures was used to identify variables that contributed independently to discriminate PSP from CBS and CBS from PD.

Results

Twenty-one patients fulfilled the diagnostic criteria of PSP (18 probable PSP, 3 possible PSP; according to the NINDS/SPSP criteria[10]), 12 fulfilled the CBS criteria. Thirteen PSP patients and 5 CBS were deceased at the time of the chart review; mean survival was 6.3 years after onset of symptoms in PSP patients and 4.4 years in CBS patients. Neuropathological confirmation of the diagnosis was not available.

Disease severity (H&Y score) at the time of lumbar puncture was significantly higher in PSP patients, when compared with both CBS and PD subgroups. Age, disease duration, and gender distribution were comparable in all 3 groups. Demographic characteristics and CSF parameters are shown in Table 1.

Table 2. ROC-analysis of CSF analysis for CBS vs. either PSP or PD

CSF variables	Cut-off point	Sensitivity	Specificity	Area under the curve (95% CI)	Youden index*	Likelihood ratio**	*P*-value***
				PSP vs. CBS			
				Univariate			
T-tau (ng/L)	>322	80.0%	63.6%	0.77 (0.61–0.95)	0.44	2.20	NS
P-tau (ng/L)	>52	63.2%	75.0%	0.76 (0.59–0.93)	0.38	2.53	NS
$A\beta_{42}$ (ng/L)	<655	73.7%	50.0%	0.53 (0.30–0.76)	0.24	1.47	<0.001
Lactate (μmol/L)	<1769	63.2%	80.0%	0.74 (0.55–0.93)	0.43	3.16	NS
Total protein (mg/L)	<539	63.2%	72.7%	0.68 (0.48–0.88)	0.36	2.32	<0.05
$A\beta_{42}$/t-tau	<3.22	68.4%	75.0%	0.72 (0.52–0.92)	0.43	2.74	<0.05
$A\beta_{42}$/p-tau	<7.76	94.7%	41.7%	0.64 (0.42–0.85)	0.36	1.62	<0.05
P-tau/t-tau	<0.18	84.2%	66.7%	0.75 (0.56–0.93)	0.51	2.53	NS
				Multivariate			
Model 1[a]	<3.95	93.8%	70.0%	0.89 (0.77–1.00)	0.64	3.13	
Model 2[b]	<1.91	68.8 %	90.0%	0.88 (0.75–1.00)	0.59	6.88	NS
				CBS vs. PD			
				Univariate			
T-tau (ng/L)	>197	75.0%	90.9%	0.91 (0.82–1.00)	0.66	8.24	
P-tau (ng/L)	>52.5	87.5%	75.0%	0.80 (0.64–0.97)	0.63	3.50	<0.05
$A\beta_{42}$ (ng/L)	<658	75.0%	50.0%	0.56 (0.34–0.77)	0.25	1.50	<0.001
$A\beta_{42}$/t-tau	<3.21	85.7%	75.0%	0.86 (0.74–0.98)	0.61	3.43	NS

*Youden index: sensitivity + specificity – 1.0.
**Likelihood ratio: sensitivity/(1 − specificity).
****P*-value for difference compared with Model 1 (PSP vs. CBS) or t-tau (CBS vs. PD) (Hanley and McNeil).
[a]Model 1: $Y = -1.539 + (0.002 \times \text{tau}) + (0.003 \times \text{lactate}) + (-0.098 \times \text{p-tau}) + (0.004 \times \text{protein})$.
[b]Model 2: $Y = 0.122 + (0.003 \times \text{lactate}) + (-0.081 \times \text{p-tau})$.
ROC-analysis, receiver operating characteristic analysis; PSP, progressive supranuclear palsy; CBS, corticobasal syndrome; PD, Parkinson's disease; CSF, cerebrospinal fluid; t-tau, total tau protein; p-tau, phosphorylated tau protein; $A\beta_{42}$, amyloid-β_{42} protein; 95 CI, 95% confidence interval.

Concentrations of t-tau were significantly higher in CBS patients than in PSP patients, PD patients, and controls ($P < 0.001$). P-tau concentrations were significantly higher in the CBS patients, when compared with PSP ($P < 0.05$) and PD ($P < 0.01$). Lactate concentrations were significantly higher in PSP than in CBS patients ($P < 0.05$). $A\beta_{42}$ and total protein concentrations did not differ between PSP and CBS patients. In addition, several ratios were calculated. As expected, the $A\beta_{42}$/t-tau ratio differed significantly between PSP and CBS (Table 1).

We neither established correlations between CSF parameters and age, disease duration, nor severity. However, mini-mental state examination (MMSE) scores correlated with $A\beta_{42}$ ($r = 0.481$, $P < 0.05$), t-tau ($r = -0.622$, $P = 0.002$), and p-tau ($r = -0.642$, $P = 0.001$) in the CBS subgroup. Similar results were obtained in the combined CBS and PSP subgroups.

Univariate logistic regression analysis was carried out to discriminate CBS from PSP revealing that neither sensitivity nor specificity exceeded 80% for individual parameters (Table 2). Therefore, multivariate logistic regression analysis was carried out to improve diagnostic accuracy in discriminating between CBS and PSP. T-tau, p-tau, lactate, and total protein concentrations were added to the selection procedure. The prediction model thus constructed reached a sensitivity of 93.8% and a specificity of 70.0%. A prediction model based on only p-tau and lactate reached a sensitivity of 68.8% and a specificity of 90.0%.

