

Rhinorrhea: A Common Nondopaminergic Feature of Parkinson's Disease

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ABSTRACT

We compared the frequency of rhinorrhea between 34 Parkinson's disease (PD) subjects and 15 normal controls (NC) and explored relationships between rhinorrhea and clinical functions, and degree of nigrostriatal dopaminergic denervation using [¹¹C]dihydrotetrabenazine (DTBZ) brain positron emission tomography imaging. Sixty-eight percent (23 of 34) of PD subjects reported rhinorrhea of any cause compared with 27% (4 of 15) of NC ($\chi^2 = 7.07$, $P = 0.008$). Rhinorrhea frequency remained higher in the PD group after excluding possible rhinitic etiologies: 35% (12 of 34) of PD versus 7% (1 of 15) of NC ($\chi^2 = 4.38$, $P = 0.04$). There were no differences in demographics, nigrostriatal dopaminergic denervation, and clinical motor or non-motor variables between PD subjects with and without rhinorrhea, except that more PD subjects with rhinorrhea complained of lightheadedness (52% vs. 9%, $\chi^2 = 5.85$, $P = 0.02$). Rhinorrhea is a common nondopaminergic feature of PD, unrelated to olfactory or motor deficits. Further investigations are needed to determine if rhinorrhea correlates with sympathetic denervation or other autonomic symptoms in PD. ©2010 Movement Disorder Society

Key Words: Parkinson's disease; rhinorrhea; PET; olfaction; dopamine transporter

Rhinorrhea, defined in previous studies involving patients with Parkinson's disease (PD) as the presence of a runny nose unrelated to allergies, respiratory infections, or sinus problems, has been reported to occur more frequently among PD patients than healthy controls.^{1,2} The two existing studies on rhinorrhea in PD have originated from only one center,^{1,2} and it remains unclear if rhinorrhea is common to all PD patients or restricted to a specific geographic region.

Friedman and coworkers have hypothesized that rhinorrhea in PD may be due to sympathetic denervation of the nasal mucosa^{1,2} but they did not investigate other autonomic signs or symptoms. The association of rhinorrhea with olfaction, motor function, and nigrostriatal dopaminergic denervation on positron emission tomography (PET) imaging has also not been explored. One study reported an association between rhinorrhea and hyposmia, but olfaction was assessed by self-report only.¹

In this study, we compare the frequency of rhinorrhea between PD subjects and normal controls (NC) and investigate the relationship between rhinorrhea and clinical (olfaction, motor, and autonomic) features, and the degree of nigrostriatal dopaminergic denervation in PD. We hypothesized that (1) rhinorrhea would be more prevalent in PD than in NC, (2) rhinorrhea would be unrelated to olfaction, motor function, or nigrostriatal dopaminergic denervation in PD patients, and (3) rhinorrhea would be related to other autonomic problems in PD.

Patients and Methods

Subjects and Patient Consents

Thirty-four men between 50–85 years of age meeting UK PD Society Brain Bank criteria for PD³ were enrolled in this study. These subjects were originally recruited for a study on falls in PD. For that study, they had to be ambulatory, nondemented (defined as Mini-Mental State Exam MMSE ≥ 25), willing to undergo brain PET imaging, and could not be taking cholinesterase inhibitors or pure anticholinergic drugs. All were recruited prospectively from the Neurology and Geriatric, Research, Education, Clinical Center clinics at the Veterans Affairs (VA) Hospital. The diagnosis of PD was confirmed by nigrostriatal dopaminergic denervation on [¹¹C]dihydrotetrabenazine (DTBZ) vesicular monoamine transporter type 2 (VMAT2) brain PET imaging. The mean Unified PD Rating Scale (UPDRS) motor score was 27.6 ± 7.3

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TABLE 1. Baseline characteristics between Parkinson's disease (PD) and normal control (NC) subjects

	PD (n = 34)	NC (n = 15)	Statistic	P value
Age (y)	68.3 ± 8.2	66.6 ± 11.7	$t = -0.59$	0.56
No. (%) with rhinorrhea (any cause)	23 (68)	4 (27)	$\chi^2 = 7.07$	0.008
No. (%) with nonrhinitic rhinorrhea	12 (35)	1 (7)	$\chi^2 = 4.38$	0.04
No. (%) on medications with potential to cause rhinorrhea	20 (59)	6 (40)	$\chi^2 = 1.48$	0.22
UPSIT scores	16.5 ± 8.3	32.2 ± 6.9	$t = 6.35$	<0.0001
MMSE	29.0 ± 1.4	29.6 ± 0.6	$t = 2.10$	0.04
VMAT2 BP _{ND} activity				
Caudate	1.02 ± 0.42	1.59 ± 0.24	$t = 5.96$	<0.0001
Anterior putamen	0.90 ± 0.40	2.20 ± 0.33	$t = 11.03$	<0.0001
Posterior putamen	0.63 ± 0.34	2.44 ± 0.35	$t = 16.68$	<0.0001
Total striatum	0.92 ± 0.31	1.95 ± 0.26	$t = 11.09$	<0.0001

(range 15–45) and mean disease duration was 6.2 ± 3.1 years (range 0.5–12). All PD subjects were on dopaminergic treatment but none on apomorphine.

Fifteen male NC were recruited from existing databases/advertisements. They had no known neurological or psychiatric illness, or family history of significant neurological or psychiatric illness in first-degree relatives. All had MMSE ≥ 25. None were taking centrally acting medications.

All participants were nonsmokers with no history of trauma or concurrent respiratory infection that could interfere with olfaction. None had clinically significant abnormalities (i.e., large vessel strokes, excessive white matter changes, or tumors) on brain MRI. Participants gave informed written consent, and the protocol was approved by the Institutional Review Boards of the University of Michigan and the Ann Arbor VA (ClinicalTrials.gov identifier: NCT00736671).

Clinical Testing Procedures

Participants were asked if they had a runny nose, allergies, sinus problems, or frequent colds/respiratory infections. Participants had rhinorrhea if they answered yes to the question “Do you have a runny nose?” A stricter definition, nonrhinitic rhinorrhea, was considered if participants had a runny nose but no allergies, sinus problems, or frequent colds/respiratory infections. This stricter definition was used in previous studies.^{1,2}

Participants were asked (using a yes/no format) if they experienced subjective lightheadedness or passed out during the past year, had a problem with “dusky hands,” or experienced bladder problems, erectile dysfunction or constipation. They were also asked about the presence of diabetes or prostate problems to rule out possible confounding factors. Orthostatic blood pressures were measured. Medications reported to cause rhinorrhea,⁴ including antihypertensives, α -antagonists, aspirin, and other nonsteroidal anti-inflammatory agents, were recorded. Olfaction was assessed using the 40-item University of Pennsylvania

Smell Identification Test (UPSIT; Sensonics, Haddon Heights, NJ).⁵ Motor severity was assessed using the UPDRS motor subsection. PD subjects were tested and imaged in the morning after withholding dopaminergic medications overnight.

Imaging Techniques and Data Analysis

DTBZ PET image acquisition and analysis has been described previously.⁶ The binding potential relative to the nondisplaceable uptake (BP_{ND}) was estimated as before,⁶ except that for this analysis, the whole neocortex was used as a reference region instead of the occipital cortex for more robust assessment. Group comparisons were performed using standard *t* tests or χ^2 analyses as appropriate. Data were analyzed using SAS, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Rhinorrhea Frequency in PD and NC

Rhinorrhea of any cause occurred more frequently in PD compared with NC (68% of PD vs. 27% of NC; $\chi^2 = 7.07$, $P = 0.008$, see Table 1). Nonrhinitic rhinorrhea was five times more prevalent in PD than NC (35% vs. 7%; $\chi^2 = 4.38$, $P = 0.04$). Mean UPSIT scores and VMAT2 BP_{ND} activity throughout the striatum were significantly lower in the PD group (Table 1). There was no difference in age or rhinorrhea medications between the patients and controls, but NC had a slightly higher MMSE score ($t = 2.10$, $P = 0.04$).

PD Subjects with and Without Rhinorrhea

There were no differences in most clinical variables, including baseline demographics, motor function, autonomic symptoms, olfactory performance, and striatal VMAT2 binding using DTBZ PET between PD subjects with and without rhinorrhea (Table 2). There

TABLE 2. Comparison of clinical variables and VMAT2 BP_{ND} activity in PD subjects with and without rhinorrhea

	Rhinorrhea + (n = 23)	Rhinorrhea - (n = 11)	t statistic	P value
Age (y)	68.8 ± 7.9	67.3 ± 9.2	-0.51	0.61
Disease duration (y)	6.2 ± 3.1	6.3 ± 3.2	0.15	0.89
No. (%) on medications with potential to cause rhinorrhea	15 (65)	5 (45)	$\chi^2 = 1.20$	0.27
MMSE	28.7 ± 1.5	29.5 ± 0.7	2.11	0.04
UPSIT score	17.5 ± 9.0	14.4 ± 6.3	-0.98	0.33
UPDRS motor	28.0 ± 8.0	26.6 ± 6.0	-0.53	0.60
No. (%) with lightheadedness	12 (52)	1 (9)	$\chi^2 = 5.85$	0.02
No. (%) who have passed out	5 (22)	0 (0)	$\chi^2 = 2.80$	0.09
SBP sitting (mm Hg)	120.0 ± 15.3	128.9 ± 11.5	1.70	0.09
DBP sitting (mm Hg)	74.7 ± 6.1	77.5 ± 9.6	1.04	0.31
Change in SBP with standing (mm Hg)	-2.9 ± 7.1	-4.3 ± 6.1	-0.56	0.58
Change in DBP with standing (mm Hg)	1.9 ± 3.9	0.5 ± 6.1	-0.80	0.43
No. (%) with bladder problems	15 (65)	7 (64)	$\chi^2 = 0.008$	0.93
No. (%) with constipation	12 (52)	3 (27)	$\chi^2 = 1.88$	0.17
No. (%) with dusky hands	2 (9)	0 (0)	$\chi^2 = 1.0$	0.31
No. (%) with impotence	15 (65)	5 (45)	$\chi^2 = 1.20$	0.27
VMAT2 BP _{ND} activity				
Caudate	1.02 ± 0.43	1.02 ± 0.41	0.02	0.99
Anterior putamen	0.93 ± 0.41	0.82 ± 0.38	-0.71	0.48
Posterior putamen	0.66 ± 0.37	0.59 ± 0.28	-0.51	0.61
Total striatum	0.92 ± 0.31	0.90 ± 0.34	-0.19	0.85

SBP, systolic blood pressure; DBP, diastolic blood pressure.

were also no differences in the frequency of diabetes or prostate problems between the 2 groups.

The rhinorrhea group had slightly lower MMSE scores ($t = 2.11$, $P = 0.04$) and complained more about lightheadedness (52% vs. 9%; $\chi^2 = 5.85$, $P = 0.02$) compared with the nonrhinorrhea PD group. There was also a trend toward a prior history of passing out ($P = 0.09$) and lower sitting systolic blood pressures ($P = 0.09$) in PD subjects with rhinorrhea. When using a stricter definition of rhinorrhea, there were no significant differences in any baseline demographic, motor, olfactory, autonomic, or PET variables, including MMSE, subjective lightheadedness, passing out, and sitting systolic blood pressures.

Discussion

Our results are consistent with the findings of Friedman and coworkers^{1,2} that a runny nose not due to allergies, sinus problems, or colds occurs more frequently in PD than non-PD subjects. We further extend these findings and demonstrate that both rhinorrhea due to any cause and nonrhinitic rhinorrhea are unrelated to severity of nigrostriatal dopaminergic activity, or olfactory and motor dysfunction in PD.

The prevalence of nonrhinitic rhinorrhea in our PD subjects (35%) was lower than previously reported (~50%).^{1,2} This could be due to our smaller sample size or the fact that our PD population was comprised of only men. Nonrhinitic rhinorrhea tends to occur more commonly in women with PD, with one study

reporting a frequency of 68% in women and 33% in men,¹ a figure more consistent with our findings.

Previous studies on rhinorrhea in PD have reported an association with olfactory impairment, but the presence of olfactory impairment was ascertained by self-report only.¹ We objectively tested odor identification using the UPSIT in this study and found no significant differences between PD patients with and without rhinorrhea, regardless of the rhinorrhea definition used. This is consistent with the observation that PD patients are hyposmic in general⁷ and suggests that rhinorrhea is unrelated to olfaction.

While nigrostriatal pathology correlates with specific motor symptoms in PD, it is now recognized that non-motor comorbidities extend beyond the loss of dopaminergic nigral neurons.⁸ We found no gross differences in striatal VMAT2 binding using DTBZ PET between PD subjects with and without rhinorrhea but our sample size was small. These findings suggest that rhinorrhea can be added to the growing list of nondopaminergic features in PD and are also consistent with the possibility that rhinorrhea may be due to sympathetic denervation.^{1,2} Sympathetic denervation in the heart has been reported in PD,⁹ but it is unclear if sympathetic dysfunction also occurs in the nasal mucosa. If it does, it may leave unopposed parasympathetic stimulation, which increases nasal secretions.² We did not perform cardiac noradrenergic PET imaging in this study but investigated other autonomic symptoms. The percentage of PD subjects with constipation, bladder problems, erectile dysfunction, or

dusky hands did not differ between the rhinorrhea and nonrhinorrhea groups, regardless of the rhinorrhea definition used. However, patients with rhinorrhea of any cause had more subjective lightheadedness and a trend toward more frequent passing out and lower systolic blood pressures when sitting. These findings were no longer significant after excluding those with a rhinitic cause of rhinorrhea, but we may have had too small a sample size to detect such a difference. Further investigations are clearly needed to see if rhinorrhea correlates with cardiac and/or cerebral sympathetic denervation or other autonomic symptoms in PD.

A limitation of the study is that we did not study women as our patients were recruited from a VA population. Another limitation is that the presence of rhinorrhea was established by questionnaire only. Anatomic causes of rhinorrhea such as nasal polyps, which are common in men and the elderly,¹⁰ cannot be completely excluded. It is possible that rhinorrhea was caused by medications in some subjects, but group comparisons showed no differences in the proportion of participants taking medications with the potential to induce rhinorrhea.

