Abstract: To investigate over time changes in striatal dopamine transporter (DAT), we performed two sequential N-α-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane single photon computed tomography (SPECT) scans in 20 subjects with essential tremor (ET), in 13 with Parkinson disease (PD) and in 23 healthy controls (HC, one scan only). We also performed an [99mTc]ethyl cysteinate dimer bicisate SPECT exam for regional brain network analysis in 9 ET, in a second group of 18 PD (9 with tremor, tPD and 9 akinetic-rigid dominant, arPD) and in 8 HC. PD subjects had a reduced DAT binding in comparison to ET and HC with an annual decline rate of 7.3% in the contralateral putamen. There were no mean uptake differences between ET and HC at baseline and no uptake loss over time in ET. A discriminant analysis grouped 30% (first scan) and 5% (second scan) of ET as PD and a partition analysis showed overlap between ET and PD for caudate nucleus uptake. Spatial covariance analysis revealed that the expression of the PD-related regional pattern separated both tPD and arPD from ET and HC. In conclusion, PD and ET do not share a common pattern of dopaminergic loss over time. However, mild impairment of dopamine transporter in the caudate nucleus may contribute to tremor onset in ET.

Key words: essential tremor; SPECT

INTRODUCTION

Essential tremor (ET) is a common movement disorder characterized by postural or kinetic tremor with a frequency of 6 to 12 Hz. Although several studies describe an abnormal olivo-cerebellar network in ET, recent evidence suggests a neurodegenerative pathophysiology and even an overlap with Parkinson disease (PD). Indeed, ET is clinically progressive and several signs, such as bradykinesia, rest tremor, and hyposmia may be observed in ET. Moreover, a population-based study revealed that patients with ET were four times more likely than unrelated healthy controls to develop incident PD. In addition, extensive structural changes in the cerebellar and midbrain white matter, as well as Lewy bodies, have been found in ET suggesting neuronal dysfunction or loss. We recently reported a mild loss of striatal dopamine transporter (DAT) in ET patients; we now repeated in some patients of the same cohort a second [I-123] N-α-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl) tropane (FP-CIT) scan after an average period of 3 years. We also performed perfusion single photon computed tomography (SPECT) to study the expression of the Parkinson disease-related covariance pattern (PDRP) in ET versus PD (with and without tremor) and healthy controls (HC).
SUBJECTS AND METHODS

Subjects

Twenty patients with ET (ETFP) and 13 PD patients (PDFP) underwent a FP-CIT SPECT twice with a time interval of at least 3 years. Baseline uptake values were compared with 23 healthy subjects (HCFP). Of the 20 ETFP patients, 9 (ETECD) had an additional SPECT scan with $[^{99m}Tc]$ethyl cysteinate dimer bicisate (ECD). Perfusion imaging data were compared with a discrete cohort of PD patients, 9 with predominant akinetic-rigid symptoms (arPD_ECD) and 9 tremor dominant (tPD_ECD). Perfusion data were also compared with a second group of 8 healthy controls (HC_ECD).

At the time of first SPECT, 7 subjects with ET were on chronic propranolol (60 $\pm$ 30 mg/day on average; range 40–80 mg/day) and 3 were on chronic clonazepam (2 mg/day), with mild to moderate benefit on tremor. The other subjects were not on neurological medications at the time of the study. There were no changes in medications at follow-up. Patients were asked to stop medication intake at least 3 days before SPECT.

ET clinical and FP-CIT imaging results at baseline were previously published.17

Inclusion Criteria and Clinical Evaluation

All ET patients had at least 5 years clinical follow-up before the first SPECT and never exhibited any additional neurological abnormalities beside postural or kinetic tremor. Diagnosis of ET was made according to the clinical criteria proposed in the Consensus Statement on Tremor by the Movement Disorders Society.1 Hyperthyroidism and other causes of tremor associated with normal radiotracer uptake18,19 were excluded.