Univariate analysis demonstrated that t-tau protein offered the best combination of sensitivity (75%) and specificity (90.9%) to differentiate between CBS and PD. The concentration of p-tau showed a sensitivity of 87.5% and a specificity of 75%. Multivariate logistic regression analysis was performed, selecting only t-tau protein as independent marker to separate CBS and PD.

Discussion

This study is one of the few to compare CSF biomarkers in patients with CBS, PSP, and PD with extended clinical follow-up. We demonstrated that the concentrations of t-tau and p-tau proteins in CSF of CBS patients were significantly elevated compared with PSP and PD. However, the diagnostic accuracy of CSF t-tau and/or p-tau seems only sufficient in the discrimination of CBS vs. PD not in discriminating CBS vs. PSP.

As the sensitivity for the initial clinical diagnosis CBS is poor,[5] the CSF profile of CBS (i.e., increased t-tau and p-tau) may increase awareness for the diagnosis CBS. A timely and correct diagnosis may result in better targeted treatment strategies, adequate patient counseling and—perhaps most important—early recognition of disease-specific complications.

In AD $A\beta_{42}$, CSF concentrations are decreased,[13] whereas in parkinsonian disorders, the data seem conflicting[14–16] presumably due to large inter-individual variability, underpowered studies, and possibly different underlying pathology as neuropathological confirmation is lacking in most studies.[17–22] T-tau and p-tau concentrations are reported to be increased in tauopathies, predominantly AD,[23] but also—in line with our results—in CBS compared with controls.[18,19,22] However, other studies failed to demonstrate such elevations in CBS,[17,21] a disparity possibly caused by enrolment of more severely cognitively affected patients in our study, possibly reflecting more cortical involvement correlating with higher CSF t-tau levels (mean MMSE score 21.3 vs. 28.0).[21]

Although both PSP and CBS are neuropathologically characterized by axonal degeneration and the accumulation of t-tau protein in the brain, only in CBS this seems to lead to an increase in CSF t-tau and p-tau concentrations.[17,18,21,22,24] The observed difference between CBS and PSP may be explained by a higher rate of atrophy, a larger brain area involved and relative proximity of the affected brain areas to the CSF compartment in CBS.[25–28] Interestingly, elevated concentrations of t-tau protein were observed previously in MSA, an α-synucleinopathy, stroke, and Creutzfeldt-Jakob disease.[11,12,29] Hence, t-tau concentrations might be a biomarker for accelerated degeneration instead of reflecting the pathological substrate.

This study has several drawbacks. First, due to the retrospective character of the study selection bias cannot be ruled out, because only patients with diagnostic uncertainty were included in this study, possibly leading to the selection of more atypical phenotypes. However, the studied population therefore closely resembles daily clinical practice in which ancillary diagnostic tests are applied in cases of diagnostic uncertainty. Second, the clinical diagnosis was not confirmed neuropathologically and therefore susceptible to misclassification. However, the final diagnosis was based on thorough clinical and ancillary investigations (including nuclear imaging and neuropsychological assessment), after extensive follow-up and according to international consensus criteria in a specialized movement disorder clinic. In addition, charts of patients with CSF parameters in overlapping ranges or with Alzheimer-like CSF profiles were re-examined to assess whether these patients exhibited clinical features suggesting potential misdiagnosis with PSP, CBS, or AD; no misdiagnosed cases were identified.

In conclusion, despite these drawbacks, our results suggest that CSF analysis could aid the diagnostic differentiation of CBS and PD. Abnormal CSF t-tau and p-tau concentrations may raise awareness for the diagnosis CBS. These results warrant validation in a prospective study with neuropathological confirmation of the diagnosis.

Acknowledgments: We thank the technicians of the Department of Laboratory Medicine (LGEM) for CSF analysis.

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Use of Smell Test Identification in Parkinson's Disease in Mexico: A Matched Case-Control Study

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ABSTRACT

Smell tests can be useful in the differential diagnosis of Parkinson's disease (PD) but are affected by cultural factors. Currently there is no smell test tailored for the Mexican population but the brief smell identification test (B-SIT) was created as a cross-cultural SIT. We have created a translation of this test into Spanish adapted to the Mexican population and have applied it to 70 PD patients and 70 age- and gender-matched controls. The B-SIT differentiated PD and controls with 71.4% sensitivity and 85.7% specificity, when subjects were divided into two age groups.

Key Words: smell; olfaction; Parkinson's disease; B-SIT

There are no reliable statistics about the prevalence of Parkinson's disease (PD) in the Mexican population but as Mexico undergoes populational aging[1] it is likely to increase significantly in the next 20 years. The diagnosis of PD is performed clinically and relies heavily on clinical expertise, although dopamine transporter scans, which have limited availability in Mexico, can be of help. Smell tests have accuracy of 70-90% in

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Additional Supporting Information may be found in the online version of this article.