In summary, rhinorrhea is a common, nondopaminergic feature in PD that is unrelated to the severity of olfactory or motor deficits. The relationship between rhinorrhea, sympathetic denervation, and autonomic dysfunction needs to be further explored using validated questionnaires. ■

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Central Oscillators in a Patient with Neuropathic Tremor: Evidence from Intraoperative Local Field Potential Recordings

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ABSTRACT

Present pathophysiological concepts of neuropathic tremor assume mistimed and defective afferent input resulting in deregulation of cerebello-thalamo-cortical motor networks. Here, we provide direct evidence of central tremor processing in a 76-year-old female who underwent bilateral deep brain stimulation of the ventral intermedial nucleus of the thalamus (Vim-DBS) because of neuropathic tremor associated with IgM paraproteinemia. Electrophysiological recordings of EEG and EMG were performed in three perioperative sessions: (1) preoperatively, (2) intraoperatively, and (3) 4 days after surgery in both rest and postural tremor conditions. Tremor-related synchronization (coherence) between motor cortex (M1) and muscles (M. extensor digitorum, M. flexor digitorum) was assessed, and additional intraoperative local field potential (LFP) recordings from Vim allowed comprehensive coherence mapping in thalamo-cortico-muscular networks. Directionality of information flow was determined by directed transfer function (DTF) and phase analyses. Stimulation effects on tremor and

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corticomuscular coherence were assessed and the patient was followed for 12 months on clinical outcome measures (Tremor Rating Scale, CADET-Score). Vim-DBS reduced tremor (59%) and improved motor functionality in daily activities (31%, CADET-A) after 12 months. Intraoperative recordings demonstrated significant coherence in the tremor frequency (4 Hz) between M1 and contralateral muscle, Vim and ipsilateral M1, Vim and contralateral muscle, but not between Vim and contralateral M1. Information flow was directed from M1 to Vim and bidirectional between M1 and muscle and between Vim and muscle, respectively. Corticomuscular coherence at tremor frequency was completely suppressed by Vim-DBS. Our case study demonstrates central oscillators underlying neuropathic tremor and implies a strong pathophysiological rationale for Vim-DBS. ©2010 *Movement Disorder Society*

Key Words: neuropathy; tremor; deep brain stimulation; coherence; local field potentials

Tremor occurs in up to 40–90% of the patients with IgM paraproteinemic neuropathy¹ and remains refractory on medical treatment in the majority of patients.^{1,3} Present pathophysiological concepts of neuropathic tremor postulate that distorted mistimed peripheral inputs reach a central processor, which is misled into producing tremor.^{3,4} Therapeutic effectivity of deep brain stimulation of the ventral intermedial nucleus of the thalamus (Vim-DBS) in a few single cases pointed to maladaptive central motor processing,² although no direct evidence was presented. To explore the present pathophysiological concept, we hypothesized on the presence of neuronal and neuromuscular synchronization (coherence) related to the tremor frequency as a substrate of central tremor processing based on intra- and perioperative recordings of oscillatory activity in a patient who underwent Vim-DBS.

Patients and Methods

Patient and Surgery

In 2005, the patient (76-year-old, female) developed bilateral kinetic and postural tremor (absent at rest) with pronunciation of the distal upper extremities paired with sensory gait disorder, loss of deep tendon reflexes, and paresthesia of the malleoli mediales. In 2007, IgM-paraproteinemia related to lymphoblastic lymphoma was diagnosed. Neurography revealed axonal demyelinating sensorimotor neuropathy (Table 1) and in 2008, IgM neuropathy was demonstrated by sural nerve biopsy. Tremor was not responsive to alcohol as documented under clinical survey and no family history for tremor was reported. Genetic analysis excluded fragile X associated tremor ataxia syndrome [fragile X tremor ataxia syndrome (FXTAS)]. Since pharmacological treatment with propranolol, primidone, gabapentin, and clonazepam did

TABLE 1. Findings and stimulation parameters

	Six months prior to DBS	One month prior to DBS	Three months after Vim-DBS (StimOff/StimOn)	Twelve months after Vim-DBS (StimOff/StimOn)
Tremor Rating Scale	not tested	20	18/6	22/9
CADET-A	not tested	60	57/34	61/42
Tibialis nerve (motor)			not tested	
DML (ms)	14.7	28.1		20.6
amplitude (mV)	0.2	0.1		0.2
conduction velocity (m/s)	14	19		13
Ulnar nerve (motor)			not tested	
DML (ms)	5.2	5.9		9.5
amplitude (mV)	6.5	6.5		7.5
conduction velocity (m/s)	23	23		26
Stimulation parameters				
Vim_left	none	none	0-1-C+, 3.7V, 60 μ s, 180 Hz	I-2-C+, 3.5V, 180 μ s, 180 Hz
Vim_right	none	none	4-5+, 3.8V, 120 μ s, 180 Hz	4-5+, 3.1V, 180 μ s, 180 Hz

Findings before, 3 months after, and 12 months after bilateral Vim-DBS. No sensory action potentials of the radial and sural nerve were obtained 6 months, 1 month before surgery and 12 months after Vim-DBS. Abbreviations: DML = distal motor latency, C = case. Bipolar stimulation parameters were used on the right Vim to avoid current spreading to capsular fibres (see details test stimulation in Supporting information Table 2).

not improve tremor, bilateral Vim-DBS was performed in 2008 awake during surgery procedure. Three intraoperative trajectories on the right Vim (T1 = central trajectory, T2 = posterior trajectory, T3 = lateral trajectory) were subjected to local field potential (LFP) recordings. Trajectory T2 yielded best clinical effectiveness upon intraoperative test stimulation and was therefore implanted. Postoperative imaging confirmed proper electrode placement (refer supporting information).

Clinical Evaluation and Paradigm

The study was approved by the local ethics committee and the patient gave informed written consent. The “Tremor Rating Scale” and “Columbia University Assessment of Disability in Essential Tremor (CADET) score, appendix A”⁵ were assessed before, 3 and 12 months after surgery, respectively. Recordings of EEG and EMG were performed (1) 1 month before surgery, (2) intraoperatively (including simultaneous Vim-LFP recordings), and (3) 4 days after surgery. In each of the sessions, recordings at rest and during postural tremor (both arms elevated separately, anteversion 90°, elbow stretched 160°, hand in supination) were performed. In the postoperative session, Vim-DBS was either stimulation turned off (StimOff) or stimulation turned on (StimOn) unilaterally both (1) ipsi- and (2) contralateral to the trembling arm.

Data Analysis

Data analysis was performed using Matlab 7.0 (The MathWorks, Natick, MA) and included (1) frequency domain analysis of muscular activity, (2) coherence analysis, and (3) directionality analysis based on directed transfer function (DTF) and the maximizing phase synchronization index. Both Vim and EEG signals were bipolarized before computing the spectral measures. Significance of the coherence spectra was tested using a 99% confidence limit. The pair of channels displaying a significant coherence maximum at tremor frequency was selected for analysis on directionality of information flow (detailed information in the supporting information).

Results

Clinical Outcome

Tremor caused severe motor impairment in daily activities at the preoperative baseline (Tremor Rating Scale: 20 points, CADET-A: 60 points). Bilateral Vim-DBS led to a marked improvement in the Tremor Rating Scale (67% at 3 months, 59% at 12 months) and in the CADET-A (40% at 3 months, 31% at 12 months), however the stimulation amplitude had to be consecutively raised within the first year to achieve

stable therapeutic effects. Neurographic parameters remained stable in the follow-up period (Table 1).

Tremor-Related Coherence

Preoperatively, significant corticomuscular coherence at tremor frequency (4 Hz) was present between left muscles and contralateral motor cortex (M1) during postural tremor (absent at rest) with maxima on C4CP4 and C2CP2. This was reproduced in intraoperative recordings and additional significant coherence between right Vim and right M1 (maximum T1T2-C4CP4) at 4 Hz and in the first harmonic frequency (8–9 Hz) was found. This coherence peak was smaller but still significant on T1T3-C4CP4 and absent on T2T3-C4CP4. No significant coherence was present between right Vim and left M1. Thalamo-muscular coherence at 4 Hz displayed a similar pattern with coherence maxima on T1T2-muscles, smaller significant coherence on T1T3-muscles, but no significant coherence on T2T3-muscles (Fig. 1). No significant coherence between Vim, M1, and muscles was found at rest.

Directionality and Conduction Time of Tremor-Related Oscillatory Activity

Phase analysis and DTF were performed on T1T2-muscle, T1T2-C4CP4, and C4CP4-muscle. There was a delay of 19 ms from muscle to Vim and of 9 ms from Vim to muscle. DTF analysis showed unidirectional feedforward flow from Vim to muscle. A unidirectional flow from C4CP4 to Vim with a delay of 7 ms was consistent with DTF analysis showing strong flow from M1 to Vim and only sparse inverse flow. Bidirectional flow delayed by 10 ms from C4CP4 to muscle and 32 ms from muscle to C4CP4 was found. DTF showed unidirectional feedforward flow from M1 to muscle (supporting information Fig. 1).

Effects of Vim-DBS on Postural Tremor

Muscular power and intermuscular coherence were marked at 4 Hz (StimOff) and reduced upon Vim-DBS, but increased between 10 and 40 Hz. No frequency shift of the tremor-related peak was observed. Corticomuscular coherence at 4 Hz (StimOff) was suppressed by stimulation (supporting information Fig. 2). If the Vim ipsilateral to the trembling muscle was stimulated (contralateral StimOff), the corticomuscular coherence remained unchanged, as expected (not shown).

Discussion

We provide evidence for central oscillators underlying neuropathic tremor. Tremor was associated with synchronous oscillatory activity at 4 Hz in a network including Vim and M1 contralateral to the trembling muscle (absent at rest). This was suppressed by Vim-DBS in parallel with a marked tremor reduction.

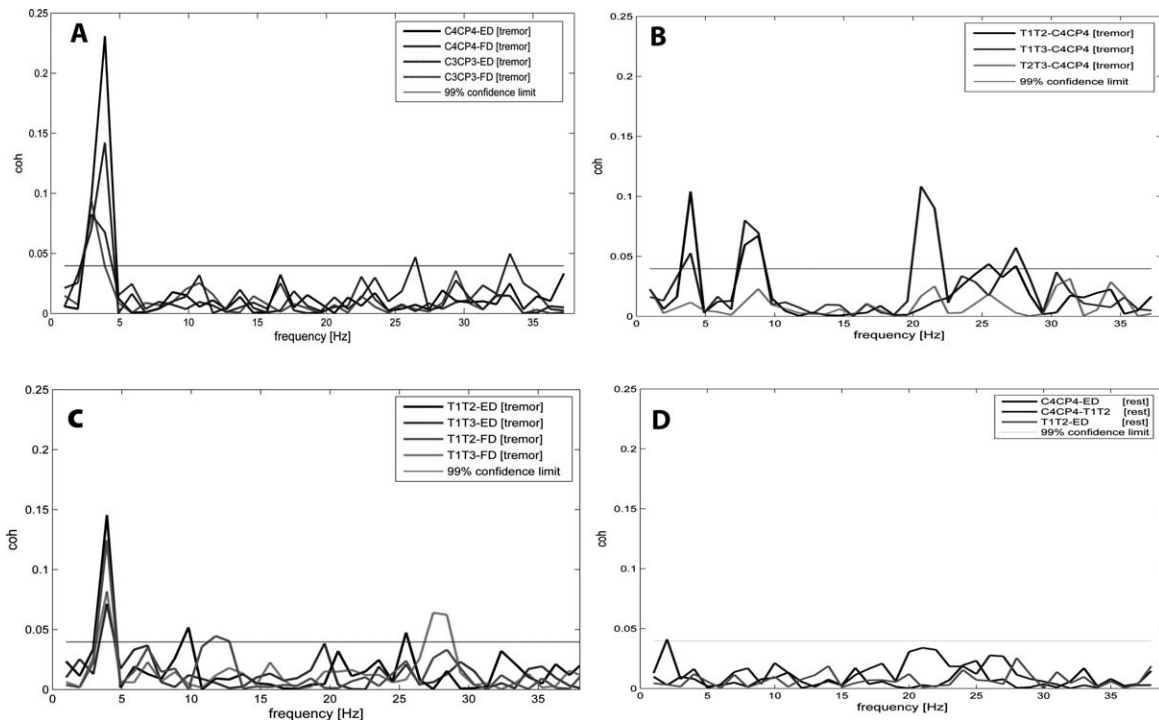


FIG. 1. Intraoperative coherence mapping between Vim, cortex, and muscle. Coherence spectra from intraoperative recordings during postural tremor of the left arm. **A** Left/right cortex–muscles. **B** Right Vim–right cortex. **C** Right Vim–left muscles. **D** coherence spectra at rest. The x-axis: frequency [Hz] and y-axis: coherence. Horizontal line: 99% confidence limit. Note that no significant coherence was present during the postural tremor condition between right Vim and left contralateral cortex (data not shown).

Several aspects of our findings support its pathophysiological significance and argue against a major influence of mechanical artefacts inevitably arising from tremor in electrophysiological recordings: (1) corticomuscular coherence at tremor frequency was paralleled by local maxima of tremor-related coherence from Vim (T1T2) to both ipsilateral M1 and contralateral muscles. One may argue, whether thalamocortical and thalamo-muscular coherence was brought in by volume conduction from corticospinal fiber tracts descending laterally from Vim. However, T3 (the most lateral trajectory) displayed weak coherence in bipolar T1T3 and no significant coherence in T2T3 indicating a local maximum on T1T2. In accordance to the topographical coherence distribution test stimulation on T2 improved tremor. Test stimulation on T3, however, failed to improve tremor but showed the lowest threshold for capsular side effects from test stimulation, as expected (supporting information Table 1). (2) Corticomuscular coherence at tremor frequency presented lateralized on the M1 contralateral to the trembling arm in each of the perioperative conditions and was suppressed by Vim-DBS together with strong reduction of tremor on both clinical and electrophysiological measures. Moreover, muscular power and intermuscular coherence increased within a broad range of the beta band, as known from physiological isometric contraction.^{6,7} (3) Directionality analysis of

tremor-related coherence revealed non-zero phase delays between Vim, M1, and muscle. Muscular activity was driven forward from both M1 and Vim and refference lagged the feedforward drive. Similar conduction delays from both Vim and M1 to muscle might raise the question whether both are coupled to a third oscillator, e.g. cerebellum.^{3,4} Alternating output from both oscillators to muscle would also explain the condition.⁸ As expected, refferent flow from muscle to M1 or Vim was not represented by the DTF analysis due to the higher signal strength in muscle.^{8,9} The phase analysis (insensitive to the amplitude of the signal) can correctly capture the driver-response relationship in neuromuscular projections whereas the DTF (sensitive to the amplitude of the signal) reliably establishes driver-response relations between EEG and VIM due to comparable signal strengths.