PD was diagnosed according to the UK Parkinson’s Disease Brain Bank criteria20 and patients evaluated with the Unified Parkinson Disease Rating Scale motor part (UPDRS-III) in drugs-off state (i.e., after overnight withdrawal of specific drugs for PD; no patients were taking long-acting dopaminergic drugs). UPDRS akinetic-rigid score was the sum of items: 18 (speech), 19 (facial expression), 22 (rigidity), 27 (arising from chair), 28 (posture), 29 (gait), 30 (postural stability), 31 (body bradykinesia); tremor score was the sum of items: 20 (tremor at rest) and 21 (action or postural tremor of hands). PD with tremor had a minimum summed limb UPDRS tremor score of four21 with at least one limb with a severity of two or higher.22 The severity of tremor symptoms in ET was evaluated according to the Fahn-Tolosa-Marin rating scale.23 Ipsilateral (or contralateral) putamen and caudate nucleus were identified based on the most affected body side at clinical investigation.

Magnetic resonance imaging (MRI) was performed before first and second FP-CIT SPECT, and only subjects with normal results (i.e., no sign of white matter lesion or atrophy) were enrolled in the study. The Hospital Ethics Committee approved the study, and all patients signed an informed consent form.

FP-CIT SPECT Data Acquisition, Reconstruction and Analysis

Intravenous administration of 110–140 MBq of FP-CIT (DaTSCAN, GE-Healthcare, UK) was performed 30–40 minutes after thyroid blockade (10–15 mg of Lugol oral solution) in all patients after overnight withdrawal of dopaminergic therapy.22 Brain SPECT was performed 3 hours later by means of a dedicated triple detector gamma-camera (Prism 3000, Philips, Eindhoven, the Netherlands) equipped with low-energy ultra-high resolution fan beam collimators (4 subsets of acquisitions, matrix size $128 \times 128$, radius of rotation 12.9–13.9 cm, continuous rotation, angular sampling: 3 degree, duration: 28 minutes) in patient and control groups.

Brain sections were reconstructed with an iterative algorithm (OSEM, 4 iterations and 15 subsets), followed by 3D filtering of sections obtained (Butterworth, order 5, cut-off 0.31 pixel-1) and attenuation correction (Chang method, factor 0.12).

The reconstructed images were then analyzed for regionally specific FP-CIT uptake using Statistical Parametric Mapping (SPM2, Wellcome Department of Imaging Neuroscience, London, UK) in conjunction with MATLAB version R2007a (The Mathworks Inc., Natick, Massachussets, USA).

A FP-CIT template was first created with SPM2 by spatially normalizing the FP-CIT images of 16 healthy subjects onto a FP-CIT MNI-based template, previously described,24 averaging the normalized images and their symmetric (mirror) image and filtering using a 3-dimensional Gaussian kernel with 8-mm full width at half maximum (FWHM).25

Using SPM2, all images of ET patients and all HC were spatially normalized to this FP-CIT images of 16 healthy subjects onto a FP-CIT MNI-based template, previously described,24 averaging the normalized images and their symmetric (mirror) image and filtering using a 3-dimensional Gaussian kernel with 8-mm full width at half maximum (FWHM).25

A reference region in the occipital cortex was defined using the VOI of superior, middle, and inferior occipital gyri and calcarine gyri of the automated anatomical labeling (AAL)26 with Wake Forest University (WFU) PickAtlas 2.4 software. The binding ratio for
each FP-CIT image was then computed in a voxel-by-voxel manner (voxel–occipital)/(occipital).

The two FP-CIT SPECT exams where performed at least 3 years apart. The annual percentage change in FP-CIT caudate and putamen binding ratios were calculated as a percentage of the baseline value, divided for the number of months between the two scans and then multiplied by 12.

Using the general linear model in voxel-based analysis of SPM2, the two-sample t-test contrasts were used to elucidate group difference between ET and HC, and paired t-tests were performed to assess the longitudinal change of DAT binding in ET patients. In every analysis, we used no global normalization, no grand mean scaling, and threshold masking absolute: 0. Clusters of at least 50 voxels with the height threshold set at $P < 0.001$ were considered as significant. Uptake was measured in each of the five regions (occipital, left caudate nucleus, right caudate nucleus, left putamen, and right putamen) delineated using the AAL VOI routine in WFU PickAtlas software.

