Systematic Review of Levodopa Dose Equivalency Reporting in Parkinson’s Disease

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Abstract: Interpretation of clinical trials comparing different drug regimens for Parkinson’s disease (PD) is complicated by the different dose intensities used: higher doses of levodopa and, possibly, other drugs produce better symptomatic control but more late complications. To address this problem, conversion factors have been calculated for antiparkinsonian drugs that yield a total daily levodopa equivalent dose (LED). LED estimates vary, so we undertook a systematic review of studies reporting LEDs to provide standardized formulae. Electronic database and hand searching of references identified 56 primary reports of LED estimates. Data were extracted and the mean and modal LEDs calculated. This yielded a standardized LED for each drug, providing a useful tool to express dose intensity of different antiparkinsonian drug regimens on a single scale. Using these conversion formulae to report LEDs would improve the consistency of reporting and assist the interpretation of clinical trials comparing different PD medications. © 2010 Movement Disorder Society

Key words: Parkinson’s disease; treatment; levodopa; levodopa equivalent dose

Levodopa (L-dopa) remains the mainstay of treatment for Parkinson’s disease (PD) over 40 years after its introduction. However, L-dopa therapy is associated with the development of motor complications—particularly at higher doses of L-dopa.1 Current practice, therefore, is to use lower doses of L-dopa to reduce motor complications, albeit at the cost of less effective symptomatic control. Alternative strategies are using monotherapy with a dopamine agonist (DA) or monoamine oxidase type B (MAOB) inhibitor or “adjuvant” therapy combining a DA, MAOB inhibitor, or a catechol-O-methyl transferase (COMT) inhibitor with low-dose L-dopa. Interpretation of randomized comparisons between these treatments is complicated by variability in the dose intensities of different regimens. To facilitate such comparisons, a number of studies have calculated a L-dopa equivalent dose (LED). The overall LED, obtained by adding together the LED for each antiparkinsonian drug, provides an artificial but practically useful summary of the total daily antiparkinsonian medication a patient is receiving. However, numerous different formulae for calculating LEDs have been developed, based on clinical trials results, summaries of product characteristics, and clinical experience, with no standard scheme being recognized.

The present systematic review documents previous LED conversion formulae and combines these providing standard formulae that can be used to compare the dose intensities of different PD treatment regimens.

MATERIALS AND METHODS

We undertook a systematic search of the published literature to identify studies that reported conversion formulae for LED using the broad search terms: “L-dopa,” “equivalent,” and “equivalency.” We searched electronic databases including Medline, Embase, and PubMed from 1980 to 2009. This was complemented with general internet and grey literature searches and hand searching.

From the search results, abstracts were screened for relevance and full papers obtained for relevant articles and for studies where the abstract did not provide sufficient information to determine if the study contained LED information. Studies published in English reporting
an original conversion formula were included. To avoid duplication of conversion formulae, we excluded any based on a previous report.

We defined the LED of a drug as that which produces the same level of symptomatic control as 100 mg of immediate release L-dopa (combined with a dopa-decarboxylase inhibitor). LED data from previous reports were converted to this system and extracted into a master table. The arithmetic mean and mode were calculated for each drug. Anticholinergics were not included in this review in view of their poor effect on akinesia and their low usage in modern practice.

### RESULTS

From the initial searches, 558 articles were identified. Of these, 75 were retrieved for a more detailed evaluation and from these 56 studies from 1990 to 2009 with original conversion formulae were included.

We presented the LED of a drug as that which produces the same level of symptomatic control as 100 mg of immediate release L-dopa (combined with a dopa-decarboxylase inhibitor). LED data from previous reports were converted to this system and extracted into a master table. The arithmetic mean and mode were calculated for each drug. Anticholinergics were not included in this review in view of their poor effect on akinesia and their low usage in modern practice.

### TABLE 1. LEDs for antiparkinsonian drugs

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Drug</th>
<th>Number of studies reporting conversion formulae</th>
<th>Total LED (mg/100 mg L-dopa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Dopa</td>
<td>L-Dopa</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>Controlled release L-dopa</td>
<td>29</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Duodopa</td>
<td>0</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>COMT inhibitors</td>
<td>Entacapone</td>
<td>8</td>
<td>LD × 0.33</td>
</tr>
<tr>
<td>Tolcapone</td>
<td>2</td>
<td>LD × 0.5</td>
<td></td>
</tr>
<tr>
<td>Nonergot-derived dopamine receptor agonists</td>
<td>Pramiptexole</td>
<td>24</td>
<td>1 mg salt</td>
</tr>
<tr>
<td>Ropinirole</td>
<td>34</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Rotigotine</td>
<td>1</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>Piribedil</td>
<td>7</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ergot-derived dopamine receptor agonists</td>
<td>Lisuride</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>30</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Pergolide</td>
<td>28</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Cabergoline</td>
<td>14</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>DHEC</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>MAOB inhibitors</td>
<td>Selegiline 10 mg (oral)</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Selegiline 1.25 mg (sublingual)</td>
<td>0</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Rasagiline</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Amantadine</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Apomorphine (infusion or intermittent injections)</td>
<td>14</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

To calculate the total LED for COMT inhibitors, the total L-dopa (including CR L-dopa if COMT inhibitor given simultaneously) amount should be calculated then multiplied by the appropriate value. For Stalevo, the L-dopa and COMT inhibitor should be split and calculated separately. The British National Formulary states that selegiline 10 mg oral is equivalent 1.25 mg sublingual.

COMT, catechol-O-methyl transferase; DHEC, dihydroergocryptine; MAOB, monoamine oxidase type B.

Reports on the COMT inhibitors entacapone and tolcapone necessarily provided a conversion ratio, rather than a LED, as the mode of action of these agents is to prolong the duration of action of the concomitant L-dopa treatment. Also, there was some variation in how the conversion ratio was reported for COMT inhibitors. For the purpose of this review, we have displayed the conversion as: total L-dopa dose × (total L-dopa dose + (total L-dopa dose × COMT inhibitor value)). Further, some studies failed to differentiate between COMT inhibitors (Supporting Information Table 1). However, most reports suggested that each dose of L-dopa was 33% more effective with entacapone. Only two reports provided a conversion factor for the other licensed COMT inhibitor tolcapone. They quote a conversion factor of 0.25 and 0.33 (Supporting Information Table 1), which seems inconsistent with a recent meta-analysis of entacapone and tolcapone trials that found tolcapone to be markedly more efficacious than entacapone (P < 0.001). L-Dopa dose was 116.5 mg/day (CI –140.6 to −92.3; P < 0.001) lower with tolcapone compared with 41.6 mg/day (CI −51.4 to −31.9; P < 0.001) lower with entacapone. Therefore, a conversion factor of 0.5 seems more appropriate.

The data on LED conversion formulae for DAs are summarized in Supporting Information Table 2. The most consistently reported LED was for bromocriptine,
with more recent reports including the newer nonergot DA’s pramipexole and ropinirole. The range of LEDs for pramipexole was wide—between 0.25 and 1.67 mg being equivalent to 100 mg of L-dopa (Supporting Information Table 2)—with a mean of 1.1 mg and a mode of 1.0 mg. This led to a final LED for pramipexole of 1.0 mg salt. Studies did not always state whether the data reported were in salt or base units, which may have explained the variation. The reported LEDs for ropinirole varied from 2.5 to 6 mg being equivalent to 100 mg of L-dopa (Supporting Information Table 2), with a mean of 4.9 mg and a mode of 5.0 mg, leading to a LED estimate for ropinirole of 5.0 mg. Only one report was available for rotigotine, with a LED of 3.3 mg being equivalent to 100 mg of L-dopa (Supporting Information Table 2) but this was based on a direct head-to-head comparison trial of rotigotine with ropinirole.19

MAOB inhibitors are given at a standard dose—increasing this does not improve efficacy—and the only two reports considering LEDs for the MAOB inhibitors provided a mean and modal LED for the standard dose of 10 mg of oral selegiline to be equivalent to 100 mg of L-dopa (Supporting Information Table 3). No data are available on the sublingual preparation of selegiline, but the British National Formulary considers 1.25 mg sublingual selegiline to be equivalent to 10 mg of oral selegiline. Similarly, there are no data on rasagiline dose equivalence, so we have taken the licensed 1-mg dose as being equivalent to 10 mg of oral selegiline and thus 100 mg of L-dopa.

The LED for apomorphine infusion (Supporting Information Table 3) varied between 1 and 20 mg equivalent to 100 mg of L-dopa (Supporting Information Table 3), with a mean of 9.1 mg and a mode of 10.0 mg.

On the basis of this, we estimate the LED for apomorphine to be 10.0 mg. Some variation in apomorphine LED dose might be due to studies not stating if they were referring to total daily infusion or intermittent injection of apomorphine. Only one study provided an LED for amantadine, but their estimate of 100 mg of amantadine being equivalent to 100 mg of L-dopa (Supporting Information Table 3) seems reasonable.

**DISCUSSION**

To our knowledge, this is the first systematic review of L-dopa dose equivalency studies in PD. For most of the commonly used drugs, sufficient data were available to reach a robust conclusion about an LED. Where data were not available, information from manufacturer’s reports and/or meta-analyses of clinical trials allowed an LED to be developed.

A limitation of this review is the paucity of direct randomized comparisons between different agents. Few of the studies identified developed their formulae from head-to-head comparator trials, or systematic reviews, and instead relied on various assumptions or clinical experience. Therefore, our LED estimates are necessarily approximations and not absolute. As such, the present review must be seen more as a consensus document than a quantitative data synthesis. Moreover, we tried to exclude reports where the authors based their LED scheme on a previous publication but this may not always have been stated in the publication. Where LEDs were stated for particular agents, there was generally good agreement on the equivalent L-dopa dose. We should also caution clinicians against using this conversion scheme precisely in individual patients; it is most appropriate for use in the context of clinical trials.

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**TABLE 2. Protocol for calculating total LED for commonly used agents with worked example**

<table>
<thead>
<tr>
<th>Actual total daily dose (mg)</th>
<th>Conversion factor</th>
<th>Subtotal LED (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate release L-dopa dose</td>
<td>400</td>
<td>×1</td>
</tr>
<tr>
<td>Controlled release L-dopa dose</td>
<td>100</td>
<td>×0.75</td>
</tr>
<tr>
<td>Entacapone (or Stalevo&lt;sup&gt;a&lt;/sup&gt;)</td>
<td>300&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LD × 0.33</td>
</tr>
<tr>
<td>Tolcapone</td>
<td>0</td>
<td>LD × 0.5</td>
</tr>
<tr>
<td>Duodopa&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>×1.11</td>
</tr>
<tr>
<td>Pramipexole (as salt)</td>
<td>0</td>
<td>×100</td>
</tr>
<tr>
<td>Ropinirole</td>
<td>20</td>
<td>×20</td>
</tr>
<tr>
<td>Rotigotine</td>
<td>0</td>
<td>×30</td>
</tr>
<tr>
<td>Selegiline—oral</td>
<td>0</td>
<td>×10</td>
</tr>
<tr>
<td>Selegiline—sublingual</td>
<td>0</td>
<td>×80</td>
</tr>
<tr>
<td>Rasagiline</td>
<td>1</td>
<td>×100</td>
</tr>
<tr>
<td>Amantadine</td>
<td>200</td>
<td>×1</td>
</tr>
<tr>
<td>Apomorphine</td>
<td>0</td>
<td>×10</td>
</tr>
<tr>
<td>Total LED</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Irrespective of the entacapone dose it is the L-dopa dose that is multiplied by 0.33 to give the subtotal LED for entacapone, this will then be added to the L-dopa dose (and other subtotal LEDs) to give the total LED.
interpretation where the medication of a large number of people is being compared.

Table 2 provides a protocol for developing a total daily LED for the commonly used medications in PD patients. This begins with logging the patient’s immediate release l-dopa dose (excluding the dopa-decarboxylase inhibitor dose), followed by adding any CR preparation with an adjustment for reduced bioavailability. The additional effects provided by COMT inhibitors are then added, based on the daily l-dopa dose. For later stage patients on enteral l-dopa infusion, 90% of the total daily infused dose is used to provide an equivalent oral l-dopa dose. DAs are then added using the conversion factors provided in Table 2. Finally, any MAOB inhibitors, amantadine, and apomorphine are added. The individual subtotal LEDs are then added to give the total daily LED.

In conclusion, the standardized LED formulae described here provide a useful tool to compare dose intensities of different antiparkinsonian medication in clinical trials. We are using these formulae in reports of the PD MED (www.pdmed.bham.ac.uk) and PD SURG (www.pdsurg.bham.ac.uk) trials and encourage other research groups to do likewise to help eliminate the inconsistencies in LED values used previously.

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REFERENCES

Restless Legs Syndrome and Parkinson’s Disease in Men

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Eilis J. O’Reilly, ScD,2,4
Hao Wang, MD, PhD,2 and
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Abstract: We examined whether men with restless legs syndrome (RLS) have a higher prevalence of Parkinson’s disease (PD) among 23,119 US participants of the Health Professional Follow-up Study who were free of diabetes and arthritis. RLS was assessed using a set of standardized questions recommended by the International RLS Study Group. PD cases were identified by self-reported questionnaires and confirmed by review of medical records. Compared to men without RLS, multivariate-adjusted odds ratios for PD were 1.1 (95% confidence interval: 0.4, 3.0) for men with RLS symptoms 5–14 times per month and 3.09 (95% confidence interval: 1.5, 6.2; \( P \) trend = 0.003) for those with symptoms 15 times or more per month, after adjusting for age, smoking, use of antidepressant, and other covariates. In conclusion, men with RLS are more likely to have concurrent PD. Prospective studies are warranted to clarify the temporal relationship between RLS and PD. © 2010 Movement Disorder Society

Key words: restless legs syndrome; Parkinson’s disease; men

Restless legs syndrome (RLS) is the most common movement disorder, affecting 5–15% adults.1,2 Because dopaminergic hypofunction in the central nervous system is involved in the disease pathophysiology of both RLS and Parkinson’s disease (PD), it has been suggested that RLS is a possible preclinical marker of PD.4 However, previous epidemiologic studies of RLS and PD generated inconsistent results.5–7 We, therefore, conducted a cross sectional analysis to examine whether men with RLS have a higher likelihood of having PD in the Health Professional Follow-up Study (HPFS), a large ongoing cohort of men.