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Funding agencies: This research was funded by the Reta Lila Weston Trust for Neurological Studies and Dr. Silveira-Moriyama is a beneficiary of a RLW fellowship. This project was funded by the National Council of Science and Technology (Consejo Nacional de Ciencia y Tecnología, CONACyT grant #87675).
Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 19 February 2010; **Revised** 21 April 2010; **Accepted** 23 June 2010
Published online 14 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23354

differentiating PD from control subjects.[2–6] Most studies in PD have used the 16-item smell identification test (SIT) from Sniffin' Sticks (SS-16),[7] the 40-item University of Pennsylvania SIT (UPSIT-40)[8] or a shorter version of the UPSIT containing 12 items claimed to be better for cross-cultural studies called brief SIT (B-SIT).[9] The B-SIT is less expensive and quicker than the UPSIT-40, and unlike the SS-16, it can be sent and returned by post to patients or subjects participating in surveys.

There are no published accounts of formal olfactory testing in parkinsonian patients in Mexico. We have applied the B-SIT to 70 PD Mexican patients and 70 matched controls.

Methods

Subjects

Seventy nondemented PD patients, who fulfilled the Queen Square Brain Bank Criteria,[10] were recruited at the movement disorder specialist clinic at the National Institute of Neurology and Neurosurgery, Mexico City, Mexico. None of the patients had undergone functional neurosurgery for PD. Seventy controls matched by gender, age, and place of residence were recruited among visitors and patients attending other clinics from the hospital. They had no symptoms or family history of neurological disorders or head trauma. No participants had any active upper respiratory tract infection, and all provided written informed consent as determined by the local ethics committee.

Sociodemographic variables collected included gender, age, age of onset, tobacco use, drug intake, and disease severity, according to Hoehn and Yahr stage (H&Y).[11] Place of residence for the last 10 years was classified according to the Mexican National Institute of Statitistics and Geography (INEGI) criteria for rural and metropolitan area.[12] Family income in an average month was quantified as an ordinal variable with four different levels. The social work classification code is a standard composite used in Mexico.[13]

Patients and controls were paired by age, gender, and living area. Mean age of subjects was 66.2 years (SD = 8.8 years); 29 (41.4%) were female in each group, 47 patients (67.1%) were residents of Mexico's Valley metropolitan area, 10 patients (14.3%) lived on other metropolitan areas, and 13 (18.6%) patients came from a rural zone in each group.

Smell Testing

We created a standard translation (see Supporting Information) for the B-SIT. Content equivalence was evaluated by a multidisciplinary panel and semantic equivalence was assessed by back translation and a pilot study. During the final experiment, the B-SIT was applied with the help of the examiner who explained the test beforehand, scratched the booklets, and marked the options indicated by the patient.

Statistical Analysis

To compare the groups, we used *t*-tests (for continuous variables) and chi-squared or Fisher's (for nominal variables). The percentage of controls and PD subjects, who answered each item correctly, was compared by McNemar tests. To evaluate the factors that affected the B-SIT in controls, we used a multiple linear regression in this group with the B-SIT as outcome variable and sociodemographic variables as covariates (age, gender, history of ever smoking, region of living as urban or rural, social work classification code, and monthly family income). To evaluate if disease duration or severity affected the B-SIT score in PD, we conducted a multiple linear regression analyses using those variables and the covariates, which showed a significant effect over the B-SIT in the control regression. To evaluate accuracy of the B-SIT, a logistic regression analysis was performed using group as outcome measure and B-SIT score and age as covariates. Assumptions for the regression analyses were confirmed by analysis of residuals. A significance level of 0.05 was used throughout, except where otherwise stated. The statistical package SPSS v.12 for Windows was used for all the analyses.

Results

In the PD group, mean disease duration was 6.7 years (SD = 5 years) and mean age of onset was 59.7 years (SD = 9 years); 26 (37%) took levodopa (L-dopa) alone, 22 (31.4%) took L-dopa and pramipexole, and 18 (26%) were on pramipexole alone; the remainder were on a selective type B monoamine oxidase inhibitor. Forty-three (61.4%) patients had mild disease (H&Y 1–2), 24 (34.2%) moderate (H&Y 2.5–3), and 3 (4.4%) severe (H&Y 4–5). Only 24 (34.3%) PD patients reported smell problems. The mean number of B-SIT items correctly identified by PD patients was 6.2 (SD = 2.5). A multiple linear regression showed that age ($P = 0.4$), H&Y ($P = 0.3$) and disease duration ($P = 0.1$) were significant determinants of the B-SIT result in the PD group.

The mean number of B-SIT items correctly identified by controls was 9.2 (SD = 1.4). The multiple linear regression in the control group using the B-SIT as the outcome variable showed that age ($P < 0.0001$, 95% CI for $\beta = -0.10$ to -0.3) was a significant covariate, but not gender ($P = 0.55$), history of ever smoking ($P = 0.39$), region of living ($P = 0.1$), social work classification code ($P = 0.14$), or monthly family income ($P = 0.16$).