ECD SPECT Data Acquisition, Reconstruction and Analysis

ECD SPECT was performed within 3 months of the second FP-CIT. Brain perfusion scans were obtained in the morning at resting condition with closed eyes and after overnight withdrawal of dopaminergic therapy. Brain SPECT studies were acquired by means of a dedicated triple detector gamma-camera (Prism 3000; Philips, Eindhoven, the Netherlands), equipped with low-energy ultra-high-resolution fan beam collimators. Six fast SPECT acquisitions were obtained with matrix size $128 \times 128$, pixel size 2.0–2.4 mm, radius of rotation 12.9–13.9 cm, continuous rotation, angular sampling: 3 degree, duration 20 min), started between 30 and 60 min. after i.v. injection of about 740 MBq of ECD (Neulite; Bristol-Myers-Squibb, New York, NY) with patients sitting with closed eyes in a quiet and dimly lit room.

SPECT data were reconstructed by iterative algorithm (OSEM, 20 iterations and 15 subsets), filtered with a 3D-Butterworth filter (order 5, cut-off 0.31 pixel-1), and corrected for attenuation according to Chang’s method (attenuation coefficient 0.1 cm$^{-1}$, elliptic fitting with separate contours for each slice).

Preprocessing of imaging data was performed by SPM99 software. Individual images were nonlinearily warped into Talairach space and were smoothed with an isotropic Gaussian kernel for all directions (full width at half maximum 14 mm) to improve the signal-to-noise ratio.

Network Analysis

We evaluated ECD SPECT scans using the (PDRP), a previously identified and validated spatial covariance pattern using $^{18}$F-fluorodeoxyglucose PET scans in 20 PD patients and 20 healthy subjects. This PDRP pattern is characterized by increased pallidothalamic and cerebello-pontine metabolic activity, with covarying metabolic reductions in the cortical motor and association regions. We used a fully automated voxel-based network quantification approach (software available at http://www.feinsteinneuroscience.org/software) to determine the PDRP network activity in each scan. The analyses were conducted on a scan-by-scan basis blind to clinical group designation.

General Statistical Methods

Statistical analyses were performed with the SPSS 9.0 statistical package. Chi Square analysis was used to test the demographic homogeneity between study groups. Non-categorical data were compared by ANOVA. We also used the Wilcoxon signed-rank test for matched pairs to compare uptake values of $PD_{FP}$ and $ET_{FP}$ at baseline and follow-up; a pair represented data obtained in the same individual at different time points. To investigate interactions effects between $PD_{FP}$ and $ET_{FP}$ uptake changes over time we used a 2 × 2 RMANOVA with post hoc correction (Bonferroni). A Pearson’s correlation coefficient was calculated to investigate the relationship between uptake values and demographic and clinical data.

We performed two discriminant analyses (with baseline results and with results at follow up) considering essential tremor, Parkinson disease and healthy subjects as three discrete cohorts. We used, both at baseline and follow up, average right and left putamen and caudate FP-CIT uptake values for healthy subjects, putamen and caudate values contralateral to the most affected body side for $PD_{FP}$ and $ET_{FP}$. The discriminant analyses were used to calculate the accuracy of FP-CIT SPECT diagnosis of PD in comparison to clinical diagnosis. Finally, a partition analysis was used to calculate uptake values (second SPECT) of contralateral caudate nucleus and putamen that would better differentiate among groups. $P$ value $< 0.05$ was considered to be statistically significant. Data are written in the text as mean ± SD, unless otherwise specified.