Clinical Aspects of Vim-DBS in Neuropathic Tremor

Absence of other well characterized tremor conditions is mandatory in order to diagnose “neuropathic tremor.” Tremor occurs with high prevalence in patients with paraproteinemic neuropathy and further clinical signs of our patient including ataxia of gait, temporally coincident onset of neuropathy, missing therapeutic response to alcohol, and negative family

history matched the clinical characteristics.³ Vim-DBS in our patient improved both postural and kinetic components of the tremor. Best tremor suppression was achieved on the most ventral (caudal) contacts on AC-PC level. This is in line with previous findings in patients with essential tremor and therapeutic effects might rather arise from crossing cerebello-thalamic projection fibers in the subthalamic white matter than from the Vim itself.¹¹ The stimulation intensity had to be increased between the 3-month and 12-month follow-up in our patient. Neurographic parameters remained stable and argue against a relevant progression of neuropathy although this cannot completely rule out a worsening of the tremor severity in the respective time range.³ The conduction delay of tremor feedback from muscle to M1 (32 ms) in our patient exceeded the phase delays in patients with essential tremor upon the same methodological approach.⁸ Interestingly, similar delays in sensory evoked potential N20 conduction times (30–48 ms) were described in patients with neuropathic tremor.³ Whether delayed reafference of the tremor phase constitutes a specific electrophysiological marker of neuropathic tremor, however, remains to be established. Our findings support the concept of maladaptive central motor processing in neuropathic tremor and therefore provide a strong pathophysiological rationale for Vim-DBS.

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Residual Striatal Dopaminergic Nerve Terminals in Very Long-Standing Parkinson's Disease: A Single Photon Emission Computed Tomography Imaging Study

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ABSTRACT

Molecular imaging studies of Parkinson's disease (PD) progression mostly focus on the first 5 years after disease onset, demonstrating rapid initial nigrostriatal neuronal loss. The fate of residual functional dopaminergic nerve terminals in patients with long-standing PD has not yet been specifically explored. Therefore, we performed [¹²³I]-FP-CIT single photon emission computed tomography (SPECT) in 15 patients with very long-standing PD (mean disease duration 20.6 ± 6.3 years). Measurable uptake of [¹²³I]-FP-CIT was still detected in the striata of all patients. As seen in early stages, reduction of tracer uptake in the putamen was more prominent than in the caudate nucleus. Asymmetry in tracer uptake between the two putamen and caudate nuclei was preserved. These findings indicate that degeneration of dopaminergic neurons in PD is not total even after many years of illness. Data should be considered in exploring underlying causes of progressive loss of nigrostriatal dopaminergic neurons and development of future novel dopaminergic therapeutic strategies in PD. © 2010 Movement Disorder Society

Key Words: single photon emission computed tomography; dopamine transporters scan; long standing; Parkinson's disease

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There is a recent increasing interest in Parkinson's disease (PD) patients with very long-standing illness. Hely et al.^{1,2} showed that after 20 years of illness, there is a shift of symptom burden from nigrostriatal to extranigral sites. This raises intriguing questions regarding the fate of dopaminergic neurons in advanced PD, i.e., are they totally lost, are there differences in survival between nigro-putaminal and nigro-caudal projections, and whether the asymmetry of neuronal loss is still maintained.

Functional imaging is a powerful approach to study the underlying neuropathology in PD. However, studies using positron emission tomography with ¹⁸F-dopa and single photon emission computed tomography (SPECT) with ligands for the presynaptic dopamine transporters (DaT), mainly focused on patients during the first 5 years after disease onset,³⁻⁶ and imaging data of patients with extreme "end stage" of PD is lacking.

We have therefore evaluated the pattern of striatal uptake of [¹²³I]-FP-CIT in PD patients with disease durations of more than 15 years.

Patients and Methods

Patients

A total of 15 patients with idiopathic PD were enrolled in the study. The study was approved by the local ethics committee and a written informed consent was obtained from all subjects. Diagnosis was based on the UK PD Society Brain Bank criteria.⁷ Disease severity was assessed according to the Unified PD Rating Scale (UPDRS), and the Hoehn and Yahr (H&Y) scale, evaluated during the "on" stage. Asymmetry index was calculated by subtracting the UPDRS score of the less affected from the more affected side. Cognitive status was assessed by the Mini Mental Status Examination. The results were compared to 11 healthy controls.

SPECT Studies

All patients received potassium iodide orally to block thyroid uptake of free radioactive iodide. A dose of 185 MBq [¹²³I]-FP-CIT was injected intravenously and imaging was performed 3 hours later. The SPECT study was performed using a dual-head γ -camera (Helix; Elscint) equipped with a low-energy, high-resolution collimator. A 20% window was centered on the 159-keV photopeak of ¹²³I. One hundred twenty frames (of 15 seconds) were acquired using a circular rotation mode into a 128 \times 128 image matrix. Transaxial, coronal, and sagittal slices 1-pixel thick were reconstructed using a third-order Metz filter set to 12-mm full width at half maximum. Attenu-

ation correction was performed with a constant linear attenuation coefficient of 0.11 cm⁻¹. As patients were not able to ambulate without medications, they were allowed to take levodopa before the SPECT procedure.

Analysis of SPECT Results

For analysis of striatal [¹²³I]-FP-CIT binding, the two transaxial slices representing the most intense striatal binding were summed and subjected to qualitative analysis of tracer activity in the striatal regions. For quantitative analysis of tracer uptake, regions-of-interest (ROIs) were constructed manually with the help of a brain atlas in areas corresponding to the right and left putamen, caudate, and overall striatum. For background evaluation, ROIs were also drawn bilaterally in areas corresponding to the medial occipital lobe. For each ROI, mean counts were measured and specific [¹²³I]-FP-CIT uptake was then calculated, according to the following formula: Specific [¹²³I]-FP-CIT uptake = (mean activity in ROI – mean activity in occipital cortex)/mean activity in occipital cortex). SPECT analysis was performed by a Nuclear Medicine expert, blinded to the patient's disease duration.

Statistical Analysis

t Test was used to compare between mean FP-CIT uptake of the putamen, caudate, and striatum of patients and controls. Paired *t* test was used to compare between mean FP-CIT uptake in both sides of the putamen and mean uptake in the caudate, and to evaluate differences within each patient between the more and less affected sides of the putamen, caudate, and overall striatum. Correlation between FP-CIT values and UPDRS and asymmetry index was assessed using Pearson test. Data are presented as mean \pm SD.

Results

Clinical Characteristics

The clinical characteristics and FP-CIT uptake values of the patients and of controls are summarized in Table 1. Mean age of the patients was 68 \pm 8.6 years (compared with 61 \pm 12 years in the control group; *P* < 0.001) and mean disease duration 20.6 \pm 6.3 years. Fourteen of fifteen patients had motor fluctuations, and 13 of 15 patients had dyskinesias. The patients had a UPDRS score at "on" of 30.4 \pm 15.5 (mean \pm SD).

FP-CIT uptake was reduced by 87% in the putamen, by 72% in the caudate, and by 78% in the striatum in patients compared with controls (Table 1). Qualitative analysis of the SPECT data revealed residual [¹²³I]-FP-CIT uptake in the striata of all patients at this advanced disease stage. The most robust uptake was observed in the caudate nucleus. Quantitative

TABLE 1. Clinical characteristics and [¹²³I]-FP-CIT uptake values in patients with advanced Parkinson's disease

Pt. No./Age/M/F	Disease duration (yr)	H & Y	UPDRS	Asym. index	MMSE	Putamen			Caudate			Striatum			P/C	
						More affected	Less affected	MMSE	More affected	Less affected	MMSE	More affected	Less affected	MMSE	More affected	Less affected
1/57/M	22	4	17	3	26	0.31	0.35	1.22	1.32	0.76	0.87	0.59	0.58			
2/73/F	17	4	47	4	30	0.39	0.48	1.1	1.36	0.85	1.03	0.66	0.62			
3/75/M	20	2	17	1	25	0.3	0.4	0.96	1.08	0.78	0.67	0.66	0.62			
4/67/F	23	5	51	10	24	0.31	0.38	0.87	1.25	0.6	0.73	0.7	0.62			
5/73/M	18	3	43	4	23	0.16	0.24	1.09	1.22	0.69	0.82	0.55	0.56			
6/73/F	17	4	17	3	29	0.31	0.51	1.22	0.99	0.57	0.69	0.66	0.68			
7/74/F	19	4	20	4	28	0.32	0.35	1.09	0.99	0.57	0.59	0.63	0.72			
8/53/M	20	4	46	8	29	0.14	0.28	0.64	0.78	0.41	0.58	0.7	0.72			
9/74/F	19	4	46	4	26	0.15	0.2	0.8	0.85	0.4	0.48	0.64	0.65			
10/72/M	20	3	26	4	NA	0.22	0.29	0.83	1.15	0.6	0.77	0.67	0.6			
11/69/F	20	3	20	6	23	0	0	0.41	0.57	0.3	0.11	0.6	0.54			
12/53/F	23	2	9	1	30	1.16	1.09	2.38	2	1.59	1.22	0.64	0.7			
13/68/M	15	3	30	2	30	0.4	0.57	1.02	1.17	0.62	0.7	0.69	0.72			
14/84/F	42	5	55	0	NA	0.34	0.54	0.59	0.63	0.44	0.58	0.76	0.81			
15/60/M	15	2	21	3	NA	0.61	0.68	1.42	1.74	0.79	1.13	0.66	0.61			
Patients mean ± SD	20.6 ± 6.3	3.5 ± 0.9	31 ± 16	3.8 ± 2.6	27 ± 2.7	0.34 ± 0.26	0.42 ± 0.24	1.0 ± 0.4	1.15 ± 0.38	0.66 ± 0.3	0.73 ± 0.27	0.65 ± 0.05	0.65 ± 0.07			
Controls mean ± SD				3.0 ± 0.52 ^a		3.0 ± 0.52 ^a	2.83 ± 0.56 ^b	4.14 ± 0.65 ^a	4.29 ± 0.68 ^b	3.41 ± 0.46 ^a	3.53 ± 0.51 ^b	0.73 ± 0.04 ^a	0.74 ± 0.03 ^b			

^aRefers to right side.^bRefers to left side.

H & Y, Hoehn and Yahr; UPDRS, unified Parkinson's disease rating scale; Asym., asymmetry; MMSE, mini-mental status examination; NA, non available.

analysis showed that the putamen is more denervated than the caudate nucleus. Mean uptake of both sides of the putamen was significantly reduced compared with mean uptake of both sides of the caudate (0.38 ± 0.24 vs. 1.16 ± 0.39 ; $P < 0.001$).

To evaluate the natural course of this asymmetry between the right and left striata in the advanced stages of the disease, we compared [¹²³I]-FP-CIT uptake in the more versus the less affected side. The putamen and caudate contralateral to the side of greater motor disability was associated with a larger reduction in tracer uptake, in comparison with the ipsilateral side. It was more prominent in the putamen (0.34 ± 0.26 in the more affected vs. 0.42 ± 0.24 in the less affected side; $P < 0.0001$) than in the caudate (1.02 ± 0.45 in the more affected vs. 1.15 ± 0.38 in the less affected side; $P < 0.01$). There was correlation between FP-CIT uptake in the putamen, caudate, and striatum and UPDRS score ($P < 0.003$, $P < 0.002$, and $P < 0.003$, respectively). However, there was no significant correlation between FP-CIT values and asymmetry index.

Discussion

This study presents the in vivo status of dopaminergic nerve terminals in the striatum of patients with very long-standing disease, with a mean duration of 21 years.

Our main finding in this unique group of patients is that there still are residual functional dopaminergic nerve terminals in the striatum, even in such advanced stage of disease. This implies that even after very long disease course, the dopaminergic nigrostriatal projections are not totally lost. Our findings are in agreement with previous imaging studies in patients with disease duration of up to 26 years, which demonstrated that ligand uptake was still detected in all parts of the striatum. Asymmetry between the more and less affected side remained, although less prominent than in early stages of the disease.⁸⁻¹¹ This study, however, expands the characterization of striatal [¹²³I]-FP-CIT uptake profile to much longer disease durations. These results seem to correlate with clinical evidence from the Sydney cohort study showing that after 15 to 20 years of illness, dopamine-dependent motor manifestations reach a plateau and nonmotor or motor nondopaminergic features supervene and become much more important at this advanced disease stage.^{1,2} Our assumption is that there is a temporal association between the stabilization of both dopamine-dependent motor symptoms and preserved striatal DaT binding.

The study also provides several perspectives on the pattern of disease progression in the various striatal regions in these patients. We have shown that even in very long-standing disease, the putamen remains more

affected than the caudate. The explanation for this phenomenon might be related to the topography of nigrostriatal connections. Postmortem studies of the substantia nigra in PD indicate that neuronal loss tends to be the greatest in the ventrolateral tier, followed by the medial ventral and dorsal tiers.^{12,13} Cytoarchitectonic analysis of the nigrostriatal pathways in cats and primates indicates that the ventrolateral tier projects mainly to the putamen and to the posterior sectors of the caudate, whereas the medial substantia nigra predominantly projects to the rostral (anterior) part of the caudate.^{14–17} Measurement of dopamine loss in brains of patients with PD showed severe reduction of dopamine levels in the putamen, while in the caudate, the only subdivision with severe dopamine reduction was in its most dorsal rostral part.¹⁸

Interestingly, asymmetry of DaT ligand uptake between the ipsilateral and contralateral sides of the caudate and putamen was still maintained many years after motor symptom onset, although less striking than known in early disease. This finding is in accordance with anatomic, clinical, and functional imaging studies of asymmetry in PD.¹⁹ The clinical and imaging data imply that by the time the disease progresses, the less affected side “catches up” with the more affected side. A possible explanation is that there is slowing of dopamine neuronal loss at later stages in the initially more affected side, rather than an acceleration of degeneration in the contralateral side. Lack of correlation between asymmetry index and ligand uptake is probably due to the reduced clinical side difference over the years or to the relatively small sample size.

One of the limitations of the study was that for ethical reasons, study design did not control for concurrent anti-PD medications received by the patients. This may theoretically increase striatal [¹²³I]-FP-CIT binding and create an apparent floor effect. Moreover, as FP-CIT also binds to serotonin transporters, the preservation of tracer uptake in our patients might reflect, in part, serotonergic neuronal function. However, the pattern of [¹²³I]-FP-CIT binding in our patients is similar to that observed in early stages. Also, the concentrations of serotonin transporters in the basal ganglia are low. Therefore, we believe that FP-CIT binding in this group of patients represents the function of dopaminergic and not serotonergic neurons. Another obvious limitation was that only a single SPECT imaging was performed. Indeed, these results warrant further studies with longitudinal serial imaging in the same patients.

In conclusion, our study demonstrates that the neurodegenerative mechanism that causes loss of nigrostriatal dopaminergic neurons is not totally destructive. These findings are encouraging, as they may imply that the nigrostriatal system is still partially

viable in some parts of the striatum even after a long course of the disease. This may have significant implications for future therapeutic strategies in PD, especially those targeting the nigrostriatal system.