There was no significant difference in terms of complete years of schooling between groups (paired *t*-test $P = 0.8$). More subjects from the PD than in the control group had never smoked (51 of 70 in PD, 36 of 70 in the control group, $P = 0.01$). The difference between the number of control and PD subjects, who correctly identified each item, is displayed in Table 1. Because age was a significant determinant of B-SIT

Table 1. Difference between control and PD subjects who correctly identified each of the 12 items of the B-SIT

Item	Percentage of subjects who correctly identified item		McNemar test *P* value
	Control group	PD group	
Cinnamon	80.0%	47.1%	<0.001*
Turpentine	52.9%	35.1%	0.052
Lemon	64.3%	50.0%	0.123
Smoke	74.3%	68.6%	0.584
Chocolate	88.6%	68.6%	0.014*
Rose	62.9%	25.7%	<0.001*
Paint thinner	71.4%	45.7%	0.006*
Banana	77.1%	38.6%	<0.001*
Pineapple	78.6%	47.1%	<0.001*
Gasoline	85.7%	62.9%	0.003*
Soap	85.7%	42.9%	<0.001*
Onion	98.6%	84.3%	0.006*

PD = Parkinson's disease.
*Significant.
Turpentine, lemon, and rose were identified by less than 70% of controls, and turpentine, lemon, and smoke failed to differentiate PD from controls.

results in controls, subjects were divided into two age groups (age < 60 or age ≥ 60), and a logistic regression showed that the B-SIT had 71.4% sensitivity and 85.7% specificity to differentiate PD from control (accuracy of 78.6%) when using a cut-off of seven or less for those aged $\geq$60 and nine or less for those aged <60. See Figure 1 for the probability curve.

Discussion

SITs can be useful in the differential diagnosis of parkinsonism,[4] and are potential surrogates for dopamine transporter SPECT,[14] which has limited availability in developing countries. Currently, there is no SIT specifically tailored for the Mexican population.

The interpretation of SITs depends on several variables including country, culture, smoking status, gender, and age. The effect of these factors is not applicable to all SITs. Gender affects the UPSIT[2] but was not a determinant of the B-SIT in our sample; this may reflect the limited power of our study, but it is also possible that shorter tests are less subjected to sex differences because a study involving more than 400 subjects found no sex difference in the SS-16 performance for subjects older than 55 years.[7] We failed to show an effect of smoking on the B-SIT result. The literature is heterogeneous about this matter[15,16] and it is possible that because of our small sample size, we were not able to detect a difference. Socioeconomic markers can influence the result of smell tests,[17] but were not significant covariates in our study. There was also no influence in the zone of living showing that the B-SIT seems to adequately discriminate controls from PD independently of place of residence. Nevertheless, our sample, from a tertiary center in a very large city is biased and only 19% of our subjects came from rural areas.

The UPSIT has sensitivity and specificity between 76 and 91% to differentiate PD subjects from controls depending on the age group.[2] The accuracy of the B-SIT in the Mexican population is below values reported for similar tests in developing countries. In Brazil, both UPSIT and Sniffin Sticks had more than 80% accuracy,[18] and in Sri Lanka, a subscale of the Sniffin Sticks had more than 90% accuracy.[19] One explanation for this is that this study had more early PD than the others and the accuracy of the clinical diagnosis of PD is higher in more advanced disease. Nevertheless, the data suggests some of the items in the test are not suited for the Mexican population because of cultural factors: lemon, turpentine, and rose, which were poorly identified by Mexican controls, were also poorly identified by controls in Brazil (respectively, 45.9, 49.5, and 51.4% of controls correctly identified the item).[18] Patients in the current Mexican study, as well in the Brazilian study, were from a limited age range (mean age [range] SD = 66.0 years [48–85 years], 8.9 years for Mexicans; 63.0 years [33–89 years], 9.8 years for Brazilians), and also came mostly from areas with high levels of pollution (Mexico City and Sao Paulo), which can cause smell impairment,[20,21] therefore, future studies involving a larger and more varied sample of subjects may be needed in order to evaluate specific items.

Our data demonstrates that one third of the items of the BSIT are not suited for the Latin American culture. Given the growing importance of smell testing in the research and practice of movement disorders, it is of great interest to identify a truly cross-cultural smell test and further research into the subject is warranted.

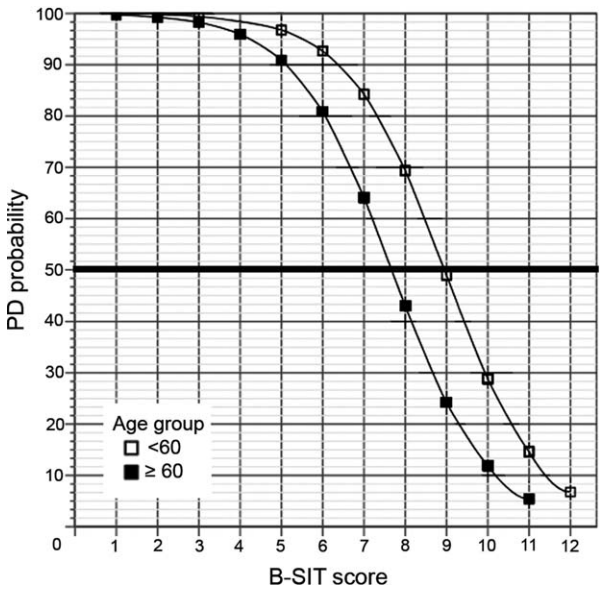

FIG. 1. Parkinson's disease (PD) probability curve for the B-SIT in Mexico. Probability of membership to PD group according to the logistic regression analysis for the brief smell identification test (B-SIT). Cut-off for determination of accuracy measures are set at a probability equal or higher than 50% of membership to the PD group.