MATERIALS AND METHODS

Study Populations

The HPFS was established in 1986, when 51,529 male US health professionals (dentists, optometrists, osteopaths, podiatrists, pharmacists, and veterinarians) aged 40–75 years completed a mailed questionnaire about their medical history and lifestyle. Follow-up questionnaires have been mailed to participants every 2 years to update information on potential risk factors and to ascertain newly diagnosed diseases in both cohorts. The institutional review board at Brigham and Women’s Hospital reviewed and approved this study, and receipt of each questionnaire implies participant’s consent.

Assessment of RLS

We asked questions in 2002 about RLS symptoms and severity based on the International RLS Study Group criteria (\( n = 37,431 \), mean age 68.7 ± 9 y) among participants who were still actively participating in the study.8,9 The following question was asked: “Do you have unpleasant leg sensations (like crawling, paraesthesia, or pain) combined with motor restlessness and an urge to move?” with the possible responses of: no; less than once/month; 2–4 times/month; 5–14 times/month; and 15 or more times per month. Those who answered that they had these feelings were asked the following two questions: (1) “Do these symptoms occur only at rest and does moving improve them?” and (2) “Are these symptoms worse in the evening/night compared with the morning?” A participant who had symptoms 5–14 times per month and answered yes to the subsequent questions was considered to have RLS for these analyses.

The questions on RLS were completed by 31,729 (85%) men. Men who did not complete the RLS questions had a similar mean age to those who did (69.0 vs. 68.6 years) and a nonsignificant slightly higher prevalence of PD (0.95 vs. 0.62%). To reduce possible misclassification of RLS, we excluded participants with diabetes and arthritis, leaving 23,119 men in primary analyses. In a secondary analysis, we further
examined the association between RLS and PD with including all participants with RLS information.

**Assessment of PD and Covariates**

Assessment of PD has been described elsewhere. Briefly, we identified new PD cases by biennial self-reported questionnaires. We then asked the treating neurologists to complete a questionnaire to confirm the diagnosis of PD or to send a copy of the medical records. A case was confirmed if a diagnosis of PD was considered definite or probable by the treating neurologist or internist, or if the medical record included either a final diagnosis of PD made by a neurologist, or evidence of at least two of the three cardinal signs (rest tremor, rigidity, bradykinesia) in the absence of features suggesting other diagnoses. Overall, the diagnosis was confirmed by the neurologist in >80% of the cases. PD cases included only confirmed definite and probable cases up to 2004.

Information on potential confounders, including age, ethnicity, smoking status, weight, height, physical activity, use of medicines, phobic anxiety scale, and history of major chronic diseases, was collected via biennial questionnaires throughout the follow-up period. Body mass index (BMI) was calculated as weight (kg)/height (m)^2. The phobic anxiety scale was assessed by the Crown-Crisp phobia index. Statistical Analyses

Statistical analyses were completed with SAS version 9.1 (SAS Institute, Inc, Cary, NC). We categorized participants into three groups: no RLS, RLS with symptoms 5–14 times per month, and RLS with symptoms 15 or more times per month. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) and to test differences in prevalence of PD across categories of RLS status. Analyses were adjusted for age (y), ethnicity (Caucasian, African-American, and Asian and others), BMI (<23, 23–24.9, 25–26.9, 27–29.9, or ≥30 kg/m^2), smoking (never smoked, former smoker, or current smoker: cigarettes/d, 1–14 or ≥15), physical activity (quintiles), use of antidepressants (yes/no), the Crown-Crisp phobic anxiety index (0–1, 2, 3, or ≥4), and presence of stroke, hypertension, or myocardial infarction (each of them, yes/no). We examined potential effect modification of the association between RLS and PD by age (< or ≥70 years, approximate median value), obesity (yes/no, based on BMI ≥30 kg/m^2), and smoking status (never vs. ever), by including multiplicative terms in the logistic regression models, with adjustment for other potential confounders.

**RESULTS**

Men with RLS were older and more likely to be whites and current smokers, to use antidepressants, score higher on the anxiety test, have been diagnosed with hypertension and stroke, and have high BMI and low exercise levels than participants without RLS (Table 1). Men with RLS had a higher prevalence of PD relative to those without RLS in each age group (Fig. 1). Compared to men without RLS, the OR for PD was 1.99 (95% CI: 1.1 to 3.6; \( P = 0.02 \) for those with RLS symptoms, after adjusting for age, smoking, and other covariates. Higher frequency of RLS symptoms, a marker for the disease severity, was associated with increased prevalence of PD (Table 2). The multivariable-adjusted ORs for PD were

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**TABLE 1. Basic characteristics according to restless legs syndrome status in 2002 in the Health Professionals Follow-up Study**

<table>
<thead>
<tr>
<th>Restless legs syndrome status in 2002</th>
<th>No RLS</th>
<th>RLS 5–14 times/mo</th>
<th>RLS 15+ times/mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>22175</td>
<td>549</td>
<td>395</td>
</tr>
<tr>
<td>Age, y</td>
<td>67.6</td>
<td>69.2</td>
<td>70.0</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>3.6</td>
<td>3.7</td>
<td>6.1</td>
</tr>
<tr>
<td>Past smokers (%)</td>
<td>52.5</td>
<td>57.4</td>
<td>55.1</td>
</tr>
<tr>
<td>African Americans (%)</td>
<td>0.6</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Asian &amp; other ethnicity (%)</td>
<td>3.0</td>
<td>1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>BMI, kg/m^2</td>
<td>25.9</td>
<td>26.4</td>
<td>26.2</td>
</tr>
<tr>
<td>Physical activity, Mets/wk</td>
<td>36.7</td>
<td>35.2</td>
<td>31.7</td>
</tr>
<tr>
<td>Phobic anxiety index</td>
<td>1.9</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Use of antidepressant (%)</td>
<td>4.4</td>
<td>8.0</td>
<td>11.3</td>
</tr>
<tr>
<td>Presence of stroke in or prior to 2002, %</td>
<td>1.3</td>
<td>2.4</td>
<td>2.9</td>
</tr>
<tr>
<td>Presence of hypertension in or prior to 2002, %</td>
<td>41.8</td>
<td>44.6</td>
<td>43.4</td>
</tr>
<tr>
<td>Presence of myocardial infarction in or prior to 2002, %</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
</tr>
</tbody>
</table>

*Values were standardized to the age distribution of the overall cohort.*
1.10 and 3.09 (95% CI: 1.5, 6.2; \( P \) for trend = 0.003) for men with RLS symptoms 5–14 times per month, and 15 or more times per month, respectively. Among participants with PD, there was a nonsignificant difference in disease duration comparing those with RLS to those without RLS (9.0 ± 4.8 vs. 7.5 ± 4.2 y; \( P = 0.7 \)).

Similar significant results were observed in several sensitivity analyses. Multivariable-adjusted ORs did not materially change after excluding participants with the highest level of phobic anxiety, with MI, stroke, PD or hypertension, or those who used antidepressant (data not shown). Further inclusion of participants with diabetes or arthritis did not change the association between RLS and PD (OR = 2.16; 95% CI: 1.2, 3.9).

We did not find significant interaction between presence of RLS and age, obesity, and smoking status (\( P \) interaction > 0.2 for all), in relation to prevalence of PD.

### Discussion

In this large cohort of men, we observed that men with RLS had a higher prevalence of PD than those without RLS, across all age groups. Compared to men without RLS, those who reported having RLS symptoms 15 or more times per month had threefold higher prevalence of PD. Strengths of this study include a large sample size, which enabled us to obtain a relatively stable estimate for the associations, and use of standardized questionnaire to assess RLS. As we did not collect information on several RLS-like syndrome (e.g., peripheral neuropathy, leg cramps, positional discomfort, radiculopathy), some misclassification in RLS assessment is possible. However, results were similar when we included or excluded men with diabetes, the most common cause of peripheral neuropathy, in our analyses. Another limitation is that we included only men, and therefore, our results cannot be generalized to women. Further, because of the cross sectional design of our study, we are not able to know whether RLS occured before onset of PD or vice versa.

Associations between RLS and PD have been noticed for long time, both conditions are associated with dopamine hypofunction in CNS. Our findings are consistent with the results of some previous epidemiological studies, but not others. In a sample of 125 PD patients in Singapore, Tan et al. reported that none of them met IRLSSG diagnostic criteria of RLS. However, recently, Loo and Tan found a marginally significant higher prevalence of RLS among PD cases (n = 400) than controls (3% vs. 0.5%; \( P = 0.07 \)) in Singapore. In a cross sectional study by Ondo et al., 20.8% of 303 PD patients had RLS symptoms. In a study examining prevalence of PD among RLS patients, Walters et al. found that 4 of 85 RLS cases (4.7%) had PD, compared to 1% PD prevalence expected among the general population over age 60. A recent report showed that in a family with a high prevalence of RLS, two (6.7%) of 30 family member with RLS also had PD. However, none of these three studies included control groups. Interestingly, a recent genome-wide association study found that MEIS1, a gene involved in embryonic development of substantia nigra, was associated with RLS risk. The relation between MEIS1 and PD risk has only been reported in one case-control study and was not significant.

### Table 2. Odds ratios (ORs) and 95% confidence interval (CI) of Parkinson’s disease according to restless legs syndrome status in the Health Professional Follow-up Study

<table>
<thead>
<tr>
<th># cases</th>
<th>No RLS (n = 22175)</th>
<th>RLS 5–14 times/mo (n = 549)</th>
<th>RLS 15+ times/mo (n = 395)</th>
<th>( P_{\text{trend}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age adjusted OR</td>
<td>1(ref.)</td>
<td>1.10 (0.40, 2.98)</td>
<td>3.24 (1.63, 6.44)</td>
<td>0.002</td>
</tr>
<tr>
<td>Multivariate adjusted OR</td>
<td>1(ref.)</td>
<td>1.10 (0.41, 3.03)</td>
<td>3.09 (1.54, 6.19)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Logistic regression models were used to calculate ORs. Adjusted for age (in years), smoking status (never smoker, former smoker, or current smoker: cigarettes/d, 1–14 or ≥15), BMI (<23, 23–24.9, 25–26.9, 27–29.9, or ≥30 kg/m²), use of antidepressant drugs (yes/no), physical activity (quintiles), the Crown-Crisp phobic anxiety index (0–1, 2, 3, or ≥4) and presence of stroke, hypertension, or myocardial infarction (each of them, yes/no).
In conclusion, we found a concurrence between RLS and PD in men. Further prospective studies are warranted to clarify whether the presence of RLS precedes onset of classic motor symptom of PD; if so, screening for RLS could help to identify individuals at high risk for PD.

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Author Roles: Gao was involved in conception, organization, and execution of research project; design and execution of statistical analysis; writing of the first draft of manuscript. Schwarzchild was involved in conception of research project, review and critique of statistical analysis, review and critique of manuscript. O’Reilly and Wang were involved in execution of research project, review and critique of statistiscal analysis, and review and critique of manuscript. Ascherio was involved in conception and organization of research project; design, review and critique of statistical analysis; review and critique of manuscript.

REFERENCES

Restless Legs Syndrome in Patients with Amyotrophic Lateral Sclerosis

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Abstract: We aimed to evaluate the frequency and determinants of restless legs syndrome (RLS) in a group of 76 patients with amyotrophic lateral sclerosis (ALS) and 100 control subjects. A diagnosis of RLS was made according to the criteria of the International RLS Study Group, and severity was assessed by the RLS severity scale. RLS was significantly more frequent in patients with ALS (ALS/RLS−) than in control subjects (25% vs. 8%; P = 0.002). Compared with control subjects, patients with ALS/RLS− showed shorter history of RLS complaints and higher frequency of symptoms occurrence. Moreover, compared with those without RLS, patients with ALS/RLS− showed increased functional impairment and more often reported sleep complaints. Multivariate logistic regression confirmed the association between RLS and functional impairment. Our findings suggest that RLS should be considered as a possible cause of disrupted sleep in patients with ALS and should be specifically investigated in these patients. © 2010 Movement Disorder Society

Key words: restless legs syndrome; amyotrophic lateral sclerosis; neurodegenerative disease; sleep; insomnia

Restless legs syndrome (RLS) is a common sensorimotor sleep disorder, which affects approximately 5 to 10% of the general population.1 RLS is defined by the presence of an urge to move the legs, usually accompanied by uncomfortable or unpleasant sensations, which begins or worsens during inactivity.2 RLS symptoms are exacerbated in the evening or at night and are partially or totally relieved by movements.2

RLS is considered an idiopathic disorder; however, it is frequently associated with iron-deficiency anemia, pregnancy, end-stage renal disease, diabetes mellitus, neuropathies, and rheumatoid arthritis.1,2 Increased RLS frequency has also been reported in many neurodegenerative disorders such as Parkinson’s disease, spinocerebellar ataxias, Huntington’s disease, and hereditary spastic paraparesis.3 However, there are no studies that have examined the association between RLS and amyotrophic lateral sclerosis (ALS).3

ALS is a rare devastating neurodegenerative disease, of unknown origin, which affects primarily the large motor neurons in the ventral spinal cord, brainstem, and motor cortex, leading to progressive muscle atrophy, paralysis, and death within 2 to 5 years of symptom onset.4 There is at present no effective cure for ALS, and great effort has to be put on alleviating symptoms and maintaining quality of life.

We noticed that RLS symptoms were reported by some patients with ALS afferent to our clinic, so, we decided to evaluate the frequency and severity of RLS in a population of consecutive patients with ALS and to investigate the factors potentially associated with its occurrence.

METHODS

A total of 76 consecutive patients with ALS (32 women, 44 men; mean age: 58.7 ± 12.8 years) were included in the study during their routine visit to our ALS Center between February 2008 and March 2009. Patients were diagnosed as definite or probable ALS according to the El-Escorial WFN revised criteria.5 The control group was composed of 100 healthy subjects, matched for age and sex to the ALS group (47 women, 53 men; mean age: 60.8 ± 13.5 years). All control subjects were not requiring any pharmacological treatment.