RESULTS

Demographic and clinical data are listed in Table 1. Uptake values are listed in Table 2. $HC_{ECD}$ were sig-
TABLE 1. Demographic data of subjects that performed two FP-CIT/SPECT studies (A) and/or ECD/SPECT (B)

<table>
<thead>
<tr>
<th></th>
<th>N. (M/F)</th>
<th>Age (at I SPECT)</th>
<th>Age at onset</th>
<th>Disease duration (at I SPECT)</th>
<th>UPDRS III at I SPECT</th>
<th>UPDRS III ak/rig score at I SPECT</th>
<th>FTMTRS at I SPECT</th>
<th>UPDRS III at II SPECT</th>
<th>UPDRS III ak/rig score at II SPECT</th>
<th>FTMTRS at II SPECT</th>
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<tbody>
<tr>
<td>A</td>
<td></td>
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<tr>
<td>ETpFP</td>
<td>20 (12/8)</td>
<td>70.4 ± 9 (59–82)</td>
<td>59 ± 15</td>
<td>10 ± 10 (1–41)</td>
<td>3.8 ± 1.8 (2–7)</td>
<td>–</td>
<td>24.1 ± 10.6 (10–42)</td>
<td>–</td>
<td>4.2 ± 2 (2–8)</td>
<td>–</td>
</tr>
<tr>
<td>PDpFP</td>
<td>13 (7/6)</td>
<td>63.4 ± 8.5 (53–75)</td>
<td>58 ± 7</td>
<td>5 ± 2.8 (3–12)</td>
<td>17.2 ± 6a (10–24)</td>
<td>1 ± 1.2 (0–2)</td>
<td>8.1 ± 3.3 (4–12)</td>
<td>–</td>
<td>23.5 ± 7.8b (10–35)</td>
<td>1.5 ± 2.4 (0–3)</td>
</tr>
<tr>
<td>HCPFP</td>
<td>23 (10/13)</td>
<td>70.5 ± 9 (42–77)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>B</td>
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<tr>
<td>ETpEC</td>
<td>9 (7/2)</td>
<td>69.8 ± 9.8 (54–83)</td>
<td>56 ± 25</td>
<td>13.8 ± 19.2 (3–44)</td>
<td>4.5 ± 2.3</td>
<td>–</td>
<td>18.1 ± 4.9 (16–26)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>tPDEC</td>
<td>9 (6/3)</td>
<td>62.7 ± 6.5 (50–69)</td>
<td>53.6 ± 5.6 (43–61)</td>
<td>9.1 ± 2.7 (5–13)</td>
<td>7.5 ± 3.8b</td>
<td>10.5 ± 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>arPDpEC</td>
<td>8 (8/1)</td>
<td>61.1 ± 5.4 (53–69)</td>
<td>52.4 ± 5.5 (44–62)</td>
<td>8.6 ± 3 (2–13)</td>
<td>1.2 ± 2</td>
<td>157.4 ± 48</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HCEC</td>
<td>8 (3/5)</td>
<td>74.3 ± 4.6 (64–79)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
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</tbody>
</table>

aUPDRS III scores at time of first FP-CIT/SPECT were significantly lower than at follow-up ($P < 0.05$).

bHealthy subjects were significantly older than PD patients, both tremor (tPD) and akinetic-rigid (arPD) sub groups ($P < 0.05$); but not than ET subjects. UPDRS III tremor score were significantly higher in tPD when compared with arPD ($P < 0.05$); but not when compared with ET subjects. ET = essential tremor subjects; PD = Parkinson patients; HC = healthy subjects.
Results of discriminant analysis are listed in Figure 1. At baseline, discriminant analysis placed 10/20 (50%) \( E_{FP} \) in the essential tremor cohort; 4/20 (20%) were grouped as \( H_{FP} \) and 6/20 (30%) as \( P_{FP} \). Four of the six \( E_{FP} \), originally placed in the \( P_{FP} \) cohort, were grouped as \( E_{FP} \) at follow up. In addition, one of the six \( E_{FP} \) originally placed in the \( P_{FP} \) cohort was grouped as \( H_{FP} \) at follow up, thus leaving only one \( E_{FP} \) subjects (5%) misclassified as \( P_{FP} \).

Based on the discriminant analysis results, FP-CIT/SPECT accuracy in discriminating PD from ET and HC was 76% at first scan and 89% at second scan.