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Reduced but Not Oxidized Cerebrospinal Fluid Glutathione Levels are Lowered in Lewy Body Diseases

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ABSTRACT

Reduced (GSH_R) but not oxidized glutathione (GSSG) has been shown to be dramatically altered in the substantia nigra (SN) of Lewy body disease (LBD) patients post mortem; but up to now, there is no convincing evidence that these changes can be monitored in vivo. We investigated GSH_R and GSSG in rapidly processed cerebrospinal fluid (CSF) and plasma samples of 80 LBD and 35 control subjects and detected reduced CSF GSH_R levels in LBD subjects. The reduction was negatively associated with age but not with disease-associated parameters. Plasma GSH_R, CSF GSSG, and plasma GSSG levels did not significantly differ between the groups. Our findings confirm the results from neuropathologic studies, which demonstrated an alteration of the glutathione system in LBD. We hypothesize that alterations of the glutathione system occur in a very early stage of the disease or may even represent a risk marker for LBD. © 2010 *Movement* Disorder Society

Key Words: age dependency; dementia with Lewy bodies; glutathione; Parkinson's disease

Additional Supporting Information may be found in the online version of this article.

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Funding Agencies: Walter Maetzler is supported by a Forschungskolleg Geriatrie Grant from the Robert Bosch Foundation, Stuttgart, Germany (Nr. 32.5.1141.0019.0), and Stefan Schmid by a German National Genome Network Grant (NGFNplus #01GS08134).
Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received 3 May 2010; **Accepted** 28 June 2010
Published online 14 September 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.23358

One of the key contributors to neurodegeneration in Lewy body diseases (LBD, encompassing Parkinson disease, PD, and dementia with Lewy bodies, DLB[1,2]) has been suggested to be oxidative stress. Glutathione is a major antioxidant that functions to maintain the redox equilibrium of a cell. The enrichment of glutathione peroxidase—this enzyme removes hydrogen peroxide by metabolizing reduced (GSH_R) to oxidized glutathione (GSSG)—in the vicinity of Lewy bodies is indicative of an involvement of the glutathione system in LBD.[3–5]

Using tissues homogenates, a selective reduction of about 40% of GSH_R levels in the substantia nigra (SN) in patients with PD compared to control subjects has been demonstrated.[6,7] Similar results have been found using mercury orange fluorescent staining and immunostaining.[8] Interestingly, the glutathione system seems to be already compromised at a very early disease stage of LBD, as a 35% loss of GSH_R levels in the SN of presymptomatic LBD subjects has been described.[9] In addition, the depletion of glutathione may be a prerequisite for other pathophysiological events in the course of LBD. A reduction of GSH_R in dopaminergic neurons potentiates their susceptibility to 1-methyl-4-phenylpyridinium (MPP+, a partial inhibitor of mitochondrial complex I activity and a selective toxin to dopaminergic neurons) both in vitro[10] and in vivo[11]. Interestingly, glutathione depletion seems not to be a consequence of such pathophysiological events: In an in vitro model, MPP+ failed to alter GSH_R levels.[12]

GSSG levels may not be representative for neurodegenerative processes in LBD, as these levels did not differ significantly between the SNs of postmortem brains of both presymptomatic and symptomatic LBD patients, in comparison to control SNs.[6,7,9]

Cerebrospinal fluid (CSF) has become one of the most frequently used biological materials for in vivo studies on neurological disorders because of its proximity to the brain. As GSH_R alterations are evident already in presymptomatic LBD, it can be hypothesized that GSH_R levels may have the potential to serve as a preclinical marker of the disease. To our knowledge, only one study investigated CSF GSH_R levels in LBD patients,[13] which included 14 untreated PD patients and 15 controls. The authors did not detect significantly different CSF GSH_R levels, but they found decreased CSF GSSG levels in the LBD group. However, a major drawback of the study, besides the low patient number, is the undefined time for processing of the CSF samples. A fast and effective processing of naïve CSF may be crucial as glutathione is rapidly oxidized and can be regenerated to the original ratio of GSH_R to GSSG within minutes.[14]

To test the hypothesis that the known changes in the glutathione redox state in LBD brains may be mirrored by changes in CSF and plasma, we here measured GSH_R and GSSG from a large cohort of LBD patients and controls. To minimize postcollection artifacts, we used rapidly processed samples.

Experimental Procedures

Subjects

Eighty LBD patients—43 non-demented PD (PDND), 16 demented PD (PDD), 21 with DLB—were recruited by movement disorders specialists at the outpatient clinic and the ward of the Neurodegenerative Department of the University of Tuebingen. All PD subjects fulfilled the UKPDS Brain Bank criteria,[15] demented PD subjects also met the DSM-IV criteria for dementia.[16] All DLB patients fulfilled the McKeith criteria.[17]

The control group consisted of 35 subjects without any sign for neurodegenerative diseases (lumbar spinal stenosis or lumbar herniated disc, 14; nonspecific symptoms such as headache, concentration deficits, and mood disturbances, 14; peripheral neuropathies, 7).