In both the ALS and control groups, a neurologist, expert in sleep medicine (D.L.C.), board certified by the Italian Association of Sleep Medicine, established the presence of RLS using the four standard diagnostic criteria proposed by the International RLS Study Group5 and evaluated the main clinical features.6 Only the patients who fulfilled all four criteria were considered affected by RLS. Moreover, a frequency of RLS symptoms ≥2 times/wk was considered mandatory for the diagnosis. When RLS criteria were met, the RLS severity scale was also applied.7 This instrument consists of 10 items assessing subjective severity of RLS-related complaints on a scale from 0 to 4, with a maximum score of 40. Presence of RLS symptoms in first-

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TABLE 1. Main demographic and clinical features of the patients with ALS and control subjects with RLS

<table>
<thead>
<tr>
<th></th>
<th>ALS/RLS† (n = 19)</th>
<th>CS/RLS† (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>59.3 ± 11.6</td>
<td>63.2 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Men/women (n)</td>
<td>11/8</td>
<td>2/6</td>
<td>NS</td>
</tr>
<tr>
<td>RLS severity scale score</td>
<td>18.1 ± 3.4</td>
<td>20 ± 2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of RLS symptoms (mo)</td>
<td>13 ± 13.3</td>
<td>36 ± 18.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Frequency of symptoms occurrence (times/wk)</td>
<td>4.9 ± 1.3</td>
<td>3.4 ± 0.9</td>
<td>0.006</td>
</tr>
<tr>
<td>Subjects with RLS symptoms ≥ 5 times/wk, n (%)</td>
<td>12 (63.2)</td>
<td>2 (25)</td>
<td>0.070</td>
</tr>
<tr>
<td>Daily duration of RLS symptoms (min)</td>
<td>64.2 ± 37.5</td>
<td>61.2 ± 26.3</td>
<td>NS</td>
</tr>
<tr>
<td>Subjects with positive RLS family history, n (%)</td>
<td>3 (15.8)</td>
<td>2 (25)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of subjects reporting insomnia, n (%)</td>
<td>9 (47.4)</td>
<td>4 (50)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of subjects with EDS, n (%)</td>
<td>7 (36.8)</td>
<td>3 (37.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Hematologic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron (normal value 28–170 µg/dl)</td>
<td>79 ± 22</td>
<td>78 ± 14</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ferritin (normal value 15–250 µg/dl)</td>
<td>167 ± 84</td>
<td>122 ± 63</td>
<td>NS</td>
</tr>
<tr>
<td>Serum transferrin (normal value 200–360 mg/dl)</td>
<td>269 ± 23</td>
<td>273 ± 24</td>
<td>NS</td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; RLS, restless legs syndrome; NS, not significant; EDS, excessive daytime somnolence.

degree relatives, insomnia, and excessive daytime somnolence were established in all patients with ALS and in control subjects. Clinical conditions that could mimic RLS symptoms (such as neuropathic pain syndromes, leg akathisia, nocturnal leg cramps, stiffness and discomfort from spasticity or prolonged fixed position, and propriospinal myoclonus) were excluded with additional questions.

We also collected the main clinical and demographic parameters for each patient with ALS, clinical or electro-physiological evidence of peripheral neuropathy or radiculopathy, and blood biochemical variables (including iron metabolism). Functional impairment due to ALS was evaluated using the ALS-Functional Rating Scale-Revised (ALSFRS-R), a 12-item, 48-point scale, which measures bulbar, upper extremity, lower extremity, and respiratory functions. Scores range from 0 (severe impairment) to 48 (normal functioning). This study was approved by the institutional review board. Informed consent was obtained from all participants.

Statistics

Continuous variables were compared with the Student’s t-test, and categorical variables were analyzed using the χ² test. The association between ALS and RLS was evaluated by a multiple logistic regression model. Univariate statistical analysis was used to investigate the risk factors for RLS in the ALS population, and the results were controlled using a multiple logistic regression model. A P value < 0.05 was regarded as statistically significant.

RESULTS

A diagnosis of RLS was made in 19 (25%) patients with ALS (ALS/RLS†) and in 8 (8%) control subjects (P = 0.002). A multivariate analysis that included as confounding variables age and sex confirmed this association and quantified the risk to be affected by RLS of 4.1 (95% confidence interval: 1.67–10.11) times greater for patients with ALS than for control subjects.

Main demographic and clinical features of the patients with ALS and control subjects with RLS are shown in Table 1. Compared with control subjects, patients with ALS/RLS† showed shorter history of RLS complaints (P = 0.001) and higher frequency of symptoms occurrence (P = 0.006).

Five patients with ALS/RLS† (26.3%) reported that RLS disturbances preceded ALS onset, whereas in the remaining patients with ALS/RLS†, RLS symptoms followed the onset of motor disturbances with a mean delay of 20 ± 11.5 months. The majority of patients with ALS/RLS† described the symptoms of RLS such as urge to move the legs associated to uncomfortable feelings (31.6%), pain (26.4%), or “creepy-crawly” sensations (21%). Four patients described their symptoms solely as urge to move (10.5%) or burning (10.5%).

As reported in Table 2, compared with those without RLS (ALS/RLS†), patients with ALS/RLS† showed increased functional disability (P = 0.003) and reported insomnia with a significant higher frequency (P = 0.041). The two mimic conditions most frequently complained by the patients with ALS/RLS† showed higher functional disability (46.1%), and positional discomfort (15.8%). Both the conditions tended to be more frequently reported by the patients with ALS/RLS† than those without RLS, even if the difference was not statistically significant (Table 2).

Logistic regression analysis—including age, sex, duration of ALS symptoms, and presence of insomnia as confounding factors—showed that ALSFRS-R score
was the only independent factor significantly associated with RLS occurrence (OR, 0.89, 95% CI: 0.81–0.97, \( P = 0.008 \)). On the contrary, the independent effect of insomnia complaints was lost (OR, 1.82, 95% CI: 0.48–6.92, \( P = 0.38 \)).

**DISCUSSION**

This is the first study that investigates the association between RLS and ALS. We found a significantly higher frequency of RLS disturbances in our population of patients with ALS (25%) compared with control subjects (8%) and with the prevalence reported in the general population, ranging from 5 to 10%.\(^1\) Patients with ALS/RLS\(^+\) reported a shorter history of RLS symptoms and higher frequency of symptoms occurrence than control subjects. Moreover, patients with ALS/RLS\(^+\) reported sleep-related complaints more often than those without RLS and showed increased functional disability.

Although previous reports have examined the frequency of RLS in many nervous system disorders,\(^1\) and especially in the neurodegenerative ones,\(^3\) there has been limited interest on its possible occurrence in patients with ALS. On the other hand, as the differential diagnosis of RLS could be difficult in patients with ALS, because of the many mimic conditions that have to be excluded, in our opinion, special attention should be put on making a diagnosis of RLS in these patients, especially from those physicians unfamiliar with sleep-related disorders. A polysomnographic examination showing periodic limb movements in sleep could be of help in selected cases.\(^2\) In this regard, 7 of the 19 patients with ALS/RLS\(^+\) underwent overnight polysomnography during their subsequent diagnostic workup, and in all cases, a periodic limb movements in sleep index \( \geq 15 \) was recorded, thus giving further support to the diagnosis (data not shown).

Even if the pathophysiology of RLS is complex and still remains to be elucidated,\(^10\) as in other neurodegenerative disorders,\(^3\) there are many findings from our study supporting a possible secondary/symptomatic RLS form: elevated frequency of RLS symptoms in patients with ALS, compared with control subjects; elevated age of onset; equal distribution between sexes; high weekly frequency of symptoms occurrence; the fact that, in most cases, the clinical onset of RLS followed that of ALS, with a mean delay of about 1.5 years; and the association of RLS with higher ALS disability. If our results are confirmed, then RLS could be considered as a long-term complication of ALS, many months after the onset of the neurodegenerative disease.

Other possible confounding variables are represented by iron deficiency and antidepressant use (especially amitriptyline),\(^10\) however, the findings from this study do not support a significant role for these factors in the pathogenesis of this form of RLS.

---

**TABLE 2. Characteristics of the patients with ALS without and with RLS**

<table>
<thead>
<tr>
<th></th>
<th>ALS/RLS(^-) (n = 57)</th>
<th>ALS/RLS(^+) (n = 19)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>58.5 ± 13.2</td>
<td>59.3 ± 11.6</td>
<td>NS</td>
</tr>
<tr>
<td>Men/women (n)</td>
<td>11/8</td>
<td>33/24</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of ALS symptoms (mo)</td>
<td>27.9 ± 13.2</td>
<td>25.3 ± 13.4</td>
<td>NS</td>
</tr>
<tr>
<td>Site of ALS onset, n (%)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Bulbar</td>
<td>14 (25)</td>
<td>3 (15.8)</td>
<td></td>
</tr>
<tr>
<td>Upper limbs</td>
<td>26 (46.4)</td>
<td>7 (36.8)</td>
<td></td>
</tr>
<tr>
<td>Lower limbs</td>
<td>16 (28.6)</td>
<td>9 (47.4)</td>
<td></td>
</tr>
<tr>
<td>Forced vital capacity (% predicted value)</td>
<td>72.6 ± 24.1</td>
<td>69.2 ± 25.2</td>
<td>NS</td>
</tr>
<tr>
<td>ALSFRS-R score</td>
<td>33.5 ± 8.3</td>
<td>26.8 ± 7.5</td>
<td>0.003</td>
</tr>
<tr>
<td>No. subjects reporting insomnia, n (%)</td>
<td>13 (22.8)</td>
<td>9 (47.4)</td>
<td>0.041</td>
</tr>
<tr>
<td>No. subjects with EDS, n (%)</td>
<td>13 (22.8)</td>
<td>7 (36.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Presence of neuropathy/radiculopathy, n (%)</td>
<td>5 (8.8)</td>
<td>1 (5.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Nocturnal leg cramps, n (%)</td>
<td>23 (40.4)</td>
<td>12 (63.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Positional discomfort, n (%)</td>
<td>7 (12.3)</td>
<td>5 (26.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Use of medications, n (%)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Riluzole</td>
<td>57 (100)</td>
<td>19 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>7 (12.3)</td>
<td>2 (10.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Hematologic parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum iron (normal value 28–170 µg/dl)</td>
<td>83 ± 31</td>
<td>79 ± 22</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ferritin (normal value 15–250 µg/dl)</td>
<td>183 ± 77</td>
<td>167 ± 84</td>
<td>NS</td>
</tr>
<tr>
<td>Serum transferrin (normal value 200–360 mg/dl)</td>
<td>248 ± 42</td>
<td>269 ± 23</td>
<td>NS</td>
</tr>
</tbody>
</table>

ALS, amyotrophic lateral sclerosis; RLS, restless legs syndrome; NS, not significant; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale—Revised; EDS, excessive daytime somnolence.
In conclusion, our findings of increased frequency of RLS symptoms in patients with ALS suggest that RLS might be a cause of disabling sleep disturbance in these patients and should be specifically investigated in the evaluation of ALS, especially in those patients with insomnia or marked physical impairment.

Acknowledgments: None.

Author Roles: Daniele Lo Coco: 1A, 1B, 1C, 2A, 2B, 2C, 3A, 3B. Federico Piccoli: 1A, 1B, 2C, 3B. Vincenzo La Bella: 1A, 1B, 2C, 3B. Daniele Lo Coco - Conception, organization and execution of research project; design, execution, review and critique of statistical analysis; writing of the first draft and review and critique of the manuscript. Federico Piccoli - Conception and organization of research project; review and critique of statistical analysis; review and critique of the manuscript. Vincenzo La Bella - Conception and organization of research project; review and critique of statistical analysis; review and critique of the manuscript.

Financial Disclosure: None.

REFERENCES


Abstract: We report a 67-year-old patient with idiopathic basal ganglia calcification (IBGC). He presented with progressive cognitive impairment, frontal lobe dysfunction, mild leg spasticity, and levodopa (l-dopa)-responsive parkinsonism. Transcranial sonography (TCS) revealed marked hyperechogenicity of the basal ganglia and periventricular spaces bilaterally. The detected signal alterations showed a fairly symmetric distribution and corresponded to the hyperintense calcifications depicted on the computer tomography brain scan. The combination of symmetric hyperechogenic areas adjacent to the lateral ventricles and of the basal ganglia may serve as an imaging marker characteristic of IBGC. Hyperechogenicity due to extended basal ganglia calcification as presented here is distinct from the pattern of hyperechogenicity caused by heavy metal accumulation, which is described to be less striking. In addition to atypical parkinsonian syndromes such as progressive supranuclear palsy and multiple system atrophy, IBGC is thus another differential diagnosis of parkinsonism with basal ganglia hyperechogenicity. © 2010 Movement Disorder Society

Key words: idiopathic basal ganglia calcification; transcranial sonography; parkinsonism; lenticular nucleus

Transcranial sonography (TCS) of the brain parenchyma has been established as an easily implementable and inexpensive noninvasive method to evaluate intracranial structures. Numerous studies have addressed sono-
graphic alterations of the lenticular (LN) and caudate nucleus (CN) in movement disorders and revealed abnormal, hyperechogenic areas within the LN and CN in patients with Huntington’s disease,1 atypical parkinsonism,3 multiple sclerosis,4 primary dystonia,5,6 and Wilson’s disease.7 In the latter, an increased deposition of copper was suggested to be responsible for the apparent TCS alterations.7 Postmortem brain studies of patients with primary adult-onset dystonia also revealed an accumulation of copper in the LN, which may contribute to basal ganglia hyperechogenicity in dystonia patients.5,6,8 The most important reason for increased basal ganglia hyperechogenicity are, however, circumscribed calcifications, which can be found in 5 to 10% of neurologically healthy subjects. Markedly increased areas of basal ganglia hyperechogenicity due to extensive calcifications were recently described in patients with bilateral striopallidodentate calcinosis, also referred to as (idiopathic) basal ganglia calcification.9,10 Small intracranial calcifications can occasionally be detected on TCS before they become evident on computer tomography (CT) or magnetic resonance (MR) imaging, respectively.7,11 Despite these recent advances, the underlying causes of the TCS basal ganglia hyperechogenicity in most of the aforementioned movement disorders remain elusive.

As mentioned above, idiopathic basal ganglia calcification (IBGC) is characterized by massive symmetrical calcification of movement-controlling areas such as the basal ganglia and other brain structures, e.g., the cerebellum and cerebral cortex.12 This disorder provides a unique opportunity to study basal ganglia alterations with a defined histopathology. We report clinical and TCS findings of a patient with sporadic IBGC and provide data to further elucidate the pathophysiological underpinnings of TCS hyperechogenicity.