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Transcutaneous Port for Continuous Duodenal Levodopa/Carbidopa Administration in Parkinson's Disease

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ABSTRACT

Motor fluctuations in Parkinson's disease (PD) can be reduced by intraduodenal infusion of levodopa-carbidopa (Duodopa®) via percutaneous endoscopic gastrojejunostomy (PEG). We applied the transcutaneous soft-tissue anchored titanium port (T-port) in 15 PD patients with motor fluctuations; 7 Duodopa-naïve (non-PEG), and 8 previously receiving Duodopa (former-PEG). Motor scores (UPDRS-III) and quality of life (QOL, PDQ-8) were assessed at baseline and 6 month follow-up. Six patients had local irritation shortly after implantation, persisting in one patient at 6 month follow-up, which led to explantation. After having finished the protocol, four T-ports were explanted in total. UPDRS-III and PDQ-8 scores improved moderately in the non-PEG patients, but remained similar in the former-PEG users. Two former-PEG users developed polyneuropathy. No obstructions, retractions, or leakages occurred. Technical and hygienic properties of the T-port were preferred by most patients. The T-port seems to be suitable for most PD patients qualifying for Duodopa therapy, although local infection may lead to explantation during longer-term follow-up. © 2010 Movement Disorder Society

Key Words: Parkinson's disease; duodopa; T-port

Most patients with Parkinson's disease (PD) develop clinical response fluctuations and dyskinesias during the course of the disease.¹ These complications can be

reduced by achieving constant plasma concentrations of levodopa, using f.i. intraduodenal infusion of levodopa-carbidopa gel (Duodopa®).² Duodopa has been accepted as an effective treatment of fluctuating levodopa-responsive PD patients irrespective of optimal oral medication,^{3–5} although technical problems with the percutaneous endoscopic gastrojejunostomy (PEG) system frequently occur.^{4,6} A soft tissue-anchored transcutaneous titanium port, the T-port (Fig. 1), has been introduced recently as an alternative to the former PEG.⁷ The T-port allows ingrowth of subcutaneous tissue, resulting in good fixation and possibly a barrier for local infection.⁸ Apart from the fixation into the abdominal wall and the attached internal tube to the duodenum, the T-port may overcome the aesthetic hurdles for patients to start with Duodopa.⁷

The aim of the current study was to extend the experiences with the third generation T-port for Duodopa infusion in a larger sample of advanced PD patients. To assess whether use of the T-port would reduce frequent tubing and connector related complications of the current PEG system, PD patients already treated with Duodopa through a PEG system ("former-PEG") were compared with PD patients that were Duodopa naïve ("non-PEG"). Second, the effects of Duodopa treatment using the T-port on motor score and quality of life (QOL) were assessed.

Patients (or Subjects) Methods

Patients

Fifteen patients with idiopathic PD (eight former-PEG, seven non-PEG) with motor fluctuations and/or dyskinesias, not optimally treated with oral drugs, participated in this study. The patient characteristics are described in Table 1. PD was diagnosed according to the UK PD Society Brain Bank criteria and was confirmed at the UMCG Neurology Movement Disorder clinic (KLL and TL). Non-PEG patients were included if they showed a satisfying clinical response to Duodopa during the test phase. Inclusion took place from September 2007 until December 2008, whereas the final visit of the last patient was in May 2009. This study was approved by the Medical Ethical Committee of the University Medical Center Groningen. All participants signed informed consent.

Design

Prior to inclusion all patients received Duodopa treatment via a naso-gastrojejunal tube; former-PEG patients to allow the PEG opening to heal, non-PEG patients to test the clinical response on Duodopa. The baseline visit was scheduled after the wound was healed in former-PEG patients. The non-PEG baseline visit took place before the test phase (i.e. while still on

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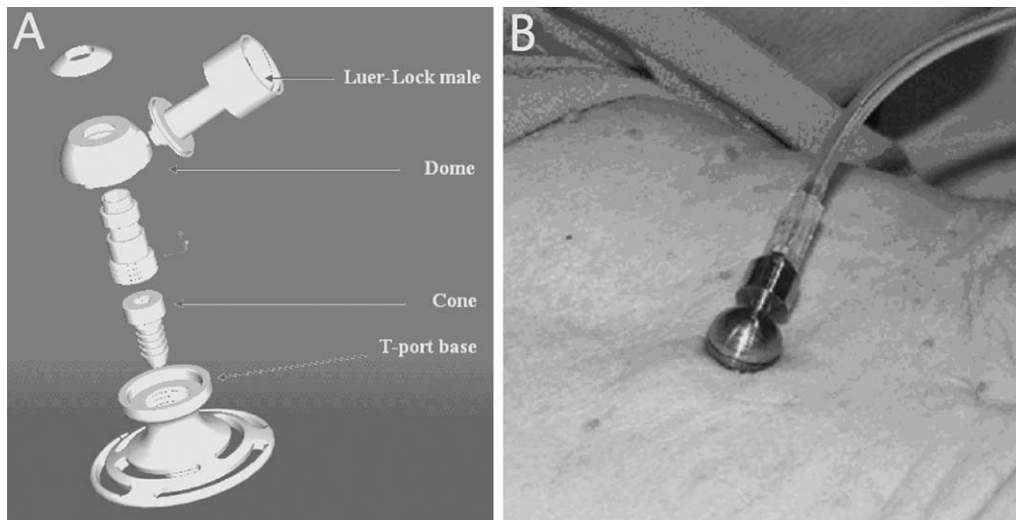


FIG. 1. A. Parts of the generation III T-port. B. T-port implanted in a patient with PD. The plastic tube is connected to a Duodopa pump and to a tube through the stomach wall with the tip in the small intestine.

oral medication). Within 1 month from baseline, the T-port was implanted. Duodopa administration was started immediately after implantation.

T-port Implantation

Interventional radiologists (RN: first two patients; TP: the others) performed all T-port (generation III) implantations and radiological gastrostomies under local anesthesia. Before implantation, patients received 1 g cefazoline intravenously. The transverse colon was radiographically visualized with contrast medium. The stomach was inflated with air via a nasogastric tube. Under local anesthesia and fluoroscopic guidance, the stomach was percutaneously punctured with an 18-gauge needle. A T-fastener (William Cook Europe, Bjaeverskov, Denmark) was positioned inside the stomach. A 4-French (F) catheter was placed at the ligament of Treitz, replaced with a stiff guide wire and the percutaneous tract dilated to 10-F. A 10-F, 60-cm-long polyurethane tube (PBN Medicals, Stenlose, Denmark) with a metallic cone at the proximal end and a pig tail curl at the distal end was inserted over the stiff guide wire with its tip beyond the ligament of Treitz. A subcutaneous pocket was created with a skin incision where the perforated flange of the T-port was placed. The intestinal tube was fixated inside the T-port. The string/needle was pulled out beside the port and fixated on gauze, allowing the T-fastener to fix the anterior wall of the stomach against the abdominal wall. The string was cut after 10–14 days. Postoperative care involved pain-relief medication, rebandaging as needed and cleaning the T-port entry-zone with water, whereas debris was removed if necessary.

Assessments

Adverse events were documented. Tolerability was defined as the number of subjects who completed the 6 months protocol. Motor scores and QOL were assessed using part III of the Unified Parkinson's Disease Rating Scale (UPDRS-III) and the PDQ-8,⁹ respectively. Mean scores with SD were calculated for baseline and 6 months follow-up visits. A paired *t*-test was used to compare means between groups.

Results

T-port Complications

T-port implantations were without technical problems and early wound recovery was uneventful. Shortly after implantation, six patients had local irritation around the port that is, more pronounced redness, pain and/or secretion. This resolved spontaneously or after application of Fucidin crème within 1 month post-implantation. One patient (non-PEG) had persisting low grade secretion, worsening over time. Bacterial culture revealed *Pseudomonas*

TABLE 1. Demographics

	Former-PEG	Non-PEG
Age	61 (14), 33–76	62 (9), 49–78
Disease duration	17.4 (7.5), 4–26	13.1 (4.7), 6–20
Men	5	5
women	3	2
UPDRS-III (baseline)	27.3 (15.7), 7–54	31.3 (12.4), 17–46
PDQ-8 (baseline)	9.6 (6.1), 0–17	10.7 (3.1), 6–15
Duodopa dose visit 6	1999 (804), 1047–3040	1702 (199), 1432–2022

Where applicable, mean, (standard deviation, SD) and range are depicted.

contamination of the T-port. No kinking, retractions, blocking, and leaking occurred.

Non-T-port Related Complications

Two former-PEG patients developed an axonal sensory polyneuropathy (combined with motor symptoms as well in one patient), confirmed by EMG. Laboratory controls revealed borderline Vitamin B12 levels. Both patients had received Duodopa for < 1 year, one patient receiving 3040 mg/day (24 hour infusion), the other 1892 mg/day (16 hour infusion). Duodopa dose was not changed after switching from PEG to T-port. Complaints in both patients improved when Duodopa dose was lowered and vitamin B12 was supplemented.

Clinical Assessments

Seven out of eight former-PEG considered the T-port system as good to very good. One patient had progressive gait disturbances and sensory axonal polyneuropathy. The other patient who developed polyneuropathy was content regarding the T-port system. Seven out of eight had experienced multiple PEG-related problems previously, like obstruction ($n = 3$), leakage ($n = 5$), dislocation ($n = 4$), and infection ($n = 2$), probably explaining the improved motor fluctuations after T-port implantation. The T-port was considered as a clear improvement, compared to the former PEG system, in terms of handling, aesthetical aspects and mechanical complications. Four of seven non-PEG patients considered the motor response to be excellent. Two patients were moderately happy, but both suffered from concomitant depression and one missed the oral dopamine agonist, which had been stopped once Duodopa was started. Another patient experienced progression of pre-existent cognitive impairments.

Mean (SD) UPDRS-III and PDQ-8 scores at baseline and 6 months follow-up were respectively 25.0 (15.5) and 21.9 (11.5), 9.1 (6.5) and 8.3 (5.4) for former-PEG and 31.3 (12.4) and 22.6 (9.8), 10.7 (3.1) and 7.1 (4.9) for non-PEG patients. Baseline and 6 month UPDRS-III and PDQ-8 scores were similar for former-PEG (respectively $t = 0.54$, $P = 0.6$ and $t = 0.089$, $P = 0.41$). Non-PEG patients showed a trend towards improvement on both scores at 6 months (UPDRS-III: $t = 2.16$, $P = 0.07$ and PDQ-8: $t = 2.12$, $P = 0.08$). When tested one-tailed, these results are significant on a $P < 0.05$ level.

Discussion

The T-port system appears to be a satisfying device in the majority of PD patients qualifying for Duodopa therapy. During the 6 month follow-up after implanta-

tion, the T-port was well tolerated in 14 of 15 patients. However, due to infection, four T-ports were explanted after having finished the protocol. So within the first 6 months, the tolerability of the T-port was 100%, which decreased to 73% after longer duration follow-up (>1 year). In one patient the infection was clearly due to a lack of hygiene. Other reasons for dissatisfaction were concomitant depression in two patients and progression of pre-existent cognitive impairments in one other patient. However, both were unrelated to the T-port device.

The total lack of obstructions, kinking, retractions, or leakage is an important improvement compared to the former PEG-systems. Newer PEG systems, f.i. the FREKA PEG tube, perform much better, becoming more comparable to the T-port. Longer follow-up is needed to get complete insight in the long-term tolerability of the T-port.

Apart from technical properties, patients preferred the aesthetic properties of the T-port. The generation III T-port is considerably better tolerated than the earlier generation T-ports.⁷

Non-T-port related adverse events included axonal polyneuropathy, not directly linked to a significant vitamin B12 deficiency, although supplementation seemed to improve patients' complaints. A recent study described that a high proportion of PD patients receiving levodopa therapy for a long time, at higher doses may develop an axonal neuropathy.¹⁰

Motor Response and QOL

Motor scores improved moderately in non-PEG, confirming earlier reports about positive effects of Duodopa on PD motor symptoms.^{2-4,11,12} Even though our Duodopa-naive group consisted of a very small number of patients, one-tailed statistical testing revealed a significant improvement of motor functions over 6 months time.

Additionally, QOL improved in non-PEG, as measured with the PDQ-8. Although we did not perform extensive cognitive and affective testing, this screening test indicates overall wellbeing, related to PD.⁹ A recent study investigating effects of Duodopa on non-motor symptoms and quality of life showed similar results, with improvement on PDQ-8 scores after 6 months follow-up.¹¹

Conclusions

The T-port system seems to be a suitable alternative to PEG tubes for PD patients who qualify for Duodopa therapy. However, the explantations during longer follow-up, due to local infections, indicate that the T-port is not the solution for all patients with

Duodopa. Longer follow-up is needed to determine the definite position of the T-port in the treatment of PD.

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Nonlinear Decline of Mini-Mental State Examination in Parkinson's Disease

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ABSTRACT

The trajectory of cognitive functioning in patients with Parkinson's disease (PD) is not known. We used a random change point model to study the individual cognitive trajectory for up to 15 years in a prevalence sample of 238 PD patients, and used the mini-mental state examination (MMSE) to assess the longitudinal cognitive course. We observed that the rate of global cognitive decline was nonlinear. Following a relatively stable period, an inflection point was identified, after which the rate of decline gained momentum with an annual decline of 2.8 points on the MMSE. The course was similar in men and women. This inflection point was estimated to occur 13.3 years (95% credible interval 11.8, 13.6) after the diagnosis of PD; however, there were wide interindividual variations in the time from onset of PD to the inflection point. © 2010 Movement Disorder Society

Key Words: Parkinson's disease; cognitive impairment; course; nonlinear

Significant cognitive decline can be detected already during the first 3 years after the diagnosis of Parkinson's disease (PD), although some patients remain stable.^{1,2} In one of the few studies assessing long-term cognitive decline,³ marked interindividual variation was observed, and interestingly, there seemed to be a time-dependent effect, i.e., patients showed a much more rapid decline in the period after dementia was

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diagnosed compared with the prodementia period. However, the statistical method used in the study did not allow for the identification of the onset of this acceleration in the sample nor the trajectory of decline.

A nonlinear decline has also been reported in elderly people developing Alzheimer's disease, where a sharp "inflection point" seems to exist, after which the rate of cognitive decline starts to increase.⁴ To our knowledge, the trajectory of cognitive decline in PD has not yet been explored in detail. Such information is important to predict clinical course in individual patients and to inform future clinical trials. Therefore, by reanalyzing data from a long-term community-based prevalence cohort, we wanted to test the hypothesis that global cognitive decline is not linear in PD, and to explore whether an inflection point for global cognitive decline can be identified. In addition, as there is evidence of marked heterogeneity in the time from onset of PD to dementia,⁵ we wanted to investigate how the time from onset PD to such an inflection point would vary between patients.