In addition to clinical testing, all patients were evaluated with the motor part of the Unified Parkinson Disease Rating Scale (UPDRS III), and all subjects underwent a Mini-Mental State Examination (MMSE). The local ethics committee approved the study, and all participants gave their informed consent.

CSF and Plasma Collection and Glutathione Assay

CSF and blood collection and determination of routine diagnostic parameters of patients and controls were performed according to standardized protocols (spinal tap between 8:00 and 10:00 a.m., subjects were required to fast overnight). The samples for determination of glutathione levels were taken immediately after acquisition by a technical assistant, transferred to the lab in a closed box on ice, centrifuged within 15 to 30 minutes after collection and stored at −70°C until analysis. Only samples of subjects with normal routine CSF diagnostics were collected. CSF and plasma albumin and immunoglobulin levels, and CSF cell count did not differ significantly between the cohorts.

GSH_R and GSSG concentrations were determined in 1:4 diluted CSF and plasma using a fluorescent detection kit (Luminos DetectX, Biotrend, Cologne, Germany) with standard curves based on dilutions of purified GSH. GSH_R was read first with a thiol detection substrate (i.e., a nonfluorescent molecule, that covalently binds to the free thiol group on GSH to yield a highly fluorescent product) followed by the addition of a reaction mixture that converted all GSSG into GSH_R. This then reacted with the excess thiol detection substrate to yield the signal related to total GSH. Tests were run in duplicate, blinded to clinical diagnosis. Four CSF GSH_R samples (3 LBD, 1 HC) and three plasma GSH_R samples (2 LBD, 1 HC) yielded

Table 1. Demographic, clinical, and biochemical data

	DLB	PDND	PDD	LBD	Controls	*P*-value
Subjects: f/m	11/10	17/26	12/4	40/40	19/16	0.69
Age (yr)	73 (50–83)	66 (53–81)	72 (63–84)	69 (50–84)	66 (50–81)	0.03
Aao first symptom (yr)	70 (49–78)	60 (30–76)	63 (57–72)	62.5 (30–78)		
Disease duration (yr)	3 (1–9)	5 (1–23)	9.5 (1–21)	5 (1–23)		
Hoehn&Yahr stage (0–5)	2.5 (2.5–3)	2 (1–4)	3 (1–4)	2.5 (1–4)		
L–Dopa equiv. dose (mg)	250 (0-700)	225 (0–900)	550 (300–950)	400 (0–950)		
UPDRS motor part (0–100)	24 (10–66)	28 (12–60)	35 (26–57)	28 (10–66)		
MMSE (0–30)	20 (11–28)	29 (26–30)	23 (14–28)	27 (11–30)	30 (27–30)	<0.0001
CSF GSH_R (μM)	0.31 (0.02–1.64)	0.28 (0.05–1.43	0.21 (0.02–0.95)	0.27 (0.02–1.64)	0.52 (0.02–1.89)	0.038*
CSF GSSG (μM)	0.17 (0.07–0.62)	0.17 (0.03–0.76)	0.19 (0.09–0.29)	0.17 (0.02–0.76)	0.20 (0.02–0.71)	0.30
Plasma GSH_R (μM)	0.31 (0.02–3.24)	0.32 (0.02–2.76)	0.62 (0.02–1.85)	0.35 (0.02–3.24)	0.58 (0.02–3.68)	0.16*
Plasma GSSG (μM)	0.33 (0.15–1.45)	0.35 (0.14–1.56)	0.29 (0.16–0.97)	0.33 (0.14–1.56)	0.39 (0.12–1.81)	0.70
CSF GSH_R/GSSG	1.34 (0.17–20.0)	1.10 (0.30–33.1)	1.86 (0.09–3.66)	1.22 (0.09–33.1)	1.78 (0.27–30.7)	0.24
Plasma GSH_R/GSSG	1.29 (0.02–16.2)	0.60 (0.10–15.8)	2.26 (0.05–8.04)	1.04 (0.02–16.2)	1.37 (0.02–17.1)	0.63

P-values (* corrected for age) were determined for the comparison of the Lewy body disease group (LBD) compared to controls using the Wilcoxon rank sum test (see also text). In addition, detailed information is provided for the subgroups, i.e., Parkinson's disease without dementia (PDND), with dementia (PDD) and dementia with Lewy bodies (DLB). Aao, age at onset; CSF, cerebrospinal fluid; GSH_R, reduced glutathione; GSSG, oxidized glutathione (glutathione disulfide); MMSE, MiniMental State Examination; UPDRS, Unified Parkinson Disease Rating Scale (performed in off state).

concentrations below the detection limit of the kit (0.04 μM) and were arbitrarily set at 0.02 μM.