PATIENTS AND METHODS

Transcranial Sonography

TCS was performed using the Siemens Antares ultrasound system (Siemens; equipped with a 2.0–2.5 MHz sector transducer; S3 probe) by an experienced sonographer (JH). Intracranial structures were examined through the temporal bone window with a penetration depth of 14 centimeters. The images were adjusted for gain power, compression, and time-gain compensation depending on the quality of the individual temporal bone window. Regions of interest were evaluated in standardized axial planes at mesencephalic, diencephalic, and cella media levels as previously described.13 The area of hyperechogenicity in the ipsilateral SN was manually encircled and measured using a computer-based analysis (Scion Image Beta 4.02 Win software package).

RESULTS

At the age of 64 years, this 67-year-old patient developed progressive cognitive impairment, emotional lability, increased irritability as well as a loss of fine motor skills of both hands. Neurological examination revealed frontal release signs, moderate dysarthria, a hypophonic speech, bilateral bradykinesia predominantly on the right, moderate leg spasticity, and a wide based, unsteady gait due to limb ataxia. Neuropsychiatric testing demonstrated moderate bradyphrenia and an impairment of short-term memory and executive functions. IQ testing demonstrated levels between 67 and 99, and he scored 25/30 on the Mini Mental State Examination test. The cognitive impairment worsened slightly over the follow-up period of 16 months. A CT brain scan showed calcification of the basal ganglia, cerebellum, and periventricular spaces bilaterally (Fig. 1). Routine blood test including calcium, phosphate, and parathyroid hormone levels were normal. CSF protein levels were slightly increased suggestive of a mild dysfunction of the brain-CSF barrier. Dopaminergic treatment resulted in distinct improvement of psychomotor speed, bradykinesia, and mobility reflected by the decline of the UPDRSIII score from 32 before treatment to 22 on treatment (100 milligrams levodopa (l-dopa)/tds) (see Supporting Information video segment 1).

TCS revealed striking LN hyperechogenicity bilaterally, in part at the same level of intensity as the contralateral skull and the calcified pineal gland (Fig. 1, see Supporting Information video segment 2). The CN head and the thalamus also demonstrated hyperechogenic areas, though to a lesser extent. On the cella media level, a marked hyperechogenicity was apparent adjacent to the lateral ventricles of both sides. The detected signal alterations showed a fairly symmetric distribution on TCS and corresponded well to the hyperintense calcifications on the CT brain scan. The area of SN hyperechogenicity was increased on the left side [0.35 cm² (>0.25)] and was not measurable on the right side due to an insufficient temporal bone window.

DISCUSSION

We report a case of IBGC presenting with l-dopa-responsive parkinsonism, progressive cognitive impairment, frontal lobe dysfunction, limb ataxia, and mild leg spasticity as well as provide TCS results which demonstrate extensive intracranial calcifications.9,10
Basal ganglia, but not periventricular hyperechogenicity, has been found repeatedly in atypical parkinsonism, especially in progressive supranuclear palsy and multiple system atrophy (MSA-P). IBGC, as reported here, represents an additional differential diagnosis of unusual parkinsonism with both hyperechogenicity of the basal ganglia and periventricular spaces.

To date, one of the main shortcomings of TCS is the unknown pathoanatomical correlate of the detected hyperechogenicity in several movement disorders with this echofeature. Although SN hyperechogenicity in Parkinson’s disease (PD) is thought to be associated with an increased content of iron, bound to proteins other than ferritin, only limited data is currently available for the LN and CN. Hyperechogenicity in these areas has been observed in various disorders and may be related to enlarged perivascular spaces, heavy metal deposition (Wilson’s disease), primary dystonia, gliosis depending on the underlying condition or simply be an artifact. The increased hyperechogenicity of deep brain structures in multiple sclerosis was suggested to be caused by iron accumulation and gliosis. In the present case, we demonstrate that the accumulation of calcium compounds is associated with hyperechogenicity and visible upon TCS.

FIG. 1. The CT brain scan revealed profound periventricular calcium deposits (A, dotted line; LV, lateral ventricle) and intense calcifications of both striata (B, →) as well as (lower) parts of the thalamus (B, *) adjacent to the third ventricle (3V), whereas brainstem structures including SN showed normal findings in CT and MRI scans (not shown). The TCS images of the cella media level (C) and diencephalic level (D) showed marked hyperechogenic areas corresponding to the hyperintense areas as shown in (A) and (B).

The striking basal ganglia hyperechogenicity in this patient showed almost the same level of intensity as the contralateral skull bone and the frequently calcified pineal gland (see Supporting Information video segment 2). Hyperechogenicity due to extended basal ganglia calcification as presented here may therefore usually be rather distinct from the pattern caused by heavy metal accumulation, which has been described to be less striking. Taken together, the basal ganglia echo features in IBGC are likely different from those seen in conditions where an increased iron content presumably contributes to the TCS hyperechogenicity. Apart from the varying intensity, also the highly symmetric distribution and the large afflicted area may be specific TCS features of IBGC.

The increased SN hyperechogenicity in our patient is a finding usually present in patients with idiopathic PD. Interestingly, a recent study revealed similar findings of increased areas of SN hyperechogenicity in patients with distinct multiple sclerosis subtypes, who additionally presented CN and LN hyperechogenicity. We cannot exclude that our patient had concurrent idiopathic PD although the clinical examination did not suggest this. In addition to parkinsonism, our patient presented with cognitive, pyramidal, and cerebellar symptoms which are common features of
IBGC. Of note, the parkinsonian signs showed a good response to dopaminergic medication in our IBGC patient. Although IBGC is suspected to induce striatal cell loss, the positive response to dopaminergic medication suggests postsynaptic preservation of neurons and basal ganglia circuits.

To summarize, the combination of extensive, symmetric hyperechogenic areas adjacent to the lateral ventricles and of the LN as well as the striking, bone-like hyperechogenicity may be a common finding in IBGC. Our data strengthen the notion that the underlying causes for TCS basal ganglia hyperechogenicity are heterogeneous. Future TCS studies in conditions with well-defined pathoanatomical correlates will help to improve the validity of this method.

LEGENDS TO THE VIDEO

The accompanying video demonstrates the clinical status before and after L-dopa-treatment (3 × 100 milligrams) with improvement of bradykinesia and even ataxia of the upper limbs. The gait is faster, less wide based, and less unsafe after dopaminergic treatment. The second part shows a video footage of his TCS examination. This demonstrates a marked increased hyperechogenicity of both lenticular nuclei.

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REFERENCES

Impaired Sense of Smell and Color Discrimination in Monogenic and Idiopathic Parkinson’s Disease

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Abstract: Olfaction is typically impaired in idiopathic Parkinson’s disease (IPD), but its role is uncertain in monogenic PD. Diminished color discrimination has been suggested as another early sign of dopaminergic dysfunction but not been systematically studied. Furthermore, it is unknown whether both deficits are linked. We examined 100 patients with IPD, 27 manifesting mutation carriers (MC), 20 nonmanifesting mutation carriers (NMC), and 110 controls. Participants underwent a standardized neurological examination, the University of Pennsylvania Smell Identification Test (UPSIT), the Farnsworth-Munsell (FM) color discrimination test, and mutation testing in known PD genes. The monogenic group consisted of 15 Parkin (6MC/9NMC), 17 PINK1 (10MC/7NMC), 8 LRRK2 (4MC/4NMC), 3 SNCA (MC), and 4 ATP13A2 (MC) carriers. Olfaction was most impaired in IPD (UPSIT percentiles 10.1 ± 13.5) compared with all other groups (MC 13.8 ± 11.9, NMC 19.6 ± 13.0, controls 33.8 ± 22.4). Within MC, carriers of two mutations in Parkin and PINK1 showed higher UPSIT percentiles than LRRK2 and SNCA carriers. Color discrimination was reduced in IPD (FM total error score 134.8 ± 92.7). In MC (122.4 ± 142.4), the reduction was most pronounced in LRRK2, NMC (80.0 ± 38.8) were comparable with controls (97.2 ± 61.1). UPSIT and FM scores were correlated in the control (r = −0.305; P = 0.002) and the IPD group (r = −0.303; P = 0.006) but not among mutation carriers.

First, we confirmed olfaction and color discrimination to be impaired in IPD and suggest olfaction to be a premotor sign. Second, olfaction differed between carriers with one and two mutations in Parkin/PINK1-associated PD. Third, olfaction and color discrimination impairment do not necessarily evolve in parallel. © 2010 Movement Disorder Society

Key words: Parkinson’s disease; sense of smell; color discrimination; monogenic parkinsonism

Although Parkinson’s disease (PD) is defined by its cardinal motor signs, nonmotor symptoms (NMS) are increasingly recognized as important features. Impaired olfaction is known to be associated with idiopathic PD (IPD). It is present in more than 80% of the patients and often precedes the motor signs. Similarly, neuropathological studies suggest changes in the olfactory system to occur early in the disease course. Little is known about olfaction in monogenic PD.

Color discrimination is also decreased in IPD and its impairment may progress with disease severity. Only few studies have been performed in IPD and no systematic studies are available in monogenic PD. Based on clinical and neuropathological knowledge it is conceivable that the impairment in olfaction, color discrimination, and motor performance in PD may be pathophysiologically linked. However, this has not been systematically studied.

To further investigate olfaction and color discrimination, we performed smell and color discrimination tests in patients with idiopathic PD and nonmanifesting mutation carriers (NMC), and controls. Furthermore, we analyzed the putative relationship of olfaction and color discrimination via correlation analysis.

**PATIENTS AND METHODS**

**Participants**

Study subjects comprised patients with IPD, individuals with mutations in one of the known genes for PD, and nonrelated healthy controls. We recruited patients among consecutive outpatients of the Movement Disorders Clinics in Luebeck, Kiel, and Hamburg and in ongoing family studies. All patients with PD were examined in the “on” status. Controls were recruited...
from nonblood relatives of patients with PD and volunteers from the general population of Luebeck. The study was approved by the local ethics committee and all participants gave written informed consent.

Diagnostic Process and Clinical Assessment

All participants underwent a uniform in-person examination for motor assessment including UPDRS part III, Hoehn and Yahr (H&Y), and Schwab and England scales. On movement disorder specialist examination (N.B., J.H., C.K.), the UK brain bank criteria were established for patients with PD with the exception that a positive family history was not regarded an exclusion criterion. All participants answered a basic demographic, a PD risk factor questionnaire, and the UPDRS part I and II. To evaluate cognitive function, we employed the Mini Mental State examination (MMSE) and the Montreal Cognitive Assessment (MoCA) (for both we defined 24–30 as normal, 0–23 as dementia).

Olfaction Testing

Olfaction was assessed using the University of Pennsylvania Smell Identification Test (Sensonics, Inc., Haddon Heights, NJ, USA) with 40 microencapsulated smell items. The UPSIT is a “scratch and sniff test” and was individually administered to each participant by a trained technician. The UPSIT test manual includes normative data that can be used to calculate age and sex adjusted percentiles from UPSIT scores.

Color Discrimination Testing

The Farnsworth-Munsell (X-Rite, Inc., Grand Rapids, MI, USA) 100 HUE test was used to rate color discrimination ability. This test is based on the proband’s performance in arranging 84 color-coded boxes according to their shade of color. For test evaluation, the number of errors and the degree of color difference between erroneously assigned boxes are taken into account using a computerized rating procedure. Test results are presented as total error score (TES). All participants with low visual acuity or color blindness were excluded. Two patients with IPD and one control person met these criteria and were excluded.

Genetic Screening

Participants were tested for the presence of mutations in the Parkin, PINK1, and ATP13A2 gene and for known mutations in the LRRK2 genes by high resolution melting analysis on the LightCycler 480 (RocheDiagnostics, Mannheim, Germany) or by direct sequencing. Furthermore, we tested for gene dosage alterations in Parkin, PINK1, ATP13A2, LRRK2, and SNCA by multiplex ligation-dependent probe amplification (MLPA, MRC-Holland, Amsterdam, The Netherlands).

Statistics

Two-tailed Mann-Whitney or Kruskal-Wallis tests were used for continuous, χ² tests for categorical data and Spearman-Rho tests for correlations. For comparison of TES, groups were matched for age (±5 years).

Two multivariate linear regression models were built. The model for olfaction included group assignment, MoCA, current smoking status, H&Y stage, and disease duration; the model for color discrimination was calculated likewise including age and gender, but without smoking status.

RESULTS

Sample Characteristics

We examined 257 participants, grouped into 100 patients with IPD, 47 carriers of mutations in different PD genes, and 110 controls. The group of mutation carriers comprised 27 manifesting carriers (MC) and 20 nonmanifesting carriers (NMC) (Table 1, Fig. 1).

Although sex distribution was similar across groups, average age at examination, age at onset (AAO), and disease duration differed. The MC had the youngest age at onset and longest disease duration (Table 1). Patients with IPD had the highest average UPDRS I (1.9), UPDRS II (9.9), and UPDRS III score (24.3). Two patients with IPD had an MMSE <24 points. In the MoCA, 27 individuals scored <24 (17 IPD, 2 MC, 1 NMC, 7 controls).