Patients and Methods

Patients were drawn from a population-based prevalence study conducted in Rogaland County, Western Norway, in 1993. Details on patient recruitment have been previously reported.⁶ In brief, from a population base of 220,000, ~400 subjects were identified through search of hospital files and information obtained from all available sources in the community and evaluated clinically by experienced movement disorders specialists. Two hundred forty-five of the subjects were diagnosed with PD according to research criteria, and 238 (51% women) fulfilled the PD diagnosis at subsequent assessments and consented to participate in a prospective longitudinal clinical observation study of PD. A subgroup of these patients has come to autopsy and all had their clinical PD diagnosis confirmed neuropathologically.⁷

The cohort was followed prospectively for up to 12 years. Patients were examined at baseline and at follow-up assessments after 4 and 8 years, and thereafter annually. Subjects not able to be transported to the outpatient clinic were examined at their homes or nursing homes. At each visit, standardized rating scales of motor, psychiatric, and cognitive symptoms were administered, as previously described.³ At all visits, global cognition was assessed using the mini-mental state examination (MMSE),⁸ which was used as the measure of global cognitive function in this study. Although this scale was designed to measure cognition in Alzheimer's disease and is not the best method to assess cognitive impairment in PD,⁹ it has shown to be sensitive to decline in PD.³

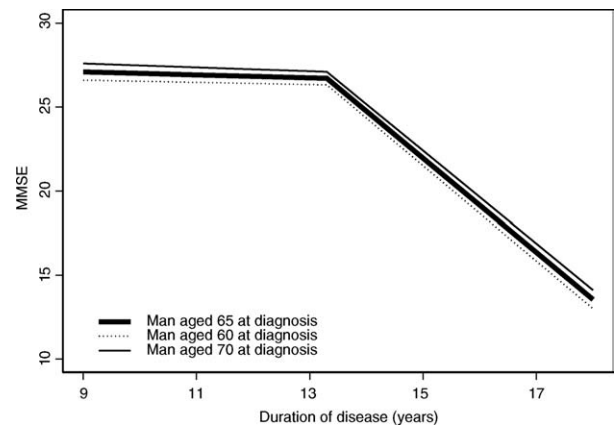


FIG. 1. Estimated mean MMSE trajectory.

Ethics

The study was approved by the Regional Committee for Medical Research Ethics, Western Norway, and patients provided written informed consent.

Statistics

As the aim of our investigation was to examine the hypothesis of nonlinear change in cognition in PD, we fitted a series of random effects models in which MMSE scores were modeled as a function of duration of disease. First, we fitted a model in which MMSE scores were described following a linear trajectory; second, we fitted a model following an accelerating trajectory. Third, we considered a model consisting of two linear phases that meet at a change point. This change point indicates the onset of change in rate of decline. By modeling the change point as a random effect, we obtained an estimate of a change point for a reference individual [man diagnosed with PD at age 65, with 95% credible interval (CI)] in addition to estimates of the change point of each individual in the sample examined. Further estimates of interest obtained from the model describe cognitive performance at 9 years after PD diagnosis and annual rate of decline before and after the onset of change in rate of decline. All models were adjusted for possible differences by gender and age at PD diagnosis. Models were fitted in a Bayesian framework using WinBUGS¹⁰ assuming a hierarchical structure. WinBUGS uses Markov Chain Monte Carlo methods to produce estimates of model parameters. Vague prior distributions were considered. To assess the dependence of results to the choice of prior distributions, sensitivity analyses were conducted varying the prior distributions considered in the model. Results from these sensitivity analyses confirmed the robustness of results reported. Missing data were assumed to be missing at random.

Models were compared using the deviance information criteria (DIC),¹² which is a generalization of Akaike's information criteria.¹³ Models with lower DIC are best supported by the data.

TABLE 1. Estimates of the MMSE scores and rate of decline according to age and gender using the random change point model

Fixed effects	Mean (SD)	95% Credible interval
Cognitive scores 9 yr after diagnosis	27.1 (0.03)	26.4, 27.8
Gender (0 = M, 1 = F)	0.4 (0.44)	-0.4, 1.3
Age at diagnosis	-0.1 (0.03)	-0.2, -0.09
Annual rate of decline before change point	-0.09 (0.07)	-0.2, -0.05
Gender (0 = M, 1 = F)	0.04 (0.09)	-0.1, 0.2
Age at diagnosis	0.005 (0.005)	-0.004, 0.02
Annual rate of decline after change point	-2.8 (0.3)	-3.4, -2.3
Gender (0 = M, 1 = F)	0.09 (0.3)	-0.6, 0.8
Age at diagnosis	-0.07 (0.01)	-0.1, -0.04
Change point	13.3 (0.8)	11.8, 13.6

Results

At baseline, mean (SD) age was 65.0(9.8), duration of disease 9.2(5.7), MMSE score 24.7(6.0), UPDRS III 28.5(15.8), and Hoehn & Yahr stage was 2.9(1.1) (range 1–5). After 4 years, 128 completed MMSE and 79 after 8 years. In further waves conducted annually, 37, 30, and 26 patients completed MMSE. The vast majority of dropouts were due to death and some due to inability to perform the MMSE, whereas less than 10% were due to refusals. At each wave, individuals who dropped out of the study were older at diagnosis than those who remained in the study (t tests, $P < 0.001$). No differences were found in age at diagnosis between dropouts and nondropouts (t tests, $P > 0.05$).

DIC values from the linear, quadratic, and random change point models were 3337.52, 3072.47, and 2681.04, respectively. These values indicate that the random change point model fitted the data best and supported the hypothesis that the decline was nonlinear (Fig. 1). Results from the random change point model fitted indicate that after the diagnosis of PD, a period with a slow rate of decline [0.09 (SD = 0.07)] points per year was observed (Table 1). This initial stable period was followed by a period with a much more rapid decline, -2.8 (SD = 0.3) points per year. The decline was similar in men and women. An inflection point was found, which was estimated to occur 13.3 years (95% CI 11.8, 13.6) after the diagnosis of PD, with a wide interindividual variation (Fig. 2). Results were similar when reanalyzed without patients with dementia at baseline (results not shown).

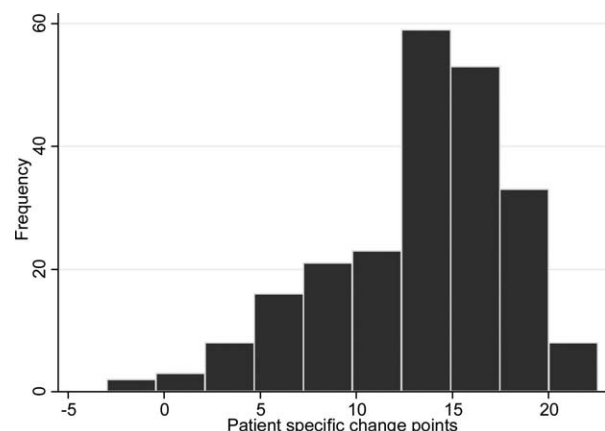
Discussion

We studied the trajectory of global cognitive decline as measured with a global cognitive rating scale in a prevalence sample of PD patients. The cohort was followed for up to 12 years after the baseline assessment.

We observed that the rate of global cognitive decline was nonlinear. Following a relatively stable period, an inflection point was identified, after which the rate of decline gained momentum with an annual decline of 2.8 points. Thus, the main hypothesis that global cognitive decline in PD is not linear was confirmed, and consistent with a cross-sectional study suggesting that transition to dementia in PD is due to the addition of cortical to subcortical deficits.¹⁴ The inflection point was estimated to occur 13 years after diagnosis of PD. There were wide interindividual variations in the duration to the inflection point.

One of the limitations of our study is that the long interval between testing during the first 8 years lead to a high dropout. Also, as only global cognition was measured, differential trajectory of decline in various cognitive domains, as previously reported in AD⁴ could not be assessed. As MMSE is less sensitive in the earliest disease stages, this might have contributed to the accelerated decline. Another limitation is that this was a prevalence sample, i.e., baseline assessment was performed mean 9 years after onset/diagnosis of PD, with wide variations, i.e., some patients were included immediately after onset of disease, whereas others had had PD for up to 20 years at inclusion. One effect of this design was that cognitive performance was variable at inclusion, with some patients being demented already at inclusion. However, the majority of those with dementia at baseline did not survive to the first follow-up assessment, but some patients might already have passed the inflection point at inclusion. It is nevertheless possible that an increased rate of decline at more cognitively impaired stages, as has been shown in Alzheimer's disease¹⁵ might have contributed to our findings.

In addition, we have previously demonstrated that the time to dementia diagnosis varies remarkably in PD, some developing dementia with the first 2 to 3 years after diagnosis, whereas others remain nondemented for decades before developing dementia.⁵ Our data suggest that this variation is primarily due to the

**FIG. 2.** Histogram of estimated patient-specific change points.

time to the inflection point, which is followed by a rapid decline rather than a variation in the slope of decline. Further methodological work is required to allow for the investigation of factors that may delay the onset of a change in rate of decline.

Our findings of nonlinear cognitive decline have relevance for patient information about prognosis, as well as for clinical trials attempting to delay the onset of dementia or reduce the rate of cognitive decline. Future studies should also include patients at time of diagnosis, and tests assessing various cognitive domains should be administered, to explore whether such an inflection point occurs for all domains and at the same or different time points. ■

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Severe Dystonic Encephalopathy Without Hyperphenylalaninemia Associated with an 18-Bp Deletion Within the Proximal *GCH1* Promoter

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ABSTRACT

In a recent *GCH1* mutation screen, an 18-bp deletion was identified within the proximal promoter in two patients with early-onset Parkinson's disease. The mutation removes cAMP response element critical for adequate GTP cyclohydrolase I activity in selected cell types, including dopaminergic neurons, but its biological significance was unclear as it was also detected in one control individual. We present an 11-year-old boy with infantile-onset severe dystonic encephalopathy without hyperphenylalaninemia whom we found compound heterozygous for the same promoter *GCH1* deletion and another common missense mutation associated with classical dopa-responsive dystonia. Extensive diagnostic work up excluded other causes of dystonia, and comprehensive mutation scan did not reveal any additional *GCH1* sequence variations, supporting the association between the promoter deletion and disease phenotype. ©2010 *Movement Disorder Society*

Key Words: GTP cyclohydrolase 1 deficiency; dopa-responsive dystonia; *GCH1*; promoter; dystonic encephalopathy

Introduction

GTP cyclohydrolase I (*GCH1*; EC 3.5.4.16) catalyzes the first step in the biosynthesis of tetrahydrobiopterin (BH₄), a cofactor of phenylalanine, tyrosine

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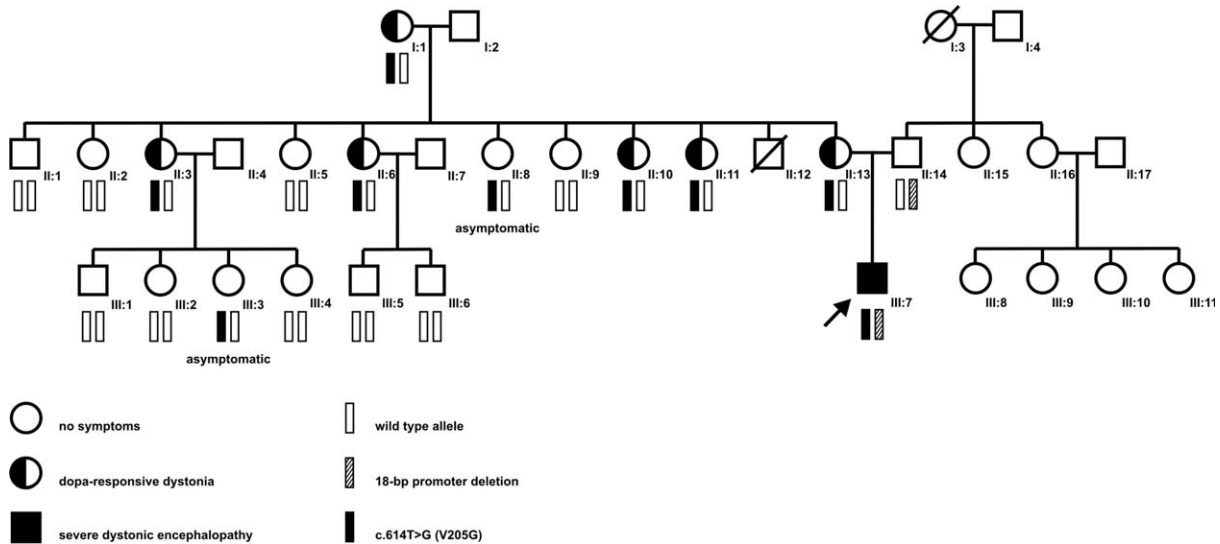


FIG. 1. The family pedigree.

and tryptophan hydroxylases, all NO synthase isoforms, and glyceryl-ether mono-oxygenase.¹ *GCH1* deficiency manifests with a spectrum of neurological deficits,² including dystonic features attributable to predominant dopamine depletion in the striatum.³ The clinical presentation depends upon the gene dosage (one vs two mutant alleles), residual normal function and possible dominant negative effects of the mutated allele, and potential modifier factors (e.g., estradiol-dependent transcription enhancement⁴). The most common syndrome is that of dopa-responsive dystonia (DRD; MIM# 128230), an autosomal dominant localized dystonia characterized by excellent response to low-dose levodopa.⁵ Mutations of both *GCH1* alleles produce a spectrum of autosomal recessive dystonic encephalopathies with or without hyperphenylalaninemia (HPA).^{6,7} Comprehensive mutation scans in *GCH1*-deficient patients revealed more than 100 distinct mutations, none of which localized to the *GCH1* promoter.^{8,9}

Due to some overlap between DRD and early-onset Parkinson's disease (EOPD) a large cohort of EOPD patients were screened for *GCH1* mutations with generally negative results except for a heterozygous 18-bp proximal promoter deletion detected in two EOPD *PRKN*-negative patients without dystonia.¹⁰ Although the same deletion was found in one control individual,¹⁰ it may have functional consequences, as it removes cAMP response element (CRE) required for cAMP-mediated transcription critical for *GCH1* activity in some cell types, including midbrain and hypothalamic dopamine neurons.¹¹

The clinical relevance of this mutation is highlighted by our case report of an 11-year-old boy with severe dystonic encephalopathy without HPA whom we found compound heterozygous for the 18-bp promoter

deletion and another common *GCH1* missense mutation.

Patients

Case History

The 11-year-old proband (III:7 in pedigree in Fig. 1), born full-term after a noneventful pregnancy and delivery is the only child of nonconsanguineous parents of Polish origin. In the first few months of life he was noted for delayed psychomotor development with axial hypotonia and dystonic posturing of the extremities. He had poor suck and difficulty swallowing, leading to relative failure to thrive. He has never been able to walk, sit unsupported, roll over, or voluntarily control sphincter function. Severe dystonia of the trunk and limbs has dominated the clinical course beyond the infantile period. Additionally, he has developed brisk deep tendon reflexes in both upper and lower extremities, bilateral striatal toe imitating extensor plantar response but without toe fanning, thoracic scoliosis, and shortening of the right leg. Prominent jaw dystonia has made him unable to fully close his mouth and produce normal speech (he only can utter a few poorly intelligible words). His parents claim he can comprehend language well, but formal neuropsychological testing is difficult to perform due to profound movement impairment.