GSSG concentrations were calculated using the formula $\frac{GSH_T - GSH_R}{2}$.[13,18]

Data Analysis

Data were analyzed with JMP software (version 7.0, SAS). Nonparametric test procedures (Wilcoxon rank sum test, Kruskal Wallis test, Spearman's rho) were applied due to non-normal distribution of some parameters such as GSH_R levels and the UPDRS and MMSE scores. Fisher's exact test was used for analyzing categorical data, and Spearmen partial correlation for evaluating confounder effects. As LBD patients were significantly older than controls, we corrected for age with a multiple regression model (2 effects: LBD yes/no, and age). The likelihood ratio was calculated to assess the significance of the model effect. Differences were assumed to be significant at $P < 0.05$ (two-sided).

Results

Subjects

Demographic and clinical information are supplied in Table 1. The LBD cohort was slightly but significantly older.

Glutathione Levels and Demographic Parameters

GSH_R (rho = −0.19, $P = 0.053$) and GSSG levels (rho = −0.20, $P = 0.048$) in the CSF showed a weak negative correlation with age. Plasma GSH_R (rho = −0.12, $P = 0.25$) and GSSG levels (rho = −0.14, $P = 0.12$) were not correlated with age. None of the evaluated glutathione levels showed a relevant association to gender ($P \geq 0.31$).

Glutathione Levels and Disease-Related Parameters

In the CSF, age-corrected GSH_R levels were lower in LBD subjects compared to controls. In plasma, there was a trend toward lower GSH_R levels in LBD patients compared to controls; however, this was relevantly influenced by age. Oxidized glutathione levels did neither differ significantly in the CSF nor in the plasma between the investigated cohorts. Table 1 and Figure 1 provide detailed information about glutathione levels in LBD and controls, and about glutathione levels in the LBD subgroups PDND, PDD, and DLB (glutathione levels did not differ significantly between these subgroups, not shown).

CSF GSH_R levels of LBD subjects were neither associated with age at onset of the disease, disease duration, UPDRS motor score, levodopa (L-Dopa) equivalent dose nor with the MMSE score. However, there was a negative correlation of CSF GSH_R to age which was only marginally influenced by disease-associated demographic parameters. CSF GSH_R levels of controls correlated positively with age; however, this result did not reach significance (Supporting Information Table).

Discussion

In this study, we found a significant reduction of GSH_R in the CSF of LBD patients. The glutathione system has an important antioxidative function in particular in the brain, and there is evidence that the