Olfaction

Decrease in olfaction was most pronounced in the IPD compared with all other groups. MC and NMC had significantly lower olfaction scores than the control group (P < 0.001, P = 0.004). Analyzing MC and NMC combined, Parkin and PINK1 mutation carriers differed significantly from IPD (P = 0.015, P < 0.001) but also from controls (P = 0.001, P = 0.035). Olfaction performance was comparable in NMC and MC (Fig. 1A, P = 0.131). For PINK1 and Parkin, MC carriers with two mutations showed higher UPSIT percentiles than carriers with one mutation (P = 0.027). Stepwise linear regression for UPSIT percentiles was overall significant (P < 0.001), including group assignment, MoCA score, smoking status, H&Y, disease duration. Group assignment was the only individual significant influence (P < 0.001) with an explained variance of 28.2% (R² = 0.282).
TABLE 1. Characteristics, olfaction, and color discrimination test results of all study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IPD (n = 100)</th>
<th>MC (n = 27)</th>
<th>NMC (n = 20)</th>
<th>Controls (n = 110)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, women/men</td>
<td>39/61</td>
<td>12/15</td>
<td>6/14</td>
<td>48/62</td>
<td>0.68</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>63.7 ± 10.3 (61.7–65.8)</td>
<td>54.5 ± 10.3 (50.4–58.6)</td>
<td>46.9 ± 10.4 (42.0–51.7)</td>
<td>58.7 ± 10.9 (56.6–60.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at onset (yr)</td>
<td>55.2 ± 13.9 (52.3–58.7)</td>
<td>42.3 ± 12.3 (35.8–48.9)</td>
<td>NA</td>
<td>NA</td>
<td>0.001</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>8.4 ± 7.4 (6.9–9.9)</td>
<td>13.8 ± 13.3 (6.7–20.8)</td>
<td>NA</td>
<td>NA</td>
<td>0.02</td>
</tr>
<tr>
<td>MMSE score</td>
<td>28.6 ± 6.6 (26.4–30.7)</td>
<td>29.1 ± 6.5 (27.4–30.8)</td>
<td>29.5 ± 6.0 (27.2–29.9)</td>
<td>29.3 ± 5.9 (29.1–29.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>MoCA score</td>
<td>26.2 ± 3.4 (25.5–26.9)</td>
<td>27.7 ± 2.8 (26.4–28.9)</td>
<td>27.7 ± 2.4 (26.4–28.9)</td>
<td>27.3 ± 2.4 (26.8–27.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Smokers currently</td>
<td>9 of 83 (10.8%)</td>
<td>11 of 26 (42.3%)</td>
<td>4 of 19 (21.3%)</td>
<td>23 of 108 (21.3%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation (95% confidence intervals) unless otherwise stated.

*Age and gender adjusted.

aP-values calculated with Pearson Chi-Square; other P-values are calculated with Kruskal-Wallis Chi-Square for nonparametric tests.

bNot included in statistic test.

cCase numbers are smaller than study participant numbers because of exclusion criteria (rhinorrhea for UPSIT or missing glasses for FM.

Abbreviations: IPD, idiopathic Parkinson’s disease; n, number of cases; MC, mutation carriers; NMC, nonmanifesting mutation carriers; NA, not applicable; UPDRS, Unified Parkinson’s Disease Rating Scale; MMSE, Mini-Mental State Exam; MoCA, Montreal Cognitive Assessment; UPSIT, University of Pennsylvania Smell Identification Test; FM, Farnsworth-Munsell color discrimination test; TES, total error score.

Color Discrimination

The TES scores were highest (indicating low color discrimination ability) in the patients with IPD and the MC, lowest in the controls and the NMC (Table 1). All analyses were age-adjusted by matching because color discrimination correlated with age (r = 0.420, P < 0.001). Color discrimination ability was comparable between MC, NMC, and controls (Fig. 1C,D), and differed between IPD and controls (P = 0.005) and between IPD and NMC (P = 0.013). Color discrimination did not differ by gene apart from LRRK2 MC (Fig. 1D), who showed the highest TES but also the highest variability.

Stepwise linear regression estimating the influence of group assignment, age, gender, MoCA score, disease duration, and H&Y stage on TES was overall significant (P < 0.001). Linear regression revealed an individual influence of MoCA score (P < 0.001) and H&Y (P = 0.047), with explained variance of 15.7% for the MoCA score (R² = 0.157), 1.8% for H&Y, and 17.5% combined.

Correlation of Olfaction and Color Discrimination

Across all groups, UPSIT and TES scores were correlated (r = -0.313; P < 0.001). Within the individual subgroups, correlation was significant in the IPD (r = -0.303; P = 0.006) and control group (r = -0.305; P = 0.002), but neither in the MC nor NMC group.

DISCUSSION

Olfaction was significantly decreased in IPD compared with controls, which is in keeping with published studies.1 Furthermore, IPD had lower olfaction than monogenic PD. Olfaction and color discrimination were correlated in IPD and controls, but not in mutation carriers independent of their clinical status.

In Parkin-associated PD, olfaction was previously reported to be less affected than in IPD.5 Similarly, Parkin-associated PD may differ neuropathologically, although this is under debate11,15 In our study, Parkin MC with two mutations showed higher UPSIT percentiles than IPD. Strikingly, Parkin MC with only one mutation had UPSIT percentiles closer to those of the IPD. A similar picture evolved in PINK1 carriers. However, case numbers are too small to draw firm conclusions. In PINK1 no pathological studies are available as yet.

Color discrimination was comparable with controls in all genetic subgroups apart from LRRK2 MC. Three of four LRRK2 MC showed poor color discrimination with
high variability compared with all other groups, whereas the LRRK2 NMC showed average performance.

The strengths of our study are the extensive genetic testing, and the comprehensive clinical assessment with all diagnoses established by a movement disorders specialist and all special tests applied by trained technicians. Limitations of our study include age differences between groups which were adjusted for by using age-matched analyses. However, we did not additionally adjust for disease duration, which also differed between the genetic and idiopathic PD groups. Another limitation is the small sample size especially in the individual groups by gene. Although the current smoking status differed between groups, especially between MC and NMC, it did not show a significant influence on UPSIT performance in the regression model.

Olfaction was decreased in NMC, further suggesting it to be a premotor feature. Despite mean disease duration of 15 years in our MC and only 8.5 years in the IPD group, MC performed better in olfaction testing. This may be linked to differences in pathology between genetic and idiopathic PD. The MC and NMC group did not differ in color discrimination, with the possible exception of LRRK2 carriers. It is tempting to speculate that color discrimination may be a “later” feature than decreased olfaction. Furthermore, linear regression showed an association of color discrimination ability and disease severity, which is in line with longitudinal observations.

In conclusion, olfaction and color discrimination do not necessarily develop in parallel and both show group differences between monogenic and idiopathic PD.
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REFERENCES
Advanced Magnetic Resonance Imaging in Benign Hereditary Chorea: Study of Two Familial Cases

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Abstract: No brain abnormalities are usually detected on conventional magnetic resonance imaging (MRI) in benign hereditary chorea (BHC); there are currently no studies with advanced techniques in literature. We investigated whether conventional and advanced MRI techniques could depict regional brain abnormalities in two familial BHC patients and 24 healthy controls. No brain abnormalities on conventional scans were detectable; also, no significant differences in fractional anisotropy of the basal nuclei were observed. Volumetric analysis showed a decreased volume of the striatum bilaterally compared with controls, whereas spectroscopy demonstrated a significant increased myo-inositol/creatine ratio bilaterally, a reduction of choline/creatine ratio bilaterally, and of N-acetyl-aspartate/creatine in the right putamen. With the limits of the small sample size in the patient group, these data show that, despite the absence of macroscopic changes on conventional MRI, volumetric and metabolic abnormalities are present in the basal nuclei of BHC patients. © 2010 Movement Disorder Society

Key words: benign hereditary chorea; MRI; spectroscopy; diffusion tensor imaging; basal ganglia

Benign hereditary chorea (BHC) is a rare autosomal dominant disorder1 caused by mutations in the thyroid transcription factor gene (TTIF-1) on chromosome 14q13,1,2 essential for the organogenesis of the lung, thyroid, and basal nuclei. The clinical presentation and phenotype of classic BHC (choreic movements, jerks, and possible cognitive dysfunction)3 have expanded as variable combinations of lung, thyroid, and neurologic atypical abnormalities,2,3 which recently led to the introduction of the term brain-thyroid-lung syndrome.4

The TTIF-1 is one of the first genes to be expressed in the developing brain,5,6 in particular in the rostrobasal telencephalon.6,7 This region eventually develops into numerous structures including the medial ganglionic eminence, a precursor of the globus pallidus,8 essential for the presence of cholinergic neurons in the striatum. These neurons normally originate in the pallidum, migrate to the lateral ganglionic eminence (the precursor of the striatum), and then to the cortex2,8 and their absence or reduction leads to reduced inhibition of the thalamus.1 It also results in loss of GABAergic neurons and calbindin-positive cells that also normally originate in the pallidum and then migrate to the cortex, as shown by pathologic studies of BHC patient.9 TTIF-1 is probably important in regulating basal ganglia formation, but not its function, once it is properly developed.10

Brain abnormalities are not usually detected on conventional magnetic resonance imaging (MRI) and on 18F-2-fluoro-2-deoxy-D glucose positron emission tomography (FDG-PET) studies in BHC.1,11 The literature contains only three reports in genetically confirmed cases with aspecific, probably occasional, cerebral abnormalities, showing respectively, a sellar cystic mass, a hypoplastic pallidum with lack of differentiation of the medial and lateral component, and a hyperintense bilateral pallidum signal with multiple small hyperintense foci in the vermis.10,12 Of these findings, it would seem that only the pallidal hypoplasia might in some way be related to the disease, whereas the signal intensity alteration detected in the pallidum is also found in various other brain pathologies.13–15 There are currently no studies in the literature focusing on the use of advanced MRI techniques in BHC.

The aim of this study is to evaluate whether conventional and advanced MRI, in particular diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), and volumetric basal nuclei analysis, could depict regional brain abnormalities in two typical BHC patients with genetically confirmed mutations in the TTIF-1 gene.

PATIENTS AND METHODS

Clinical Data

The patients evaluated were two women, mother and daughter, aged 37 and 18 years at the time of MRI
analysis, respectively, showing the typical clinical picture of BHC, as board-based unsteady gait without altered consciousness, intentional tremor and choreic movements, in addition associated with hypodontia and slightly reduced levels of thyroid hormones. Detailed clinical findings and molecular investigation demonstrating the presence of a deletion including the TITF-1 and PAX9 genes were previously described.\textsuperscript{16,17}

**MRI Protocol**

Both patients underwent a conventional and advanced MRI protocol on a 1.5-Tesla Philips Intera Gyroscan scanner (Koninklijke Philips Electronics, the Netherlands). Twenty-four age-matched healthy volunteers (14 females/10 males; median age = 28.2 years; range 17–55 years) were selected. Although they all underwent the conventional MRI protocol, DTI, and T1-weighted (-w) 3D fast field echo for volumetric analysis, only 8 controls (2 females/6 males; median age = 29.7 years; range 23–46 years) agreed, through written informed consent to undergo MRS due to the time length of the examination.

The conventional MRI sequences (5-mm thick) comprised: coronal T1-w inversion recovery (TR = 3.43 s, TE = 15 ms), sagittal T2-w spin echo (SE; TR = 4.91 s, TE = 110 ms), axial T2-w fast SE (TR = 4.846 s, TE = 100 ms), and axial fluid attenuated inversion recovery (TR = 11,000 ms, TE = 140 ms). The advanced MRI sequences comprised: axial T1-w 3D fast field echo (TR = 25 ms, TE = 4.6 ms, voxel size = 0.98 mm\(^3\)), axial 2-mm thick DTI (TR = 15.3 s, TE = 70 ms, no. directions = 15, b-value = 0–900), and single voxel MRS (voxel size = 30/15/20 mm\(^3\), TR = 1,800 ms, TE = 25 ms), centred on left and right striatum, in view of the documented association between striatum involvement and the BHC-related mutation in TITF-1.\textsuperscript{7,9}

**Postanalysis**

Postanalysis was performed at two dedicated workstations using MatLab r2008A (The MathWorks, Inc.) and Statistical Parametric Mapping (SPM5 - Functional Imaging Laboratory [FIL]).\textsuperscript{18} We estimated the fractional anisotropy (FA) maps using the 15 diffusion weighted images and the early T2-w DTI image.

Given the small sample size in the patient group, we considered more appropriate to perform a region of interest-based analysis selecting those regions that may play a role in BHC (striatum, pallidum, and thalamus bilaterally).\textsuperscript{7,9} One of the operators (A.P., who had 10 years of experience in interpreting brain MRI) determined the volumes of the left and right caudate, putamen, pallidum, and thalamus nuclei by using a manual segmentation technique both on FA maps and 3D T1-w images.\textsuperscript{19} Two other operators (F.P., who had 5 years of experience in MRI postanalysis, and G.M., who had 4 years experience in interpreting brain MRI) computed diffusion measurement and calculated the intracranial volume. Specifically, an estimation of the intracranial portion of the images was obtained as the sum of the three tissue compartments, which are provided by SPM5 (gray matter, white matter, and cerebrospinal fluid).\textsuperscript{20} A hard cut-off was used to exclude any voxel whose probability of belonging to any of the three classes was less than an iteratively determined threshold. To calculate the intracranial volume with the SPM-tissue class method, the number of surviving voxels was obtained and multiplied by the volume of a single voxel. Volumes of deep nuclei were then normalized to each subject’s intracranial volume to correct for individual size differences as previously performed by Mascalchi et al.\textsuperscript{19}

Single voxel MRS postprocessing was performed using the Java based Magnetic Resonance User Interface software package (jMRUI - The MRUI Project)\textsuperscript{21} and MatLab. Corrections were made for chemical shift artefact and partial voluming effects associated with different gray matter and white matter content, as proposed by Stanley and coworkers.\textsuperscript{22,23} N-acetyl aspartate/creatine (NAA/Cr), choline/creatine (Cho/Cr), and myo-inositol/creatine (Myo/Cr) ratios were calculated on left and right striatum regions.

We found high intraobserver repeatability and high interobserver reproducibility (two different rates) for manual volume measurements and FA measures of the right caudate nucleus and right putamen and measurements of intracranial volumes. Both tests showed similar interclass correlation coefficient values (0.95) for what concerns all measures used in this study.

**Statistical Analysis**

Considering the small sample size in the patient group, no statistical analysis was performed or general linear model applied to the volumetric analysis. We considered more appropriate to report patients’ MRS metabolite ratios, FA, and volume values as single values. A reference range was estimated for the control group and considered as between the 5th and 95th percentile. Each patient was compared with the control group to determine whether it fitted within or outside the reference range.

**RESULTS**

Both the patients and all 24 healthy control subjects successfully underwent the conventional MRI and
advanced MRI protocol. The images from the two patients with BHC, due to their neurologic symptoms, showed slight movement artefacts, but the quality of the sequences obtained, including the MRS sequences, was nevertheless good; in particular, an operator (F.P.), coregistered T1-w with b0 DTI images, and the realignment analysis showed values of translation of 0.4 mm and of rotation of 1.1°.
No grey or white brain matter abnormalities, specifically no morphological or signal alterations in the structures of the basal nuclei, were observed on the conventional sequences in either patient. MRS analysis is shown in Figure 1A, in particular we observed a higher Myo/Cr and a reduced Cho/Cr ratio in the striatum bilaterally and a reduced NAA/Cr ratio in the right striatum in patients compared with controls.