Magnetic resonance imaging (MRI) of the brain, nerve conduction velocities and electromyography, and ophthalmological examination were normal. Laboratory studies, including blood count, routine biochemistry, full plasma amino acid profiles, serum lactate, ammonia, copper and ceruloplasmin, serum tandem mass spectrometry, urine gas chromatography-

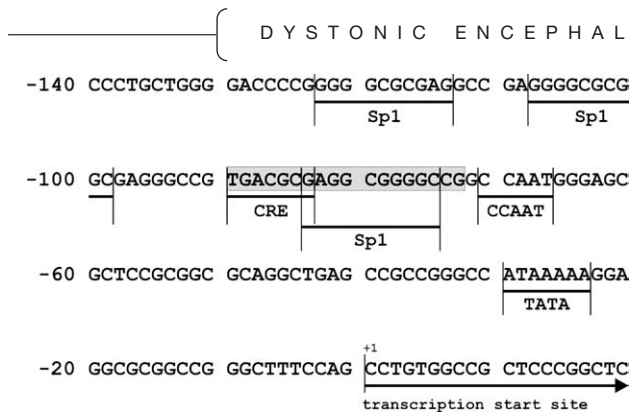


FIG. 2. The sequence of the *GCH1* proximal promoter with key transcriptional motifs. The deleted nucleotides are marked with shading. CCAAT = CCAAT box; CRE = cAMP response element; Sp1 = binding site of transcription factor Sp1; TATA = TATA box.

mass spectrometry, serum biotinidase, peripheral leukocyte lysosomal enzymes, and transferrin isoforms were all negative or normal. Specifically, plasma phenylalanine levels were consistently well within a reference range (26–98 $\mu\text{mol/L}$). Lumbar puncture was not performed due to lack of parental consent. Karyotype was normal male (46,XY). The *TOR1A* assay excluded a common c.904-906delGAG mutation responsible for early-onset primary dystonia (DYT1).

Levodopa therapy (50 mg/day), initiated based on the results of the genetic testing (see below), has led to moderate but clinically significant improvement. He can close his mouth, chew, and swallow more effectively, which has led to considerably better food intake, and has started moving his limbs more vigorously. However, further dose increase has been prevented by emerging dyskinesia.

Family History

The patient's mother (II:13) had an insidious onset of walking difficulty in childhood with slowly progressive dystonic posturing and hyperreflexia of the lower limbs, bilateral pes cavus, and mild postural hand tremor. The signs and symptoms were asymmetrical with left predominance and relative shortening of the left leg. MRI of the brain, ophthalmological examination, and neuropsychological assessment were all normal. She showed an excellent response to low-dose (200 mg/day) levodopa therapy with complete resolution of neurological deficits. Her mother (I:1) and four of her sisters (II:3, II:6, II:10, and II:11) were similarly diagnosed with DRD and successfully treated with levodopa (100–300 mg/day).

The patient's father (II:14), aged 35, has a nonprogressive spastic right hemiparesis following infantile tuberculous meningoencephalitis, but no indication of dystonia or parkinsonism. His mother (I:3) died in older age without signs or symptoms of a neurological disease. His father (I:4), two younger sisters (II:15 and

II:16), and four daughters (III:8–11) of one of them are reportedly healthy, though have not been available for clinical evaluation or genetic testing.

Results

The *GCH1* gene was analyzed in the proband, his parents, and his mother's relatives (Fig. 1) with bidirectional sequencing. The analysis included all coding exons with flanking intronic fragments, two alternative splicing isoforms of the final coding exon, 5'UTR, and circa 500 bp of the proximal promoter.

The proband (III:7) was found compound heterozygous for the missense c.614T>G (V205G) mutation, inherited from his mother (II:13), and 18-bp promoter deletion (Fig. 2), transmitted by the father (II:14). All symptomatic members of the mother's family were heterozygous carriers of the same V205G substitution. We also detected it in two asymptomatic individuals (one of the mother's sisters, and a daughter of one symptomatic sister). The V205G mutation was previously associated with DRD,¹² and a lack of clinical impairment in the two asymptomatic carriers may reflect either a reduced penetrance or presymptomatic phase.

We sequenced the proximal *GCH1* promoter region, encompassing the site of the 18-bp deletion, in 100 healthy, nonconsanguineous control individuals (or 200 chromosomes), representing general adult population of Southern Poland, and did not find any sequence variations.

Discussion

In this report, we present evidence for the clinical relevance of the 18-bp *GCH1* promoter deletion in humans. This mutation has recently been identified in a heterozygous state in two patients with EOPD without dystonia, but also in one control individual, which made the association unclear.¹⁰ The deletion overlaps the CRE and Sp1 consensus motifs (Fig. 2). CRE and adjacent CCAAT-box mediate the effects of cAMP on *GCH1* expression, enabling the interaction between CRE binding protein, CCAAT-enhancer binding protein beta, and nuclear factor-Y.¹³ The *GCH1* expression is regulated in a tissue-specific manner with cAMP being an enhancer in a limited number of cell types, including midbrain and hypothalamic dopamine neurons,¹¹ adrenal medullary cells,¹⁴ and mesangial cells.¹⁵

The proband, compound heterozygous for the promoter deletion and common missense mutation, presented with a phenotype largely similar to other reported cases of severe *GCH1* deficiency resulting from mutations in both alleles.^{6,7} The promoter mutation very likely reduces the rate of *GCH1* expression in a couple of tissues critically dependent on cAMP, such as dopaminergic neurons, but it does not affect the protein product itself, and should not lead to enzyme

deficiency in other cell types, explaining the lack of HPA. Moreover, the accompanying V205G missense mutation may confer a lower HPA risk, which is suggested by the lack of HPA in a boy with a homozygous mutation in the adjacent codon (V206A).⁷

The important limitation of our report is that we were unable to measure pterin levels in the cerebrospinal fluid or *GCH1* activity in the patient's cells. Such tests are highly specialized and not readily available; besides, we had no parental consent for lumbar puncture. It should be stressed, however, that the transcriptional insufficiency caused by the loss of CRE in the promoter is likely limited to few cell populations, which are normally inaccessible for testing in a living person. Nevertheless, the evidence for the pathogenic nature of the 18-bp promoter deletion is compelling: consistent clinical presentation, mutation segregation with the autosomal recessive phenotype, no additional *GCH1* sequence variants, extensive exclusion of alternative etiologies, and clear response to levodopa in combination with the previous *in vitro* data on the physiology of the *GCH1* proximal promoter.¹³

The improvement following the administration of levodopa was significant but partial, which could be attributed to at least three factors: (1) we have not been able to start the patient on concomitant BH₄ therapy (as recommended by several authors)^{7,14} because of high cost and administrative barriers (lack of drug registration for this indication), (2) we could not use levodopa in doses higher than 50 mg/day due to poorly tolerated dyskinesia, and (3) the longstanding dopaminergic deficit in the developing brain may have led to persistent and potentially irreversible neuronal dysfunction.

Although this case suggests the 18-bp promoter deletion may become symptomatic in combination with a missense mutation of the other allele, it remains to be determined if it can also be pathogenic in heterozygous carriers, and, specifically, whether it can cause Parkinson's disease (PD). At age 35, the patient's father has no dystonic or parkinsonian features, and nonprogressive hemiparesis can well be explained by infantile neuroinfection. However, we monitor his neurological status continuously, as the two reported men with EOPD and the same mutation were aged 33 and 36 at onset.¹⁰

The mechanism whereby the *GCH1* promoter mutation could increase the PD risk or lead to the development of PD-like phenotypes will remain a matter of speculation until functional studies are performed on promoter knockout cell line and animal models. It has recently been hypothesized that PD and DRD could be differentiated by complementary dopamine deficiency patterns in the striatum with predominant dopamine loss in the matrix and striosome compartments in PD and DRD, respectively.¹⁶ In DRD, neuropathological studies¹⁷ have revealed loss of tyrosine hydroxylase in nigrostriatal neurons but, unlike in PD, without signs of their degeneration. The *GCH1*

promoter mutation may result in relative dopamine deficiency in certain neuronal subpopulations, and thus create environment for development of parkinsonism in response to secondary insults. ■

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A Pilot Open-Label Trial of Zonisamide in Unverricht-Lundborg Disease

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ABSTRACT

Action myoclonus frequently remains the primary cause of disability in Unverricht-Lundborg disease (EPM1) patients. Pharmacological treatment of myoclonus in these patients continues to be challenging; indeed conventional AEDs may be poorly effective in monotherapy or even in combination. We carried out a pilot, open-label trial of add-on zonisamide (ZNS) in patients with EPM1. Twelve EPM1 patients with epilepsy and action myoclonus were included in the study. Oral ZNS was gradually titrated until the target dose of 6 mg/Kg/day. Unified Myoclonus Rating Scale was obtained in each subject before and after ZNS add-on. A significant reduction of myoclonus severity was reached after ZNS introduction. ZNS was generally well tolerated and only two patients withdrew due to mild adverse effects. Our trial suggests that ZNS may be a valuable therapeutic option in EPM1 patients. © 2010 Movement Disorder Society

Key Words: zonisamide; Unverricht-Lundborg disease; epilepsy; myoclonus

Action myoclonus may be due to multiple etiological factors enhancing motor cortex excitability. It is often intractable and related to severe functional disability.¹ Unverricht-Lundborg disease (EPM1) is the most com-

mon form of progressive myoclonus epilepsies. Symptoms stabilize in adulthood but action myoclonus frequently remains the primary cause of disability in affected.²

Zonisamide (ZNS), a new-generation antiepileptic drug (AED) with broad spectrum and multiple potential mechanisms, is licensed for adjunctive treatment of partial seizures in adults.³ Only few reports suggest the potential efficacy of ZNS in patients with myoclonus, but systematic studies on a specific type of progressive myoclonus epilepsy (PME) have not been performed yet.⁴⁻⁶

We carried out a pragmatic, pilot, open-label trial to evaluate the tolerability and the antimyoclonic efficacy of ZNS in patients with EPM1.

Patients and Methods

We included 12 patients with homozygous expansion of CSTB promoter and chronic (without remission for at least 1 year), refractory (partially responsive or unresponsive to at least two conventional antimyoclonic AEDs) action myoclonus, confirmed by electrophysiological investigations (Table 1). All patients were asked to sign a written informed consent, and the local ethical committees approved the study. Twelve patients (7 men) aged from 22 to 70 years (mean: 42.6 ± 13.6) were recruited. Duration of myoclonus ranged from 12 to 60 years (mean: 30.8 ± 12.3). Six patients also showed negative myoclonus. All patients but one was on polytherapy (Table 1). ZNS was orally administered at a starting dose of 25 mg twice/day for 1 week and up-titrated to 50 mg twice/day for the next 2 weeks, followed by increments of 50 mg twice/day each week until to the target dose of ~6 mg/Kg/day. Titration phase ranged 4 to 6 weeks. To assess myoclonus severity, all patients underwent Unified Myoclonus Rating Scale (UMRS)⁷ before ZNS add-on and 2 weeks after the end of titration phase. Dosage of concomitant AEDs remained constant for at least 3 months before the beginning to the end of the trial and their plasma levels were monitored monthly. Mean pretreatment and post-treatment scores for each UMRS section were compared. Statistical analyses were carried out by using χ^2 -test, Wilcoxon matched-pairs test, and Spearman test.

Results

All patients completed the trial. The duration of the study ranged from 6 to 8 weeks. The ZNS reached dose was between 300 and 500 mg/day (mean: 391.6 ± 66.8). ZNS add-on was associated with clinical improvement of myoclonus and lowering of mean scores on all UMRS sections except Section 6 (physician's assessment of disability) ($P < 0.05$; Fig. 1). No correlation between duration of disease and ZNS

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TABLE 1. Clinical features of the 12 EPM1 patients

Patient/gender/age (yr)	Disease duration (yr)	Concomitant AEDs (mg/day)	ZNS dose (mg/day)	Trial duration (wk)	Adverse effects
1/F/25	24	VPA 1750	300	6	Paresthesias, irritability
2/M/49	38	VPA 1000, CLZ 6, ACZ 250	400	7	–
3/F/37	29	VPA 1500, LEV 3500, PB 100	500	8	–
4/F/43	31	LEV 1000, VPA 1000	400	7	–
5/M/39	30	VPA 750, ACZ 250, PB 100, CLZ 4, LEV 4000	400	7	–
6/M/52	31	VPA 2000, CLZ, PB	400	7	–
7/F/52	33	VPA 1500, CLZ, PB	400	7	–
8/M/27	12	VPA 1000	400	7	–
9/M/48	33	LEV 2000, CLZ 5, VPA 1200, PRM 250	400	7	–
10/M/48	36	VPA 1800, LEV 3500, PB 100, CLZ 6	300	6	Drowsiness
11/F/22	13	LEV 4500, CLZ 18, CLZ 6, PB 100	300	6	Drowsiness
12/M/70	60	CLZ 6, PB100	500	8	–

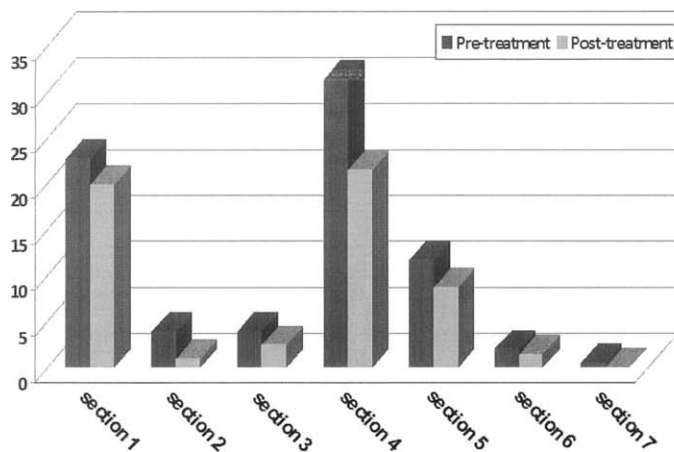
VPA, valproate; CLZ, clonazepam; ACZ, acetazolamide; LEV, levetiracetam; PB, phenobarbital; PRM, primidone.

response was found. UMRS scores after ZNS add-on did not statistically differ in patients with and without levetiracetam (LEV). No change in the plasma level of concomitant AEDs occurred during the trial. ZNS was well tolerated by all the subjects except from 2 patients who exhibited drowsiness (10, 11) and another one (1) who reported paresthesias and irritability (Table 1). However, these adverse effects were of mild or moderate severity and discontinuation of the drug is not needed. Two subjects (2,3) withdrew ZNS a few days after final UMRS evaluation due to loss of efficacy. The remaining 10 subjects were clinically reevaluated at ~3-months intervals (mean follow-up: 23 ± 9.3 months; range 6–32) and all are still on ZNS, exhibiting sustained antimyoclonic effect.