The partition analysis results are listed in Table 3. Contralateral uptake values providing the best split between groups were 0.29 for the putamen and 0.23 for the caudate nucleus.
for the caudate nucleus. An uptake value <1.89 for the contralateral putamen was diagnostic for PDFP (100% probability that a subject with uptake values <1.89 has clinical signs of PD); whereas the best split for the uptake value of the contralateral caudate nucleus (<1.29) did not clearly discriminate ETFP (38% probability) and PDFP (52% probability).

UPDRS ratings for akinesia and rigidity in the PDFP group correlated negatively with contralateral putaminal uptake at first SPECT (P < 0.05) and with uptake values of all brain regions (i.e. ipsilateral and contralateral putamen and caudate nucleus) at second SPECT (P < 0.05). At second SPECT, total UPDRS-III score also correlated negatively with uptake values (P < 0.05).

Group mean PDRP expression of ET_{ECD} (0.5 ± 0.4) did not differ from HC_{ECD} (0 ± 1), but was different (P < 0.05) from arPD_{ECD} (2.2 ± 1.4) and tPD_{ECD} (2.0 ± 1.1) (P < 0.05). No difference was found between arPD_{ECD} and tPD_{ECD} (Fig. 2). Three PD patients with the lowest PDRP scores overlapped with the ET patients in terms of pattern expression. For discriminating PD from ET, the optimal cutoff value was determined to be a PDRP score of 1.00, representing the upper limit of pattern expression for the ET group. This corresponded to a specificity of 100% and a sensitivity of 83.3%.

### DISCUSSION

Mean uptake values of ET_{FP} subjects did not differ between baseline and 3-year follow-up and when compared to HC_{FP}. Still, a discriminant analysis clustered 70% of ET_{FP} in a distinct cohort and a partition analysis revealed a four times higher probability for ET_{FP} (in comparison to HC_{FP}) to have an uptake value in the contralateral caudate nucleus below 1.29 (Table 3).

Other imaging studies also support abnormalities in dopamine nerve terminals in patients with ET\textsuperscript{22,29,30} but the role of caudate nucleus in the pathophysiology of tremor is not well established. Tremor can be induced by intra-caudate injections of bretarily, tetra-benazine or mescaline and further suppressed by local injections of catecholamines (dopamine and epinephrine).\textsuperscript{31} Patients with contralateral infarction of the caudate nucleus showed delayed onset of parkinsonian tremor but not bradykinesia nor rigidity.\textsuperscript{32} Pathological studies showed a more prominent degeneration of the medial substantia nigra pars compacta that project mainly to the caudate nucleus, in tremulous variant PD (tremor-PD)\textsuperscript{33} and we recently demonstrated more selective caudate nucleus FP-CIT uptake loss in tremor-PD.\textsuperscript{17} It could be speculated that patients with tremor-PD and ET may have a very selective dysfunction of caudate nucleus-thalamic pathway. Eventually, with dopamine depletion in the caudate nucleus, thalamic targets will fire in a burst oscillatory fashion and act as a pacemaker.\textsuperscript{34–36} Alternatively, the caudate-cerebellar pathway, via the inferior olive,\textsuperscript{37,38} may be responsible for the tremor development. In this case, the loss of inhibitory dopaminergic innervation of the caudate nucleus will determine a release of the excitatory caudate-olive pathway and trigger abnormal oscillations of inferior olive.\textsuperscript{39}

Taken together, these results suggest that subjects with ET might have a lower dopaminergic tone in the caudate nucleus that may trigger tremor onset and pos-

### TABLE 3. Recursively partition of uptake values of contralateral putamen and caudate nucleus

<table>
<thead>
<tr>
<th>Putamen contralateral (left side for HC_{FP})</th>
<th>Subject group</th>
<th>Uptake value</th>
<th>(&lt;1.89)</th>
<th>(\geq1.89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETFP</td>
<td>0%</td>
<td>43%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDFP</td>
<td>100%</td>
<td>8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCFP</td>
<td>0%</td>
<td>49%</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Caudate nucleus contralateral (left side for HC_{FP})</th>
<th>Subject group</th>
<th>Uptake value</th>
<th>(&lt;1.29)</th>
<th>(\geq1.29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETFP</td>
<td>38%</td>
<td>34%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDFP</td>
<td>52%</td>
<td>6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCFP</td>
<td>10%</td>
<td>60%</td>
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</tbody>
</table>