Figure 1B,C shows the FAs (B) and volumes (C) for the right and left caudate, putamen, pallidum, and thalamus nuclei in BHC patients as single values and in healthy control subjects as reference range (5th–95th percentile). The analysis showed no significant differences in FA values and thalamic volumes, whereas the right and left caudate and left putamen volumes were significantly reduced in both patients compared with controls. The right putamen and the right pallidum volumes were significantly reduced respectively only in the mother and in the daughter, compared with controls. The intracranial volumes were 1,345.42 cm³ in the daughter and 1,101.87 cm³ in the mother (healthy controls reference range: 1,227.31–1,619.10 cm³).

DISCUSSION

The literature contains few reports of patients affected by BHC submitted to conventional MRI protocols and, to date, none of patients evaluated using advanced MRI techniques.

Conventional brain MRI of our two BHC patients appeared normal; it may be hypothesised that in this genetic disease, there is no specific pattern of macroscopic abnormalities detectable on conventional MRI and that the few lesions observed in literature are to be considered, for the most part, occasional findings. More data from a larger patient series would be needed to clarify this issue.

The advanced MRI techniques can provide information, which goes beyond the presence of macroscopic damage detectable on conventional imaging, specifically on cellular integrity, structural volume, and metabolic-biochemical abnormalities. By applying advanced MRI techniques in our two patients, we found that volumetric analysis and MRS are sensitive enough to detect nonmacroscopic brain abnormalities. Volumetric comparison has showed volume reduction of the striatum, predominantly in the caudate. MRS was, at the same time, particularly sensitive, showing an increased Myo/Cr ratio in the striatum bilaterally, a decreased Cho/Cr ratio bilaterally and reduced NAA/Cr in the right striatum. Myo is usually considered a glial marker and is taken as an index of gliosis. Molina et al. reported similar findings in choreacanthocytosis, which, however, were accompanied by abnormal signal hyperintensity the striatum. The biochemical abnormalities described in this study are, of course, aspecific findings, which can result in different pathologic conditions, as in multiple sclerosis or in Alzheimer’s disease.

Conversely, our DTI findings, which are known to reflect brain structural integrity, were not significantly altered. It is probable that in BHC, unlike choreacanthocytosis, there is a reduction in the number of cells (which volumetric analysis is able to document), but no structural changes visible on DTI. On the other hand, the magnet strength (1.5 Tesla) and the number of directions used (15) in our study for the DTI imaging might be not sufficient to detect structural abnormalities, even when these were present, especially if we consider the small size of the targets of interest.

Finally, aware that the very small number of patients studied could preclude the correct evaluation of the data, we sought to overcome this limitation by selecting a large number of healthy controls. The data presented here, although referring to two patients with BHC, are the first results of the application of advanced MRI tools in this disease, and they show that, despite the absence of macroscopic changes on conventional MRI, volumetric and metabolic abnormalities are present in the basal nuclei. The reported abnormal findings are certainly an indication to extend the same diagnostic imaging procedure to a larger series of BHC patients carrying TITF-1 mutations.

Acknowledgments: We thank Dr. Catherine Wrenn for her precious English revision of the manuscript.

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Author Roles:

REFERENCES


What Is the Functional Significance of Nondominant Arm Tremor in Essential Tremor?

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Abstract: Tremor in the dominant arm is often the focus of clinical attention in essential tremor (ET) yet many daily activities require both arms. The functional relevance of nondominant arm tremor has rarely been studied. In 181 right-handed patients with ET, action tremor in each arm was rated using a clinical rating scale. Tremor disability was self-reported and a performance-based test of function was administered. Independently of tremor on the right, greater tremor severity on the left was associated with greater self-reported disability (P = 0.02) and greater performance-based dysfunction (P < 0.001). In 5.0% of patients, tremor was largely re-stricted to the nondominant arm. Nondominant arm tremor, independent of dominant arm tremor, had a signif-icant functional correlate, contributing to both greater perceived and greater observable functional difficulty. In 5% of patients, tremor in the nondominant arm was the

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likely motivator for seeking care, which is another indication of its functional significance. © 2010 Movement Disorder Society

Key words: essential tremor; clinical; function; disability

Although arm tremor in essential tremor (ET) is typically asymmetric,\textsuperscript{1–3} it is rarely unilateral.\textsuperscript{4–6} Therefore, in the large majority of patients, both arms are typically involved. Dominant arm tremor is often the focus of therapeutic interventions. Yet there are patients with ET who present mainly with nondominant arm tremor.\textsuperscript{7,8} Also, while many daily activities require only the dominant arm (e.g., holding a pen and putting a key in a lock), other daily tasks require both arms (e.g., holding a knife and a fork, typing, washing dishes, and tying shoe laces),\textsuperscript{9,10} suggesting that tremor in the nondominant arm might be of quantifiable functional relevance in most patients with ET.

How functionally relevant is nondominant arm tremor in ET? To our knowledge, there has been only one prior attempt to systematically study this issue in a sample of 30 patients with ET.\textsuperscript{11}

We explored this issue across a group of 181 patients with ET. We hypothesized that (1) nondominant arm tremor would correlate significantly with several measures of functional difficulty, (2) its association with functional difficulty would be independent of dominant arm tremor, and (3) in some patients, whose proportion we will estimate using this sample, tremor in the nondominant arm is the sole motivator of seeking neurological care.

We hope that these data will provide a more complete understanding of all sources of disability in ET. The clinical relevance of these results is that disability may be an important motivator of health-seeking behavior.

PATIENTS AND METHODS

Subjects

As described previously,\textsuperscript{12} patients with ET (≥18 years of age) seen at the Neurological Institute of New York, Columbia University Medical Center (CUMC), were enrolled in an ongoing clinical–epidemiological study. Each signed informed written consent approved by the CUMC Institutional Review Board. Two-hundred-eleven patients with ET qualified for a diagnosis of ET using published diagnostic criteria;\textsuperscript{12} none had Parkinson’s disease or dystonia. Of these, 192 were right handed. We excluded 11 (5.7%) who underwent surgery (all deep brain stimulation [DBS], including 3 right brain, 6 left brain, 2 bilateral), and hence, 181 patients remained.

Evaluation

Demographic and medical histories were obtained, including all medications used to treat tremor. The Cumulative Illness Rating Scale was used to quantify medical comorbidity in 14 body systems [0–3 ratings in each system, range = 0–42 (maximum morbidity)].\textsuperscript{13} A brief 10-item version of a validated tremor disability questionnaire was administered.\textsuperscript{14} Using this questionnaire, difficulty was self-reported (0 = none; 1 = need to modify or loss of efficiency; 2 = disability) on a range of daily activities (e.g., signing name, dialing a telephone, tying shoe laces, cutting nails, and carrying a cup); the score ranged from 0 to 20 (most impaired).\textsuperscript{14}

In a subsample of 122 patients enrolled before 2006, a valid performance-based test of function in ET was also performed; the test included 15 items (e.g., signing name, dialing a telephone, carrying a cup and saucer, threading needle, and placing bills in a wallet) that were rated from 0 (no difficulty) to 4 (unable to perform the task), and the score ranged from 0 to 60 (most impaired).\textsuperscript{15} A videotaped neurological examination was performed on all patients (arm extension, pouring, using spoon, drinking, finger-nose-finger, drawing spirals with each arm, 12 tests total). A neurologist specializing in movement disorders (E.D.L.) used a reliable\textsuperscript{16} and validated\textsuperscript{15} clinical rating scale to rate tremor during each test: 0 (none), 1 (mild or intermittent), 2 (moderate and usually present), and 3 (severe). These ratings resulted in a tremor score for each arm (range = 0–18) and a total tremor score (range = 0–36).\textsuperscript{12} Head (neck), voice, and jaw tremors were noted to be present or absent on videotaped examination.

Statistical Analyses

Analyses were performed in SPSS (version 17). Tremor severity on the left, tremor severity on the right, tremor disability score, and performance-based test score were all normally distributed. Pearson’s correlation coefficients (r) were used to assess correlations. We also stratified right tremor score into quartiles and, in a linear regression analysis, examined the association between increasing quartile (independent variable) and tremor disability score. We repeated the analyses, using quartiles of left tremor score. These two analyses were repeated using performance-based test score rather than tremor disability score as the outcome variable. In a multivariate linear regression analysis, we examined the independent effects of tremor...
TABLE 1. Clinical characteristics of 181 patients with ET

<table>
<thead>
<tr>
<th>Age (yr) (range)</th>
<th>67.5 ± 16.1 (18–95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>96 (53.0)</td>
</tr>
<tr>
<td>Duration of tremor (yr)</td>
<td>19.6 ± 17.5</td>
</tr>
<tr>
<td>Age of tremor onset (yr)</td>
<td>47.9 ± 21.7</td>
</tr>
<tr>
<td>Cumulative Illness Rating Scale Score (range = 0–42)</td>
<td>5.2 ± 3.7</td>
</tr>
<tr>
<td>Left tremor score quartile</td>
<td>9.1 ± 4.2</td>
</tr>
<tr>
<td>Right tremor score quartile</td>
<td>9.7 ± 4.2</td>
</tr>
<tr>
<td>Total tremor score (right and left arms)</td>
<td>19.6 ± 7.3</td>
</tr>
<tr>
<td>Tremor disability score (range = 0–20)</td>
<td>10.1 ± 5.7</td>
</tr>
<tr>
<td>Performance-based test score (range = 0–60)</td>
<td>18.7 ± 12.6</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation or proportions (percentage).

severity on the left, tremor severity on the right, age, gender, presence of any cranial tremor (neck, voice, jaw), Cumulative Illness Rating Scale Score, and use of tremor medication (currently takes an ET medication; yes vs. no) on tremor disability score (dependent variable) or on performance-based test score (dependent variable in another model).

RESULTS

General

There were 181 patients with ET (Table 1). The majority (106 or 58.6%) was currently taking ET medication. Tremor severity on the right was associated with that on the left (r = 0.52, P < 0.001).

Self-Reported Tremor Disability

Greater tremor severity on the right (r = 0.61, P < 0.001) and left (r = 0.44, P < 0.001) were each associated with increased tremor disability scores. We stratified the right and left tremor scores into quartiles (Table 2) and, in tests for trend (linear regression models), increasingly higher quartile was associated with increasingly greater disability in both the right arm (P < 0.001) and the left arm (P < 0.001). Twenty-eight patients had no or only mild right arm tremor (i.e., all postural and kinetic tremor ratings on right = 0 or 1); in these 28 patients, greater tremor severity on the left was marginally associated with increased tremor disability scores (r = 0.26, P = 0.19), but greater tremor severity on the right was not associated with increased tremor disability scores (r = 0.11, P = 0.59).

In a multivariate linear regression analysis, tremor severity on the left (β = 0.23, P = 0.02) and tremor severity on the right (β = 0.73, P < 0.001) were independent predictors of tremor disability score, but, age, gender, presence of any cranial tremor, Cumulative Illness Rating Scale Score, and use of tremor medication were not predictors of tremor disability score. The β value of 0.23 (left arm tremor) indicated that for every 10-point increase in the left arm tremor score, self-reported functional disability increased by 2.3 points.

Performance-Based Test of Function

Greater tremor severity on the right (r = 0.75, P < 0.001) and left (r = 0.60, P < 0.001) were associated with greater performance-based test scores (more dysfunction). In tests for trend, increasingly higher tremor score quartile was associated with increasingly higher performance-based test score in both the right (P < 0.001) and left arms (P < 0.001). In the 28 patients with no or only mild right arm tremor, greater tremor severity on the left (r = 0.56, P = 0.01) but not the right (r = 0.13, P = 0.60) was associated with increased performance-based test scores.

In a multivariate linear regression analysis, tremor severity on the left (β = 0.73, P < 0.001), tremor severity on the right (β = 1.48, P < 0.001) and age (β = 0.22, P < 0.001) were independent predictors of tremor disability score, but gender, cranial tremor, Cumulative Illness Rating Scale Score, and use of tremor medication were not. The β value of 0.73 (left arm tremor) indicated that for every 10-point increase in left arm tremor score, self-reported functional disability increased to 7.3 points.

TABLE 2. Function by tremor severity quartiles

<table>
<thead>
<tr>
<th>Tremor severity quartile</th>
<th>Performance-based test score</th>
<th>Performance-based test score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest quartile (N = 43)</td>
<td>5.3 ± 5.0</td>
<td>8.5 ± 8.1</td>
</tr>
<tr>
<td>Second quartile (7–10)</td>
<td>8.9 ± 4.6</td>
<td>14.8 ± 8.5</td>
</tr>
<tr>
<td>Third quartile (11–12)</td>
<td>11.7 ± 4.4</td>
<td>19.4 ± 6.8</td>
</tr>
<tr>
<td>Highest quartile (≥13)</td>
<td>14.9 ± 4.2</td>
<td>32.3 ± 11.1</td>
</tr>
</tbody>
</table>

In tests for trend, higher quartile was associated with higher tremor disability score on both the right (β = 3.1, P < 0.001) as well as the left (β = 2.2, P < 0.001). In the 28 patients, higher quartile was associated with higher performance-based test score on both the right (β = 7.8, P < 0.001) as well as the left (β = 6.4, P < 0.001).
**Patients with Tremor Largely Restricted to the Nondominant Arm**

There were nine (5.0%) patients whose likely motivation for seeking treatment at CUMC was nondominant arm tremor. On examination, they had moderate or greater kinetic tremor in the left arm (i.e., at least one kinetic tremor rating $\geq 2$), yet none or only mild right arm tremor (i.e., all postural and kinetic tremor ratings $= 0$ or $1$) and no cranial (neck, voice, jaw) tremor.

**DISCUSSION**

Nondominant arm tremor contributed to both greater self-reported disability and poorer performance-based function in this sample of 181 patients with ET. Moreover, in statistical models, its contribution to functional difficulty was independent from that of the tremor in the dominant arm. For 1 in 20 patients with ET, nondominant arm tremor seemed to be the main motivation behind seeking neurological care.

We observed that every 10-point increase in left arm tremor score was associated with an approximate 2.3-point increase in self-reported disability. Given the observation that our average patient had a tremor score on the left that was 10.1, in functional terms, this tremor would likely contribute to a loss of efficiency in two to three additional daily activities. We also showed that every 10-point increase in left arm tremor score was associated with a 7.3-point increase in performance-based test score (i.e., either additional mild difficulty on approximately seven daily tasks or moderate difficulty on three to four or severe difficulty on approximately two daily tasks).