Discussion

So far, therapy of epilepsy and action myoclonus in EPM1 patients continues to be challenging. Conventional AEDs may be poorly effective in monotherapy or even in combination.¹ Systematic controlled studies

about the effects of new AEDs on myoclonus in PMEs patients are lacking. An open-label study of 13 EPM1 patients demonstrated the effectiveness of LEV in reducing myoclonus severity, especially in younger subjects.⁸ Topiramate may also be useful but its effectiveness is not permanent possibly due to the development of drug tolerance.⁹ Lamotrigine may even aggravate myoclonus in EPM1 patients.¹⁰ The only existing open-label trial on PMEs patients receiving adjunctive ZNS (≤6 mg/kg/day) therapy for 16 weeks showed a 50% and a 75% reduction of “myoclonic seizures” in 36 and 21% patients.⁶ This study included 7 EPM1 patients, but the efficacy of ZNS on this specific group of patients was not reported. In addition, in our study, we used UMRS, the most appropriate statistically validated clinical instrument to assess myoclonus severity.⁷ After the end of trial, mean post-treatment scores were significantly lowered for all UMRS section except for global disability score (Section 6). However, the latter is a subjective measure prone to possible bias in an open-label trial particularly in such severely compromised patients with a



UMRS section	Pre-treatment UMRS score (mean)	Post-treatment UMRS score (mean)	p
section 1	22.75	19.83	0.0269
section 2	3.83	1.00	0.0010
section 3	3.92	2.42	0.0488
section 4	31.25	21.50	0.0210
section 5	11.58	8.75	0.0420
section 6	2.00	1.42	0.0674
section 7	0.50	0.00	0.0312

FIG. 1. Mean UMRS scores before and after the trial compared by using the Wilcoxon signed rank test. ZNS treatment was associated with a significant reduction in the Sections 1–5 of UMRS.

long history of disease. ZNS was generally well tolerated and only 2 patients withdrew at follow-up due to mild adverse effects. Moreover, only 2 patients showed attenuation of ZNS efficacy at follow-up. This is in agreement with the results of a previous work reporting that ZNS may decrease its antimyoclonic efficacy over time due to the natural worsening of the disease or tolerance development.⁵

In a long-term follow-up study, Magaouda et al.² found that EPM1 exhibits a self-limiting progression with symptom stabilization after ~10 years of disease. In our study, we did not find any significant correlation between the duration of disease and ZNS response. Therefore, we can suppose that the loss of ZNS antimyoclonic effect observed in 2 of our EPM1 subjects, may be due to tolerance development rather than the natural worsening of disease. Moreover, ZNS significantly reduced myoclonus severity even in patients were already on LEV.

This study includes the largest series of EPM1 patients on ZNS so far reported. Our data suggest that this drug may be a valuable therapeutic option in these patients. Prospective randomized, double-blind studies are needed to confirm these data. ■

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Oligoclonal Bands in Cerebrospinal Fluid in Patients with Tourette's Syndrome

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ABSTRACT

Since a postinfectious or autoimmune etiology is suggested to be involved in the pathogenesis of Tourette's syndrome (TS), we investigated oligoclonal bands (OB) of immunoglobulin G (IgG) in cerebrospinal fluid (CSF), indicating a humoral immune response in the central nervous system. CSF examinations including isoelectric focusing to analyze the presence of OB were performed in 21 TS patients [17 men/4 women, mean age = 29 ± 12 (SD) years]. Isoelectric focusing showed the presence of positive OB in 6, borderline bands in 2, and serum and CSF bands ("mirrored pattern") in another 2 patients. Clinical data did not correlate with CSF findings. Thus, 38% (8 of 21) of our patients exhibited pathological CSF bands. Since none of them suffered from another disease known to be associated with OB, our results suggest an association with the pathogenesis of TS itself and point to an involvement of immunological mechanisms in TS pathology. © 2010 Movement Disorder Society

Key Words: tics and Tourette's syndrome; neuroimmunology

Tourette's syndrome (TS) is a chronic disorder characterized by multiple, waxing and waning, motor and vocal tics. Genetic as well as environmental factors have been associated with the pathogenesis of TS.^{1–3} Because Sydenham's chorea (SC), an immune-mediated disease caused by group A β-hemolytic streptococci (GABHS), and TS both are characterized by hyperkinetic movements and behavioral alteration and

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TABLE 1. Clinical details of TS patients

No.	Age at LP/sex	Medication	Age of onset	STSS	follow-up (years)
1	46/W	BZD	unknown	4	7
2	9/M	–	6	2	8
3	33/M	NL, CBZ	6	5	
4	41/M	–	4	3	7
5	29/M	NL, SRI	17	2	2
6	20/M	SRI	4	5	8
7	51/M	–	11	3	4
8	17/M	NL, BZD	4	1	
9	20/M	NL	8	2	
10	20/M	NL, BIP, VLP	8	5	14
11	37/M	–	7	5	14
12	24/M	–	5	2	
13	29/M	NL, BZD	3	3	14
14	21/W	NL	9	5	5
15	38/M	SRI	7	3	4
16a	34/W	NL	6	5	
16b	36/W	NL, SRI	6	5	
17	13/M	NL, BZD	9	5	8
18	13/M	NL, BZD	8	5	9
19	32/W	NL	5	5	
20	34/M	NL, SRI	7	4	
21	40/M	–	6	2	

W = woman, M = man, BZD = benzodiazepine, NL = neuroleptics, CBZ = carbamazepine, SRI = serotonin re-uptake inhibitor, VLA = valproate, BIP = biperidene, STSS = Shapiro Tourette syndrome severity scale, No.16a/No.16b = in this woman lumbar puncture was performed twice at a 2-year interval, No.17/No.18 = monozygotic twins.

affect comparable anatomical areas, a common etiology has been suggested in these disorders.⁴ Accordingly, the term *Pediatric autoimmune neuropsychiatric disorder associated with streptococcal infections* (PANDAS) has been suggested for patients suffering from tics or obsessive-compulsive disorder (OCD) in which a GABHS infection preceded symptoms' onset. In PANDAS it is proposed that GABHS antibodies cross-react with basal ganglia antigens and trigger an immunological process.⁴ Although several data support an immunological dysregulation in TS, the etiologic significance of GABHS infection as well as the exact mechanisms remain in doubt.^{5–7} Extensive research on antistreptococcal and antineuronal antibodies has led to different results.^{8–10}

So far, extensive research has focused on serological examinations in TS, but little is known about immunological findings in cerebrospinal fluid (CSF). Detection of oligoclonal bands (OB) of immunoglobulin G (IgG) in CSF by isoelectric focusing provides a sensitive method for qualitative detection of humoral immune response. Whereas OB in CSF (but not in serum) indicate a local antibody synthesis (e.g., in multiple sclerosis or encephalitis), identical (“mirrored”) bands in CSF and serum point to a systemic immune response (e.g., in Guillain-Barré-Syndrome or rheumatologic diseases).¹¹

Here, we report the results of CSF analyses in 21 TS patients as CSF findings seem particularly interesting and might contribute further details of immune dysregulation.

Methods

Patients

Between 1993 and 2004 a lumbar puncture (LP) was performed in 21 patients [17 men, 4 women, mean age = 29 ± 12 (SD) years, range, 9–51] with TS according to DSM-III R criteria attending our Tourette outpatient clinic for a thorough diagnostic assessment. Tic severity according to the Shapiro Tourette Syndrome Severity Scale (STSS)¹² was 3.6 ± 1.4 (range, 1–5). The mean age of tic onset was 7.0 ± 3.1 years (range, 3–17). Further clinical details are summarized in Table 1. Neurological and general examinations, routine blood and urine tests, microbiological and virological investigations in serum and CSF, and cranial MRI were normal in all patients. None of the patients had a history of SC or any other disease suggesting the diagnosis of secondary tics or suffered from a concomitant disease known to be associated with OB. At the time of LP none of the patients suffered from an acute infection. Thirteen of 21 patients underwent follow up after 2 to 14 years (mean, 8.9 ± 4.0). All patients delivered written informed consent before entering the study. Ethics approval had been obtained.

CSF Examinations

CSF examinations were performed at the CSF laboratory, Hannover Medical School, and included cell count and cytology differentials. Total protein was determined with a Coomassie-Blue dye binding method, while albumin and IgG were estimated by nephelometry in paired serum and CSF samples. The state of the blood-CSF-barrier was evaluated by the albumin quotient (albumin[CSF]/albumin[serum]) with age-corrected cut-offs [(age/15)+4)] as well as the hyperbolic formula of Reiber. Quantitative intrathecal IgG production was calculated by the IgG index [(IgG[CSF] × albumin[serum])/(IgG[serum] × albumin[CSF])]. Isoelectric focusing (IEF) was performed on macro polyacrylamide gels with automated silver staining to analyze the presence of OB as described previously.^{11,13} All IEF analyses were performed by the same person (UW). Positive OB was defined as ≥ 4 bands, borderline bands as 2–3, and negative bands as < 1 band. IEF was done simultaneously in CSF and serum samples to differentiate identical (“mirrored”) patterns of bands in both serum and CSF from oligoclonal IgG responses in CSF.

Statistical Analysis

The significance of differences in tic severity (STSS), age of tic onset, and medication between patients with and without OB was assessed using independent samples *t* test. A value of $P < 0.05$ was regarded as statistically significant.

TABLE 2. CSF analyses (cell count, cytology, total protein, albumin quotient, IgG index, OB) and antistreptolysin O (ASO) titers in TS patients (n = 21)

No.	Cell count/mm ³ (normal, 0–4)	Cytological analysis	Total protein [g/l ⁻¹]; (normal <0.50)	Alb (CSF)/Alb (serum) (normal, <(age/15) +4)	IgG Index (normal, <0.7)	OB	ASO [IE/ml ⁻¹]; (normal, <200)
1	1.3	normal	0.297	3.6	0.535	0	–
2	2.7	normal	0.309	3.3	0.471	+CSF	–
3	1.7	normal	0.393	4.1	0.516	(+)CSF	–
4	4.7	normal	0.402	4.6	0.420	(+)CSF	84
5	1.3	normal	0.514	5.1	0.449	0	–
6	1.0	normal	0.307	2.7	0.472	0	–
7	1.0	normal	0.396	4.4	0.495	0	–
8	1.7	normal	0.521	5.7	0.521	0	–
9	3.7	normal	0.506	6.2	0.542	0	–
10	2.3	normal	0.291	2.9	0.492	0	990
11	6.3	normal	0.512	7.6	0.482	identical	–
12	5.0	normal	0.342	3.4	0.502	+CSF	–
13	2.7	normal	0.485	5.9	0.484	0	106
14	3.3	normal	0.304	3.9	0.434	+CSF	214
15	1.7	normal	0.443	5.6	0.455	identical	94
16a	2.7	normal	0.323	3.2	0.546	(+)CSF plus identical	–
16b	2.0	normal	0.352	3.2	0.490	+CSF plus identical	–
17	1.7	normal	0.292	2.4	0.480	0	–
18	2.0	normal	0.276	2.3	0.546	+CSF	–
19	0.7	normal	0.325	4.3	0.431	0	410
20	0.7	normal	0.430	6.0	0.513	0	61
21	2.3	normal	0.550	6.0	0.609	+CSF	–

Alb = albumin, +CSF = CSF-restricted OB, (+)CSF = borderline bands in CSF, identical = identical (mirrored) bands in serum and CSF, IgG = immunoglobulin G, OB = oligoclonal bands, IEF = isoelectric focusing, ASO = antistreptolysin O.

Results

Three patients demonstrated a marginal pleocytosis, whereas cytology differentials were normal in all patients (Table 2). Total protein was slightly elevated in 5 patients. With age-corrected cut-offs 3 patients displayed minor alterations of the albumin quotient indicating a minimal disturbance of the blood-CSF-barrier. The IgG index as well as the hyperbolic formula of Reiber as a measure of quantitative intrathecal IgG synthesis was normal in all patients. In contrast, the more sensitive qualitative method of oligoclonal IgG detection by IEF showed positive OB in 6 patients, borderline bands in 2, and a mirrored pattern in serum and CSF in 2 patients (Table 2).

When correlating clinical data to CSF findings no association with age, age at tic onset, sex, medication, and tic severity (STSS) could be demonstrated.

In 1 patient (no. 16) LP was done twice, at the age of 34, respectively 36 years. Whereas the first examination exhibited borderline bands plus identical bands in serum and CSF, the second analysis demonstrated positive OB plus identical bands.

CSF analyses in a pair of identical twins (13-year-old boys) showed positive OB in one (no. 18) but no intrathecal OB synthesis in the other twin (no. 17), (Table 2). The twin exhibiting positive OB had an earlier age of onset.

In 7 patients antistreptolysin O (ASO)-titers had been investigated in serum samples at time of LP and were significantly increased in 3 patients. Two of them (no. 10, 19) showed normal CSF findings; only one exhibited positive OB (no. 14). Patients exhibiting normal ASO-titers showed either borderline bands (no. 4), a mirrored pattern (no. 15), or a normal CSF analysis (no. 13, 20), (Table 2).

Discussion

This is the first study investigating OB in CSF in patients suffering from TS. Whereas cell count, cytology examination, albumin quotient, and IgG index showed no significant alterations, we found positive (≥ 4) or borderline (2–3) bands in 8 of 21 (38%) patients and a mirrored pattern in another 2 patients. Neither additional investigations nor the past medical history or follow-up investigations (over a period of 2–14 years) suggested the coexistence of other diseases known to be associated with OB in one of these patients. Since OB provide evidence for the occurrence of a humoral immune response, our results strongly point to an autoimmune process in at least a subgroup of TS patients. Moreover, our data suggest that an intrathecal as well as a systemic immune response may occur in TS, even in a single patient. It can be ruled out that CSF bands are without any significance

because in healthy control subjects they occur only in a minor degree of ~3%.¹⁴

Follow-up investigation in 1 patient showed reproducible intrathecal IgG synthesis and, therefore, provides evidence that OB might be a permanent rather than a temporary phenomenon in TS.

Remarkably, CSF analyses in a pair of identical twins yielded different results with positive OB in one, but no CSF abnormalities in the other twin. These findings suggest that OB are due to environmental and not to genetic factors. It is remarkable that the twin exhibiting OB had an earlier onset of tics (8 years) compared to his brother (9 years) suggesting that immunologic factors might contribute to TS as an epigenetic factor mediating tic onset.