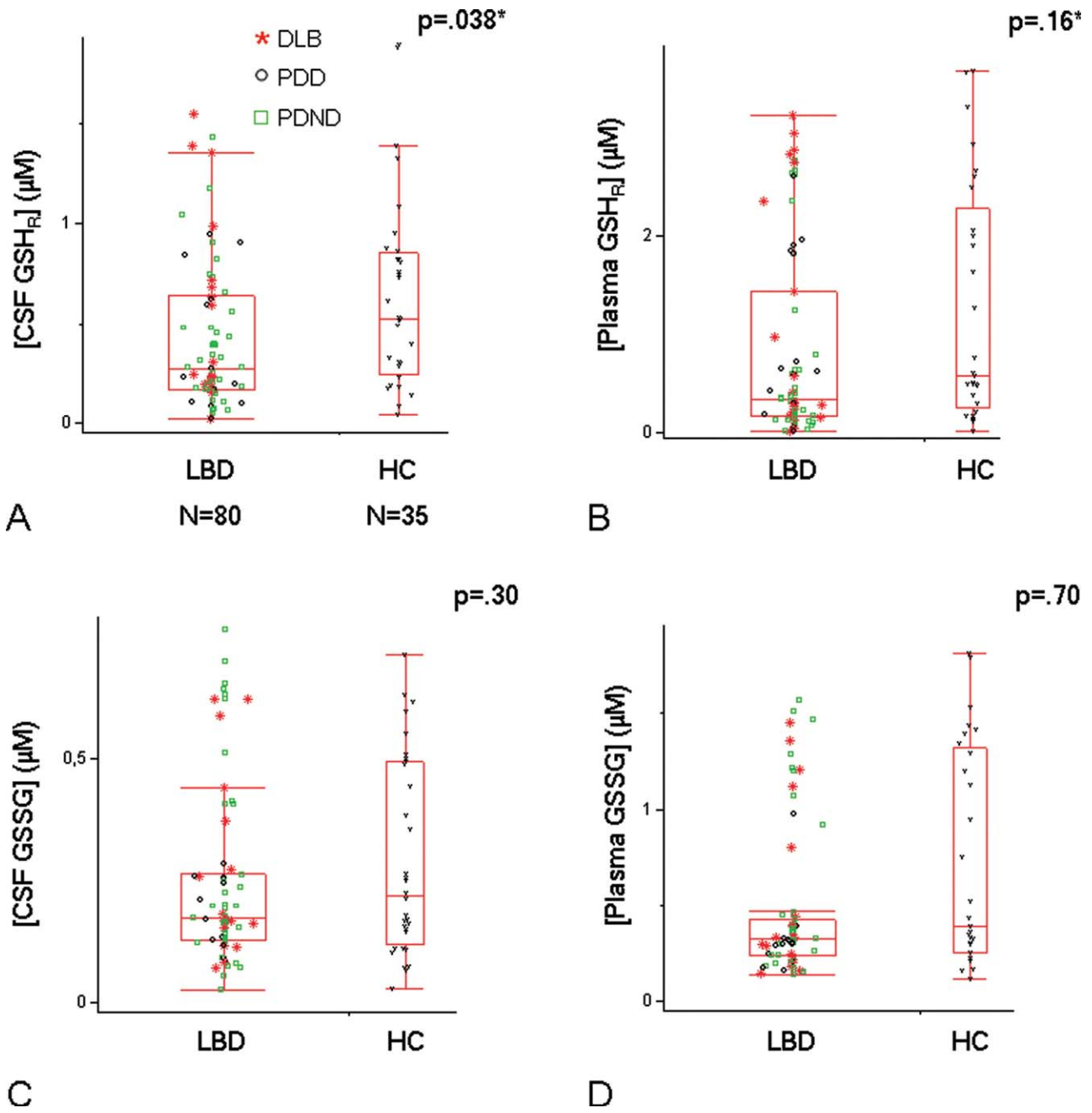

FIG. 1. Glutathione levels in Lewy body diseases compared to controls. (**A**) Significantly lower reduced glutathione (GSH_R) levels were detectable in the cerebrospinal fluid (CSF) of Lewy body disease (LBD) patients compared to control subjects (HC). (**B**) In the plasma, the same trend was observable. (**C**) Oxidized glutathione (GSSG) levels differed neither in the CSF (C) nor in the plasma (**D**) significantly between LBD patients and controls. *P*-values were performed by the Wilcoxon rank test. DLB, dementia with Lewy bodies; PDD, Parkinson's disease with dementia; PDND, Parkinson's disease non demented. **P*-values corrected for age.

glutathione system is compromised in LBD. In particular, GSH_R levels have been found to be dramatically reduced, to about 60 to 65% of normal SN levels.[6–9] Using samples from LBD and control subjects which were processed according to highest available standards to minimize postcollection bias, we detected a median reduction of CSF GSH_R levels in LBD subjects in a range comparable to postmortem studies. Also comparable to these studies,[6–9] CSF levels of the oxidized form were not relevantly altered. Thus, the CSF compartment may in fact mirror changes of the glutathione system as they occur in the parenchyma of LBD patients. Interestingly, glutathione levels did not differ relevantly between the LBD subgroups, i.e., PDND, PDD, and DLB (Table 1). This argues for a specific, Lewy body-associated phenomenon, and against a relevant influence of, e.g., co-occurring Alzheimer's pathology[19,20] on glutathione levels in LBD.

The lack of a significant difference of plasma GSH_R and GSSG levels between LBD and control subjects is reasonable: LBD is primarily a central disease, and glutathione does not cross the blood brain barrier.[21]

Interestingly, the GSH_R over GSSG ratios were not significantly different between the groups, neither in CSF nor in plasma (Table 1). To our eyes, this does not argue against a defective glutathione system per se and may, e.g., be due to the following reasons, (i) the GSH production and/or release in astrocytes[22] is altered in LBD, (ii) the redox state of the CSF is altered in LBD (e.g., due to increased levels of reactive oxidative species[23]), making an increased turnover of the (extracellular) detoxification machinery necessary, and/or (iii) the neuronal uptake of glutathione precursors[21] is altered in LBD.

CSF GSH_R levels of LBD subjects correlated negatively with age, a finding that was not relevantly influenced by age at onset of the disease and disease duration and was not (or rather reciprocally, see Supporting Information Table) observable in the control group. In addition, we did not detect any relevant association between CSF GSH_R levels and clinical parameters including the UPDRS motor part and the MMSE, and L-Dopa equivalent dose. It may be that we missed given associations due to the limited number of study participants, and the association of CSF GSH_R levels with age in LBD is unspecific. However, we argue that it is more probable that alterations of the glutathione system are an early event in the neurodegenerative process. This hypothesis is supported by results from biochemical ex vivo,[9] in vitro,[10,12] and in vivo studies[11] which basically propose that a depletion of glutathione is probably not induced by (and therefore not a consequence of) pathomechanisms typically occurring in the course of LBD (e.g., mitochondrial impairment), but is itself able to induce or accelerate such mechanisms. The occurrence of a layer of glutathione peroxidase around (nascent) Lewy bodies that generate hydrogen peroxide[3] may underscore the importance of a well-functioning neuronal glutathione system in the context of LBD. However, it must be kept in mind that a selective reduction of glutathione alone appears not to be responsible for the nigrostriatal damage in LBD, as shown by chronic infusion of a glutathione inhibitor into rat brain (which, at least in part, may explain the low CSF GSH_R levels observed in some of the healthy volunteers, see Fig. 1).[24] The glutathione depletion may rather enhance the susceptibility of affected cells against additional harmful events, e.g., mitochondrial impairment.[25] Vice versa, as a considerable number of LBD patients had relatively high, "control-like," CSF GSH_R levels it must be assumed that a compromised glutathione system is not the only prerequisite for the development of LBD. Future studies should focus on investigating reasons for this high range of CSF GSH_R levels in LBD and may, with larger cohorts, also include age-associated diseases as covariables.

In conclusion, the age-related reduction of CSF GSH_R levels in LBD subjects argues for an alteration of the glutathione system in a very early stage of the disease, or even for the hypothesis that an altered glutathione system is a risk marker for LBD. ■

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