In an interesting study of 30 patients with ET whose upper limb function was assessed with three timed functional tests, greater severity of tremor in the nondominant arm was associated with greater time to complete these tasks using that arm.11 The impact of this nondominant arm tremor was not assessed relative to daily tasks (e.g., tying shoe laces and typing). No other studies have addressed this issue.

Tremor was not measured using accelerometry. Nevertheless, the use of clinician-based ratings ensured that the observed increases in tremor severity were ones that were clinically detectable and relevant. In our analyses, we included medication use as a covariate in an attempt to assess the association between tremor severity and disability independent of such use. Nevertheless, nearly 60% of our cases were using tremor medications and they were not asked to withhold these medications before evaluation. The use of these medications could have reduced tremor causing us to underestimate the impact of tremor severity on disability. It is also possible that a patient with very severe right arm tremor might be using the left (nondominant) arm to facilitate daily activities. For this reason, our multivariate statistical models included a term for both right and left arm tremors so that we could examine the independent effects of each on disability. The study had a number of strengths including the large sample size, the use of both self-reported and performance-based measures of function, and the use of functional measures that were specifically designed to assess the effects of tremor in ET.

In summary, nondominant arm tremor, independent of dominant arm tremor, seemed to have a significant functional correlate in ET, contributing to greater perceived as well as observable functional difficulty with daily tasks. In 5% of patients, tremor in the nondominant arm seemed to be the motivator for seeking treatment, which is another indication of its importance.

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**Author Roles:** Elan D. Louis: Research project conception, organization and execution; statistical analyses design and execution; manuscript writing (writing the first draft and making subsequent revisions); and statistical analyses. Mary Mullaney: Research project execution and manuscript writing (making subsequent revisions). Marina Gerbin: Research project execution and manuscript writing (making subsequent revisions).

**REFERENCES**


**POLG, but not PEO1, is a Frequent Cause of Cerebellar Ataxia in Central Europe**

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**Abstract:** Nuclear genes, in particular mitochondrial polymerase gamma (POLG) and PEO1, have been increasingly recognized to cause mitochondrial diseases. Both genes assume a complementary role as part of the mitochondrial DNA (mtDNA) replication fork and, accordingly, seem to present with largely overlapping phenotypical spectra. We assessed the frequency and phenotypical spectrum of PEO1 compared to POLG mutations in a cohort of 80 patients with cerebellar ataxia for which common repeat expansion diseases had been excluded. Patients were selected to present additional features previously described for PEO1 mutations, namely early age of onset, progressive external ophthalmoplegia (PEO), or epilepsy. Whereas PEO1 mutations were not found in our cohort, POLG frequently caused ataxia with PEO (47%), psychiatric comorbidities (20%) and, more rarely, with epilepsy (14%). Thus, PEO1 is rare in Central Europe even in those patients displaying characteristic phenotypic features. In contrast, POLG is rather common in Central European ataxia patients. It should be particularly considered in ataxia patients with PEO, psychiatric comorbidities, and/or sensory neuropathy, even if characteristic mitochondrial extra-CNS features are absent. © 2010 Movement Disorder Society

**Key words:** ataxia; mitochondrial; genetics; epilepsy

Mitochondrial diseases can be caused by mutations in mitochondrial DNA (mtDNA) or in nuclear genes coding for mitochondrial proteins. Recently, the nuclear genes PEO1 and mitochondrial polymerase gamma (POLG) have come into focus as they are essential in mtDNA maintenance. PEO1, as a mitochondrial replicative helicase, and POLG, as the only DNA polymerase in mitochondria, are both part of the mtDNA replication machinery. Based on this close functional association, it is not surprising that the respective phenotypical spectra are widely overlapping.1 In particular, the following common phenotypic entities have been delineated: progressive external ophthalmoplegia (PEO)—either in isolation or in association with additional symptoms like psychiatric disorders, ataxia or parkinsonism—and sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO) are known as teenage to adult onset phenotypes for both genes.2,3–5 Infantile onset spinocerebellar ataxia (IOSCA) for PEO1 mutations,6,7 and mitochondrial infantile recessive ataxia syndrome (MIRAS) for POLG mutations,8 respectively, as well as mitochondrial depletion syndromes with hepatocencephalopathy (MDS) for both genes9,10 represent common early-onset manifestations with severe phenotype.

Whereas these syndromes present with a high degree of clinical pleomorphism and at highly variable age of onset in POLG,11 such information is still rare for PEO1. As ataxia seems to present one of the key features, we here assess the frequency and phenotypic spectrum of PEO1 mutations as compared to POLG mutations in a cohort of 80 patients with cerebellar ataxia in which common repeat expansions had been excluded.

**PATIENTS AND METHODS**

80 index patients were recruited from the ataxia clinic in Tübingen. Inclusion criteria represented key features indicative of both PEO1 and POLG mutations:
Progressive ataxia with (1) early onset (<25 years of age) and/or (2) epilepsy and/or (3) external ophthalmoplegia. At least one of these criteria had to be fulfilled for inclusion in this study (which means that, e.g., patients with late-onset ataxia only were not included). All index patients were negative for repeat expansions causing SCA 1, 2, 3, 6, 7, and 17. Additionally, in patients with onset of ataxia before 25 years of age GAA repeat expansions causing Friedreich’s ataxia were excluded. The entire coding region and adjacent intronic regions of PEO1 (Twinkle, C10orf2) and POLG were screened by direct sequencing. Primer sequences are noted in the Supporting Information Table. Patients underwent standardized clinical examination by movement disorders specialists (J.S., M.S., L.S.).

RESULTS

Following the Harding criteria,12 patients were allocated as early onset cerebellar ataxia (EOCA, n = 63) or late onset cerebellar ataxia (LOCA, n = 17) with a threshold of ataxia onset at 25 years of age. EOCA and LOCA were subgrouped according to the presence of additional features as either pure EOCA (n = 45), EOCA with PEO (n = 3), EOCA with epilepsy (n = 14), EOCA with PEO and epilepsy (n = 1), LOCA with PEO (n = 10), LOCA with epilepsy (n = 6), or LOCA with PEO and epilepsy (n = 1) (see Table 1).

Sequencing of POLG and PEO1

Genetic analysis of PEO1 did not reveal a pathogenic mutation in any of the 80 index patients. In contrast, sequencing of POLG identified a total of 9 patients with established pathogenic mutations (9/80; 11.3%). In the EOCA with PEO group, 2 of 3 index patients (66%) revealed a POLG mutation, harbouring compound heterozygous A467T/W748S and R627Q/G848S; R627Q with insA c.3594). Additionally, one heterozygous G517V sequence carrier was identified in this group. None of the LOCA patients with epilepsy (0/7) carried a POLG mutation.

Phenotypical Characterization

Clinical features of all patients are shown in Table 1. Age of onset varied from 0.5 to 25 years (mean: 15.8 y) in the EOCA group and from 28 to 61 years (mean: 43.9 y) in the LOCA group. In none of the POLG mutation carriers ataxia started before 12 years of age. All POLG patients (9/9) suffered from sensory neuropathy which was exclusively of the axonal type. Among all ataxia patients with sensory neuropathy, 16.7% (5/30) of the EOCA and 40% (4/10) of the LOCA patients with sensory neuropathy were POLG mutation carriers. In 3 of 9 POLG patients psychiatric features, especially depression, were observed, which accounted for 20% (3/15) of all ataxia patients with psychiatric comorbidities. Additionally two G517V het carriers suffered from psychiatric disorders. Epilepsy was observed in 3 of 9 POLG patients (=13.6% [3/22] of all ataxia patients with epilepsy), only one of them presenting occipital lobe epilepsy, myoclonic seizures with epilepsia partialis continua, and frequent convulsive status epilepticus, which has recently been described as a characteristic finding in POLG patients.15 Interestingly, characteristic mitochondrial extra-CNS features seem to be rather infrequent in POLG patients: none of them suffered from diabetes mellitus or optic atrophy and only one patient presented with hypacusis. Moreover, liver pathology does not seem to be frequent in those POLG patients who do not primarily present with Alpers syndrome: in this study, none of the ataxia patients carrying a POLG mutation showed elevated liver enzymes.

DISCUSSION

It is increasingly acknowledged that mutations in POLG are a major cause of heterogeneous neurological diseases16,17 that present not in totally discrete clinical categories but within a continuous spectrum of disease.11 In contrast, little is known about the frequency and phenotypic spectrum of mutations in PEO1—a nuclear gene that assumes a complimentary role of mtDNA maintenance and might thus present with similar characteristics.1 So far, mutations in PEO1 have been described for four main clinical entities, namely MDS,10 IOSCA,6 SANDO,3 and PEO.2 Although each of these entities seems to be restricted to rather specific age groups, phenotypic overlap is large as all of them often include, inter alia, ataxia, dysarthria, ophthalmoplegia, or epi-

Movement Disorders, Vol. 25, No. 15, 2010
<table>
<thead>
<tr>
<th>(a) EOCA patients</th>
<th>Age at onset</th>
<th>Dysarthria</th>
<th>Ptosis</th>
<th>Sensory neuropathy</th>
<th>Pyramidal tract signs</th>
<th>Psychiatric disorders</th>
<th>Additional features</th>
<th>MRI findings</th>
<th>Established mutation</th>
<th>Potential mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EOCA</strong></td>
<td>All n = 45</td>
<td>0.5–25 y</td>
<td>82.2%</td>
<td>4.4%</td>
<td>42.2%</td>
<td>40%</td>
<td>13.3%</td>
<td>Intellectual disability: 7</td>
<td>Extrapyramidal signs: 14</td>
<td>Cerebellar atrophy: 27</td>
</tr>
<tr>
<td>POLG n = 2</td>
<td>20–23 y</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
<td>50%</td>
<td>Extrapyr. signs: 1</td>
<td>Cerebellar atrophy: 1</td>
<td>Brain stem atrophy: 1</td>
<td>G517V het</td>
</tr>
<tr>
<td><strong>EOCA + PEO</strong></td>
<td>All n = 3</td>
<td>18–25 y</td>
<td>100%</td>
<td>33.3%</td>
<td>100%</td>
<td>33.3%</td>
<td>33.3%</td>
<td>Proximal myopathy: 1</td>
<td>Hypacusis: 2</td>
<td>Cerebellar atrophy: 1</td>
</tr>
<tr>
<td>POLG n = 2</td>
<td>18–25 y</td>
<td>100%</td>
<td>50%</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>Proximal myopathy: 1</td>
<td>Hypacusis: 1</td>
<td>Cerebellar atrophy: 1</td>
<td></td>
</tr>
<tr>
<td><strong>EOCA + epilepsy</strong></td>
<td>All n = 14</td>
<td>1–24 y</td>
<td>64.3%</td>
<td>7.1%</td>
<td>50%</td>
<td>50%</td>
<td>21.4%</td>
<td>Intellectual disability: 11</td>
<td>Proximal myopathy: 3</td>
<td>Cerebellar atrophy: 7</td>
</tr>
<tr>
<td>POLG n = 2</td>
<td>12–13 y</td>
<td>50%</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>50%</td>
<td>Proximal myopathy: 1</td>
<td>Extrapyr. signs: 2</td>
<td>Intellectual disability: 1</td>
</tr>
<tr>
<td><strong>EOCA + PEO and epilepsy</strong></td>
<td>All = POLG n = 1</td>
<td>22 y</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>Cerebellar atrophy: 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(b) LOCA Patients

<table>
<thead>
<tr>
<th>LOCA + PEO</th>
<th>Age at onset</th>
<th>Dysarthria</th>
<th>Ptosis</th>
<th>Sensory neuropathy</th>
<th>Pyramidal tract signs</th>
<th>Psychiatric disorders</th>
<th>Additional features</th>
<th>MRI findings</th>
<th>Established mutation</th>
<th>Potential mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>All n = 10</td>
<td>28–51 y</td>
<td>90%</td>
<td>50%</td>
<td>60%</td>
<td>30%</td>
<td>40%</td>
<td>Proximal myopathy: 2</td>
<td>Extrapyr. signs: 1</td>
<td>Cerebellar atrophy: 5</td>
<td></td>
</tr>
<tr>
<td>POLG n = 4</td>
<td>29–41 y</td>
<td>100%</td>
<td>50%</td>
<td>100%</td>
<td>0%</td>
<td>50%</td>
<td>Proximal myopathy: 1</td>
<td>Extrapyr. signs: 1</td>
<td>Serum lactate increase: 2</td>
<td>Cerebellar atrophy: 2</td>
</tr>
<tr>
<td>POLG n = 1</td>
<td>44 y</td>
<td>100%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td>G517V het</td>
</tr>
<tr>
<td><strong>LOCA + epilepsy</strong></td>
<td>All n = 6</td>
<td>45–61 y</td>
<td>66.6%</td>
<td>0%</td>
<td>66.6%</td>
<td>33.3%</td>
<td>16.6%</td>
<td>Dementia: 3</td>
<td>Cerebellar atrophy: 3</td>
<td>Brain stem atrophy: 1</td>
</tr>
<tr>
<td>POLG n = 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LOCA + PEO and epilepsy</strong></td>
<td>All n = 1</td>
<td>28 y</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>Dementia: 1</td>
<td>Cerebellar atrophy: 1</td>
<td>White matter changes: 1</td>
</tr>
<tr>
<td>POLG n = 0</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

EOCA, early-onset cerebellar ataxia; LOCA, late-onset cerebellar ataxia; PEO, progressive external ophthalmoplegia; POLG, mitochondrial polymerase gamma; ↑, increase above reference values.
Clinical characteristics of our cohort are summarized in Table 1. In contrast to PEO1, POLG mutations were rather frequent in our ataxia population (11.3%), in particular in those phenotypic groups closely resembling already established PEO1-associated entities: 11 of our patients displayed core features of IOSCA (namely age at onset of ataxia with 9 to 18 months and muscle hypotonia$^5$), and 8 patients exhibited a SANDO phenotype (namely age of onset in teens and additional SANDO$^1,3$), but none of them carried a PEO1 mutation. As we did not find any PEO1 mutations in patients with ataxia and epilepsy, the finding of PEO1-induced epileptic encephalopathy$^7$ might be restricted to typical IOSCA patients only, but not be common in other ataxia patients. Thus, the high prevalence of PEO1-associated IOSCA in Scandinavian countries$^6$ could be due to a founder effect in these regions.