Comparable to our data, Church et al.,¹⁵ demonstrated oligoclonal IgG responses in 2 of 13 patients (= 15%) with acute SC. This relatively small number of positive responses—despite proven underlying immune mechanism—has been explained by the small sample size and the fact that inflammation in SC is restricted to a focal brain region.¹⁵

Although ASO-titers had been analyzed in only 7 of our patients at the time of lumbar puncture, our data do not suggest an association between OB and ASO-titers. This observation is in line with a report by Kiesling et al.¹⁶ They described a patient suffering from tics and comorbid attention deficit hyperactivity disorder (ADHD) who exhibited positive streptococcal titers and elevated antineuronal antibodies in CSF and serum, but no intrathecal OB synthesis.

Some limitations of our study have to be addressed: first, the sample size is relatively small. However, to the best of our knowledge, this is the first report of OB in TS. Second, follow-up LP was performed in only 1 patient. Third, frequency of OB depends heavily on the chosen limit of bands: according to an international consensus, two bands in CSF are sufficient to assume local IgG synthesis; in our laboratory two to three bands are rated as borderline, and four or more as positive. Thus, high sensitivity and specificity are retained; however, a smaller number of positive results are obtained. Fourth, ASO-titers were measured in only one third of our patients and merely once at time of lumbar puncture. Therefore an association cannot entirely be excluded.

In conclusion, our results demonstrate that oligoclonal CSF bands are a common feature in TS. Our data provide evidence that the intrathecal synthesis of OB might be a permanent phenomenon caused by environmental rather than genetic factors. It remains unclear whether autoimmune mechanisms may contribute to TS pathology (at least in a subgroup of

patients) or represent an epiphenomenon to another—so far unknown—etiologic factor. ■

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Subtle Rapid Eye Movement Sleep Abnormalities in Presymptomatic Spinocerebellar Ataxia Type 2 Gene Carriers

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ABSTRACT

Rapid eye movement (REM) sleep disorders are commonly associated to patients with spinocerebellar ataxia type 2 (SCA2); however, these abnormalities have not been studied in presymptomatic gene carriers. To determine whether the REM sleep pathology is detectable before clinical manifestation of SCA2 and evaluate it as a preclinical biomarker, we studied 36 presymptomatic SCA2 individuals and 36 controls by video-polysomnography (VPSG) and sleep questionnaires. Presymptomatic subjects showed significant decrease of REM sleep percentage, REMs density, total sleep time, and sleep efficiency. Aging effect on REM sleep percentage was significant in both groups. There was no correlation between cytosine-adenine-guanine (CAG) repeat length and REM sleep. Our findings identified the REM sleep pathology as a prominent herald sign of SCA2, conferring a special importance to VPSG as a sensitive neurophysiological tool to detect early changes associated with SCA2, which contributes to the understanding of disease pathophysiology and the development of therapeutic trials focused on the preclinical disease stage. © 2010 *Movement Disorder Society*

Key Words: spinocerebellar ataxias; REM sleep; sleep disorders; biomarkers

Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominant cerebellar ataxia caused by the expansion of CAG repeats in the *ATXN2* gene, and therefore, the expression of an expanded polyglutamine tract in the ataxin 2 protein is responsible for the neurodegeneration of cerebellum, brainstem, substantia nigra, thalamus, and other structures.¹ The disease is characterized by a progressive cerebellar syndrome,² saccadic slowing,^{3,4} sensory peripheral neuropathy,^{5,6} cognitive dysfunction,⁷ and sleep disorders.^{8,9} Worldwide, the highest prevalence of SCA2 is in the Holguín province (Cuba), where there are more than 400 patients and 2,000 first-degree relatives, resulting from a founder effect.¹⁰

Although sleep disorders have been documented in patients with SCA2,^{8,9} there are no clues whether these abnormalities appear before the ataxia onset. In view of the early pons atrophy¹ in SCA2 and the relevance of this brain area for rapid eye movement (REM) sleep,¹¹ we assessed sleep performance in 36 presymptomatic SCA2 individuals and 36 age- and sex-matched control individuals with the aim to identify sleep abnormalities that could serve as potential preclinical biomarkers.

Subjects and Methods

Subjects

Thirty-six presymptomatic SCA2 gene carriers (11 males and 25 females) with ages ranging from 20 to 59 years [mean: 34.9; standard deviation (SD): 9.9], CAG repeat lengths from 32 to 43 units (mean: 36.5; SD: 2.9), and scores of Scale for the Assessment and Rating of Ataxia (SARA)¹² ranging from 0 to 3 (mean: 0.54; SD: 0.92) were admitted to the Center for the Research and Rehabilitation of Hereditary Ataxias in Holguín for this study. Thirty-six healthy individuals (aged from 18 to 63 years, mean: 36.0; SD: 10.7; and SARA scores from 0 to 1, mean: 0.11; SD: 0.36) were randomly chosen as age- and sex-matched controls. No subject was under any medication that would have interfered with sleep or polysomnographic findings. The study was approved by the Institutional Ethics Committee and was conducted according to the Declaration of Helsinki. Each subject gave written informed consent for the participation in the study.

Sleep Quality Measures

All subjects completed the Pittsburgh Sleep Questionnaire Inventory (PSQI)¹³ to assess sleep quality and the Epworth Sleepiness Scale (ESS)¹⁴ to document daytime sleepiness. Also, the subjects and/or their bed partners were interviewed to diagnose the REM behavior disorder (RBD), restless legs syndrome (RLS), and nocturnal cramps.

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TABLE 1. Video-polysomnographical (VPSG) variables in SCA2 presymptomatics (n = 36) compared with matched controls (n = 36)

PSG sleep variables	SCA2 presymptomatics	Healthy controls	P value
Total record time (TRT) (hr)	7.61 ± 0.69	7.89 ± 0.44	0.096
Sleep period time (SPT) (hr)	7.13 ± 0.61	7.61 ± 0.44	<0.001
Total sleep time (TST) (hr)	6.00 ± 1.02	7.09 ± 0.68	<0.001
Sleep efficiency (%)	81.71 ± 9.14	91.19 ± 3.33	<0.001
Arousal index	13.32 ± 10.18	11.69 ± 9.89	0.119
Sleep onset latency (min)	14.83 ± 11.81	13.5 ± 8.5	0.603
REM sleep onset latency (min)	88.73 ± 31.58	82.83 ± 34.43	0.476
Wake (%)	12.85 ± 5.91	8.58 ± 7.91	0.127
Stage 1 non-REM (%)	8.26 ± 3.99	7.35 ± 4.58	0.371
Stage 2 non-REM (%)	46.12 ± 10.09	47.80 ± 8.17	0.444
Stage 3 non-REM (%)	5.20 ± 1.90	5.21 ± 1.76	0.981
Stage 4 non-REM (%)	10.31 ± 6.32	12.32 ± 6.34	0.186
REM sleep (%)	13.50 ± 4.86	19.82 ± 4.11	<0.001
REMs density (%)	13.57 ± 6.69	29.66 ± 11.92	<0.001

Values are means ± SD. Student's *t* test (two tailed). The bold values indicates mean significant differences between both groups.

Video-Polysomnographic Recordings

All subjects underwent video-polysomnographic (VPSG) recordings for two consecutive nights during their routine sleeping hours. Polysomnography was performed with a digital polygraph (MEDICID V. Neuronica S.A, Havana, Cuba) using an standard montage, which included electroencephalographical (EEG) recording by F3, F4, C3, C4, O1, O2, A1, and A2 electrodes, vertical and horizontal electroculography (EOG), electrocardiography, and electromyography (EMG) of mental and both tibialis anterior muscles. Respiration monitoring included nasal airflow, tracheal microphone, thoracic and abdominal respiratory effort, and oxygen saturation. To exclude first night effects, results of the second night were only analyzed. Sleep was scored according to the criteria of Rechtschaffen and Kales.¹⁵ As presymptomatic SCA2 subjects showed slowing of saccadic movements,⁴ which could affect the REM sleep scoring, we paid special attention to the EMG and EEG features of REM sleep, particularly to the apparition of sawtooth waves as evidence of REM sleep because these EEG waves are closely related with the bursts of REMs. Also, the REMs were visually counted from minimum peak-to-trough amplitudes of 25 μ V. REMs density was calculated as the percentage of 3-second miniepochs of REM sleep with at least one REM.⁸ The tonic and phasic components of REM sleep were scored separately as suggested by Lapierre and Montplaisir.¹⁶ The evaluation of respiratory variables¹⁷ and periodic leg movements (PLMs)¹⁸ were performed using standard criteria.

Statistical Analysis

Statistical analyses were done using the Kolmogorov-Smirnov test to assess the normality of all variables, *t* test to compare the means of clinical and VPSG

variables between subjects and controls, χ^2 test to compare the frequency of REM sleep abnormalities between groups, and correlation analyses to determine factors influencing sleep disorders.

Results

Nineteen (52.8%) of 36 presymptomatic SCA2 individuals and 29 (80.6%) of 36 controls reported good subjective sleep quality estimated by PSQI scale; nevertheless, the remaining 7 controls reached the minimum abnormal score (5 points). PSQI total score ranged from 0 to 14 points in the presymptomatic subjects (mean: 4.94; SD: 3.11) and 0 to 5 in the controls (means: 3.06; SD: 1.45), with significant intergroup differences ($P = 0.002$).

None of the subject had a history of RBD or RLS. One (2.79%) and 10 (27.88%) of 36 presymptomatic SCA2 individuals reported a history of bruxism and nocturnal cramps, respectively. There were no significant differences between both groups for the ESS scores (presymptomatic subjects: mean: 6.58, SD: 3.06; controls: mean: 5.36, SD: 2.49; $P = 0.592$).

The VPSG variables measured in both groups are shown in Table 1. The SCA2 mutation carriers showed significant decrease of REM sleep percentage and REMs density, as well as reduction of sleep period time (SPT), total sleep time (TST), and sleep efficiency when compared with controls. The remaining VPSG variables were normal in presymptomatic subjects.

Nineteen (52.8%) of 36 presymptomatic SCA2 individuals had REM sleep percentages below normal limits (15% of SPT), whereas only 3 controls (8.33%) showed abnormal values of REM sleep amounts ($\chi^2 = 16.76$; $P < 0.0001$). The EEG and EMG features of REM sleep in presymptomatic SCA2 subjects were normal, which allowed us to adequately score the

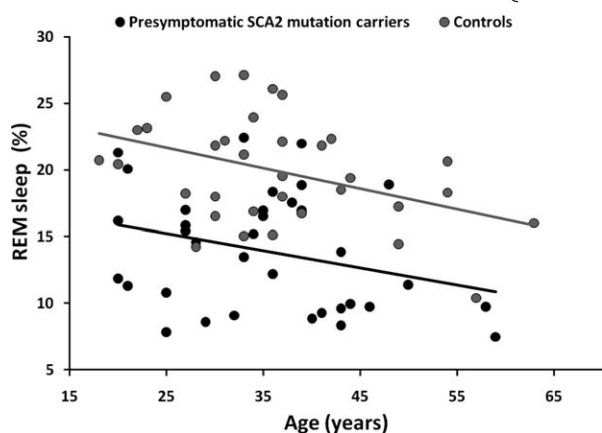


FIG. 1. Effect of age on REM sleep percentages in presymptomatic SCA2 mutation carriers (black circles) and controls (gray circles).

REM sleep epochs in spite of the reduced EOG activity.

In both groups, age showed a significant correlation with REM sleep percentage (presymptomatic subjects: $r = -0.36$; $P = 0.035$; controls: $r = -0.37$; $P = 0.033$; Fig. 1) and TST (presymptomatic subjects: $r = -0.52$; $P = 0.002$; controls: $r = -0.35$; $P = 0.048$), as well as with sleep efficiency ($r = -0.61$; $P < 0.001$) and percentage of wake periods ($r = 0.61$; $P < 0.001$) in presymptomatic subjects. No sleep variable correlated either with PSQI, ESS, or SARA scores in both groups or with CAG repeats in presymptomatic subjects.

PLMs were observed in 1 (2.78%) of 36 presymptomatic SCA2 individuals without intergroup differences. Also, no differences were found on sleep respiratory variables.

Discussion

This is the first study that investigated the sleep disorders in a large cohort of presymptomatic SCA2 mutation carriers and their sex- and age-matched controls by sleep questionnaires and VPSG. Neurological examinations could not differentiate among presymptomatic SCA2 individuals and controls, confirming the preclinical status of the presymptomatic cohort and coinciding with a previous study that found normal subjects showing SARA scores as high as 7.¹²

Subjective sleep quality measured by PSQI and ESS scales revealed no considerable sleep symptomatology in presymptomatic SCA2 carriers, which conferred a special importance to VPSG analysis. Indeed, we have demonstrated that presymptomatic SCA2 mutation carriers show early REM sleep disorders manifested by the decrease of REM sleep percentage and REMs density, which had been previously observed in patients with SCA2.^{8,9} The reduction of REM sleep in the presymptomatic subjects contributed to the decrease of TST and sleep efficiency.

Correlation analyses disclosed a significant effect of age on REM sleep percentage in presymptomatic SCA2 individuals and controls, coinciding with previous reports.^{19,20} In both groups, the correlation coefficients between age and REM sleep were very similar, which suggest that the age-dependent decrease of REM sleep in the presymptomatic cohort is not an important cause of this preclinical sleep disorder.

We considered that the decrease of REM sleep in presymptomatic SCA2 subjects was not caused by detection failures resulting from the reduced eye velocity,⁴ because the REMs were scored from a minimum amplitude of 25 μV and the EEG and EMG features of REM sleep were easily detectable. Also, considering that saccade velocity is strongly influenced by the CAG repeat size,⁴ it was expected that subjects with larger CAG repeats showed the lowest REM sleep amounts; however, no significant correlation between these variables was observed.

The REM sleep abnormalities showed by presymptomatic SCA2 individuals suggest an early dysfunction or neurodegeneration of REM-ON neurons in the pons. Nevertheless, consistent neuropathological and imaging studies in presymptomatic SCA2 subjects are mandatory to confirm the preclinical pontine involvement in SCA2, because pontine dysfunction in preclinical SCA2 has been demonstrated only in one subject by positron emission tomography.²¹

The reductions of REM sleep percentage and REMs density before the ataxia onset have not been reported in other SCA subtypes. Nonetheless, we cannot assure that these sleep abnormalities are SCA2 specific, because the lack of VPSG studies could underestimate these REM sleep disorders in asymptomatic carriers of these mutations. In fact, other REM sleep disorder, such as RBD, was previously observed in three asymptomatic SCA3 mutation carriers.^{22,23}

In conclusion, this work indicates that the decrease of REM sleep percentage and REMs density are prominent sleep disorders that herald the onset of SCA2 in presymptomatic mutation carriers. Therefore, VPSG is a sensitive neurophysiological tool to detect early changes associated with SCA2, and consequently, it may be useful to understand the disease pathophysiology from asymptomatic stages, which acquires a fundamental relevance for the development of future therapeutic options designed to delay the clinical disease onset. ■

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