Cut-off of 1.89 for putamen and 1.29 for caudate nucleus resulted to better split the subjects groups according to their uptake values. Percentages were calculated comparing predicted by expected diagnosis. For contralateral putamen uptake values <1.89 we found a probability of 100% to find a subject with Parkinson disease (PD_{FP}). A great overlap was found instead for contralateral caudate nucleus between essential tremor (ET_{FP}) and PD_{FP}, but not healthy subjects (HC_{FP}).

### FIG. 2.

Brain network. Box-plot diagram of scores for the previously identified PD-related pattern (PDRP) of essential tremor subjects (ET_{ECD}), healthy subjects (HC_{ECD}) and patients with Parkinson disease tremor type (tPD_{ECD}) or akinetic-rigid type (arPD_{ECD}). Both PD sub-groups scores significantly higher that ET_{ECD} and HC_{ECD} (P < 0.05). No difference was found between ET_{ECD} and HC_{ECD} and comparing the two PD sub-groups. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
possibly being a risk factor for PD development. Although we could not perform a second SPECT in the control group, we can reasonably assume that a lack of dopaminergic loss over 3 years might be considered within the normal range and the test-retest reliability of FP-CIT SPECT. Given this, the absence of a PD-like progression loss of the dopaminergic innervation in subjects with ET clearly distinguish, on a pathophysiology basis, PD and ET.

The mean reduction of mean reduction of \([^{[123]}I]β-CIT\) binding in PD patients versus healthy controls has been reported as 5.8% over 15 months (5.6%/year).\(^{40}\) Further studies confirmed an annual loss ranging between 5 and 8%\(^ {41,42}\) although Marek et al reported a higher rate of 11.2%/year.\(^ {43}\) One \([^{[123]}I]IPT\) study reported a striatal binding loss of 6.6% in the first year and a further 5.3% in the second year.\(^ {44}\) In line with those results we found an annual rate of uptake loss ranging from 5.6% in the contralateral caudate nucleus to 7.3% in the contralateral putamen. Interestingly, the accuracy of FP-CIT/SPECT consistency with PD clinical diagnosis of PD improves at about 4% per year of disease.

Brain network analysis with PDRP showed a clear cut difference between ET\(_{ECD}\) and PD\(_{ECD}\) (both tPD\(_{ECD}\) and arPD\(_{ECD}\) groups), supporting a different pathogenesis for these two diseases. Furthermore, PDRP expression separated individual PD\(_{ECD}\) patients from ET\(_{ECD}\) subjects with excellent specificity, comparable to the level of discrimination from FP-CIT SPECT scans at the second time point. This suggests that PDRP quantification with ECD SPECT may be useful in conjunction with FP-CIT SPECT in enhancing the accuracy of discriminating between PD and ET patients at early clinical stages of these disorders. Importantly, this study did not investigate the anatomic- and network-related substrates of tremor generation in PD or ET. By contrast, we demonstrate similar PDRP elevations in the tPD\(_{ECD}\) and arPD\(_{ECD}\) groups, which had comparable levels of bradykinesia and rigidity. These findings are compatible with those of an earlier FDG PET study showing that elevated pattern scores in PD patients are not attributable to the presence of tremor.\(^ {41}\)

In conclusion, PD and ET do not share a common dopaminergic decline over time and the PDRP clearly differentiates ET from arPD and tPD. Still, mild dopaminergic nerve terminal loss in the caudate nucleus may prompt tremor onset.

**Author Roles:** IUI and AA participated in the conception of the study. IUI, GM, SH, MC, RB, AR, RC gathered and analyzed the data. IUI, GM, SH, CT, GP, DE, AA participated in the redaction of the paper. All authors have seen and approved the final version.

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