In summary, our results indicate a much lower frequency of PEO1 compared to POLG in Central European ataxia patients. Despite the large phenotypic variability, sequencing of POLG might be particularly considered in non-SCA and non-FRDA ataxia patients with PEO, psychiatric comorbidities, and/or axonal sensory neuropathy—even if characteristic mitochondrial extra-CNS features are absent. In contrast, sequencing of POLG cannot be corroborated in patients with onset of ataxia in the first decade and without further indicative features.

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Author Roles: Schicks was involved in research project: conception, organization, execution; manuscript: writing of the first draft. Synofzik was involved in research project: conception, organization, execution; manuscript: writing of the first draft. Schulte was involved in research project: conception, organization, execution; manuscript: writing of the first draft. Schulte served as an editorial board member of Movement Disorders; received research support from Santhera Pharmaceuticals [MICONOS trial (Co-PI)], the Deutsche Forschungsgemeinschaft [SCHO754/3-1 (PI) and SCHO754/4-1 (PI)], the German Research Council (BMBF)[#01GM0644 to Leukonet (PI), #01GM0603 to GeNeMove (PI) and #01GM0864 to mitoNET (PI)], the EU [#LSHM-CT-2004-503304 to EUROSCA (Co-investigator)], the E-RARE program [#01GM0807 to EUROSQA (PI) and #01GM0820 to RISCA-Co-PI], and the HSP-Selbsthilfegruppe Deutschland eV.

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Impairment, Not Dementia (PD-CIND). We quantified disease and Dementia (PD-D) or with PD and Cognitive Impairment (AD) is present in a subset of individuals with Parkinson’s disease and one-half of PD-D patients had the biomarker signature associated with Alzheimer’s disease.

Abstract:
We tested the hypothesis that the CSF biomarker signature associated with Alzheimer’s disease (AD) is present in a subset of individuals with Parkinson’s disease and Dementia (PD-D) or with PD and Cognitive Impairment, Not Dementia (PD-CIND). We quantified CSF Aβ42, total tau (T-tau), and phospho-tau (P181-tau) using commercially available kits. Samples were from 345 individuals in seven groups (n): Controls <50 years (35), Controls >50 years (115), amnestic Mild Cognitive Impairment (aMCI) (24), AD (49), PD (49), PD-CIND (62), and PD-D (11). We observed expected changes in AD or aMCI compared with age-matched or younger controls. CSF Aβ42 was reduced in PD-CIND (P < 0.05) and PD-D (P < 0.01), whereas average CSF T-tau and P181-tau were unchanged or decreased. One-third of PD-CIND and one-half of PD-D patients had the biomarker signature of AD. Abnormal metabolism of Aβ42 may be a common feature of PD-CIND and PD-D. © 2010 Movement Disorder Society

Key words: Parkinson’s disease; cognitive impairment; CSF biomarkers; Aβ42 tau

CSF Aβ42 and tau in Parkinson’s Disease with Cognitive Impairment

Thomas J. Montine, MD, PhD, *1 Min Shi, MD, 1 Joseph F. Quinn, MD, 2 Elaine R. Peskind, MD, 3,4 Suzanne Craft, PhD, 3,5 Carmen Ginghina, PhD, 1 Kathryn A. Chung, MD, 2 Hojoong Kim, MD, 5,6 Douglas R. Galasko, MD, 7 Joseph Jankovic, MD, 8 Cyrus P. Zabetian, MD, MS, 5,6 James B. Leverenz, MD, 4,6 and Jing Zhang, MD, PhD 1

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Recent focus on biomarkers for Alzheimer’s disease (AD) is fueled by now abundant data showing that processes of this disease start years before dementia or less severe forms of cognitive impairment (CI); the latter is commonly defined clinically as amnestic Mild CI (aMCI). Currently, the most successful biomarker candidates for AD are PET imaging for fibrillar amyloid beta (Aβ), structural MRI, and quantification of cerebrospinal fluid (CSF) Aβ42, total tau (T-tau), and tau phosphorylated at amino acid 181 (P181-tau; reviewed in Ref. 2). Indeed, increased CSF T-tau and P181-tau and decreased CSF Aβ42 are characteristic of AD or aMCI in cross-sectional studies (reviewed in Refs. 2 and 3), and similar changes are present in older individuals without CI who are at increased risk of conversion to MCI or AD. Emerging data suggest that neuroimaging and quantification of these CSF proteins are comparable in detecting processes of AD in preclinical settings.

Parkinson’s disease (PD) is now recognized commonly to include dementia (PD-D) and less severe forms of CI; in the PD research community, the latter is commonly defined by a set of clinical criteria for “CI, Not Dementia” (CIND). Indeed, the point prevalence of dementia among patients with PD is approximately one-third, and ~75% of patients with PD develop dementia over 10 years. Several hypotheses for CI in PD have been proposed, including subcortical processes, extension of Lewy body disease from brainstem to isocortical structures, and co-morbid AD; however, the extent to which each or a combination of these processes contributes CI in PD is unclear. The ability to identify patients with PD-CIND or PD-D deriving from AD versus other causes of dementia likely will be critical to organizing clinical trials and...
managing patients once disease-modifying therapies are developed. This issue has been addressed only in a few individuals by neuroimaging and in CSF-based studies of limited number of relatively small disease groups that yielded conflicting conclusions. Here, we quantified CSF Aβ42, T-tau, and P181-tau in 345 individuals to estimate the prevalence of co-morbid AD among patients with PD who did or did not have CI at the time of lumbar puncture.

**PATIENTS AND METHODS**

The Human Subject Institutional Review Boards of Baylor College of Medicine, Oregon Health & Science University, the University of California at San Diego, VA Puget Sound Health Care System, and the University of Washington approved this study. All individuals provided informed consent, and underwent evaluation that consisted of medical history, physical and neurologic examinations, laboratory tests, and neuropsychological assessment. Laboratory evaluation included complete blood count (serum electrolytes, blood urea nitrogen, creatinine, glucose, vitamin B12, and thyroid stimulating hormone); all results were within normal limits. Exclusion criteria included moderate or heavy cigarette smoking (more than 10 packs/year), alcohol use other than social, and any psychotherapeutic drug use other than for treatment of AD or PD.

Controls were healthy volunteers who had normal cognitive performance on a battery of neuropsychological tests at the time of lumbar puncture as previously described. All controls had at least 1 year of follow-up (median of 3 years) without demonstrating any symptoms or signs of neurologic disease. AD, PD, and aMCI were diagnosed by established criteria. The diagnosis of PD-D was determined by established criteria and included the “one-year rule” for differentiation from Dementia with Lewy Bodies, viz., dementia must occur 1 year after onset of motor parkinsonism in PD-D. The diagnosis of PD-CIND was made in subjects with a diagnosis of PD and a clinical dementia rating of 0.5 but without dementia as determined by PD-D criteria.

All CSF was obtained by lumbar puncture in the morning, was free of visual contamination by blood, had hemoglobin levels <6.0 μg/mL and was flash frozen and then stored at −80°C in polypropylene cryovials until used. All CSF samples from individuals in research cohorts at our institutions that met the above criteria were assayed for T-tau, P181-tau, and Aβ42 concentrations using AlzBio3 Luminex kits from InnoGenetics (Alpharetta, GA) by following exactly the manufacturer’s instructions and were within the range of values reported by others. Coded samples were analyzed by individuals who did not know any corresponding clinical information. Statistical analyses were performed with GraphPad Prism (San Diego, CA).

**RESULTS**

To match age among disease groups and individuals with aMCI, we excluded 12 PD patients who were ≤50 years of age and divided Controls into ≤ or >50 years old. There was no significant difference in concentrations (pg/mL) of any of the three CSF analytes between Controls >50 year and Controls ≤50 year. Table 1 summarizes data from the remaining 333 CSF samples used in primary analyses. As expected, the AD group had significantly decreased CSF Aβ42 (P < 0.001) and significantly increased CSF T-tau (P < 0.001) and P181-tau (P < 0.001) concentrations compared to Controls >50 year. Similar to others, we observed increased CSF T-tau (P < 0.05) and P181-tau (P < 0.001) concentrations in individuals with aMCI. Figure 1A plots individuals’ CSF Aβ42 vs. P181-tau levels for both Control groups, aMCI, and AD.

---

**TABLE 1. Age and biomarker results for each group**

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>Age (yr) Mean ± SD</th>
<th>Disease duration (yr) Mean ± SD</th>
<th>CDR global median</th>
<th>Hoehn-Yahr score median</th>
<th>Concentration in CSF (pg/mL) median (25th to 75th interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &gt;50 yr (115)</td>
<td>68 ± 10</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>386 (284–514) T-tau (82* (54–123) P181-tau (66*** (46–112)</td>
</tr>
<tr>
<td>MCI (24)</td>
<td>68 ± 7</td>
<td>10 ± 5</td>
<td>2</td>
<td>–</td>
<td>216*** (158–247) T-tau (88*** (67–126) P181-tau (51*** (39–72)</td>
</tr>
<tr>
<td>PD (41)</td>
<td>64 ± 10</td>
<td>8 ± 6</td>
<td>0</td>
<td>2</td>
<td>322 (282–401) T-tau (54 (44–67) P181-tau (20* (17–25)</td>
</tr>
<tr>
<td>PD-CIND (58)</td>
<td>66 ± 8</td>
<td>8 ± 7</td>
<td>0.5</td>
<td>2.5</td>
<td>313* (225–389) T-tau (53 (44–66) P181-tau (20* (17–22)</td>
</tr>
<tr>
<td>PD-D (11)</td>
<td>71 ± 8</td>
<td>16 ± 6</td>
<td>2</td>
<td>3.5</td>
<td>220** (187–275) T-tau (33 (30–72) P181-tau (17 (12–30)</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test comparing six groups (Controls >50 yr and five disease groups) for Aβ42, T-tau, or P181-tau concentrations (pg/mL) had P < 0.0001 for all three comparisons. Dunn’s corrected repeat paired comparisons with Controls >50 yr had ***P < 0.001, **P < 0.01, or *P < 0.05.

CDR, clinical dementia rating; MCI, mild cognitive impairment; AD, Alzheimer’s disease; PD, Parkinson’s disease; CIND, cognitive impairment not dementia; D, dementia.
CSF P181-tau/Aβ42 is a convenient means to summarize the coincidental increase in CSF P181-tau and decrease in CSF Aβ42 that is characteristic of AD. Assuming that clinically silent AD is uncommon in Controls ≤50 years, we and others have previously used this ratio in Controls ≤50 years to define an upper cutoff value for normal CSF P181-tau/Aβ42. Using this approach, 3% of Controls ≤50 years, 25% of Controls >50 years, 92% of individuals with aMCI, and 96% of patients with AD had abnormally increased CSF P181-tau/Aβ42. Similar results were obtained using CSF T-tau/Aβ42 (not shown). These results validated this method for detecting preclinical and clinical AD.

Among the groups of patients with PD, CSF Aβ42 levels ranged from normal in those without CI, to progressively lower values in patients with PD-CIND or PD-D. Unlike MCI and both groups with dementia, there were 8 patients (age range) with PD (35 to 50 years) and 4 patients with PD-CIND (40 to 50 years) who were ≤50 years old. None of the CSF concentrations for any of the three analytes was out of the range reported for the corresponding group of older patients.

**FIG. 1.** Scatter plots of CSF Aβ42 and P181-tau concentrations. Individuals' CSF Aβ42 and P181-tau concentrations for (A) both Control groups, subjects with amnestic Mild Cognitive Impairment (MCI), and patients with Alzheimer’s Disease (AD), and for (B) patients with Parkinson’s disease (PD) without cognitive impairment (CI), with CI but Not Dementia (PD-CIND), or dementia (PD-D).

**DISCUSSION**

Quantification of CSF Aβ42, T-tau, and P181-tau provides a validated means to assess processes of AD in patients with dementia or CI, and even in older individuals who are cognitively normal. Applying this tool to 119 patients with PD and a spectrum of CI, we observed progressively lower CSF Aβ42 concentrations in patients with PD, PD-CIND, or PD-D. In combination with exquisite studies of others, these data suggest that progressive CI in patients with PD may be associated with increased deposition of fibrillar Aβ in cerebrum and that this process might be demonstrable with PET imaging.

T-tau increases in CSF in AD and other degenerative and destructive diseases of brain and is widely thought to signify damage to neurons. Although CSF T-tau levels trended to lower values in the PD-D group, these were not significant and thereby concordant with the results of some but not others who observed an increase in average CSF T-tau in patients with PD-D. In contrast, we observed that average P181-tau concentrations in PD and PD-CIND groups were significantly 20% lower than age-matched controls, and this result differs from others who have reported no difference or increased average CSF P181-tau in these groups. CSF P181-tau is more difficult to interpret than T-tau as its levels presumably reflect at least two potentially related mechanisms, cellular processes that lead to phosphorylation and release from damaged neurons. The reasons for these discrepant results among studies are not clear. However, one interpretation of our results is that patients with PD, PD-CIND, and PD-D may have less neuron damage than patients with aMCI or AD and may
have suppression of those biochemical processes that lead to threonine-181 phosphorylation on tau.

We estimated that approximately one-third of patients with PD-CIND and slightly less than one-half of patients with PD-D had abnormally increased CSF P181-tau/Abeta_42, although there was no distinct pattern of subpopulations (see Fig. 1B). This stands in sharp contrast to the >90% of patients with aMCI or AD who had abnormally increased CSF P181-tau/Abeta_42. One possible explanation for these results is that the majority of patients with PD and CI do not have co-morbid AD.

Our results suggest that validated CSF biomarkers for processes of AD may be helpful in identifying those patients with PD and co-morbid AD. Further investigation of the neuropsychological profile and possible risk from inheritance of the e4 allele of APOE is needed for this group of patients with PD and CI. However, our results also indicate that this is a minority of patients with PD-CIND or PD-D, and underscore the need for further research into other more common causes of CI and dementia in patients with PD.

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Author Roles: Montine was involved in conception and organization of research project; design, execution, review and critique of statistical analysis; writing of the first draft, review and critique of manuscript. Shi was involved in organization and execution of research project, review and critique of manuscript. Craft was involved in organization and execution of research project, review and critique of statistical analysis, review and critique of manuscript. Zhang was involved in conception, organization, and execution of research project; review and critique of manuscript.

**REFERENCES**