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Progression of Tremor in early stages of Parkinson’s disease: A clinical and neuroimaging study

Jacopo Pasquini1, Roberto Ceravolo1, Zahi Qamhawi2, Jee-Young Lee2,3, Günther Deuschl4, David James Brooks5,6, Ubaldo Bonuccelli1 and Nicola Pavese5,6

Author Affiliations
1. Dipartimento di medicina clinica e sperimentale – Pisa University, Italy
2. Division of Brain Sciences, Neurology Imaging Unit, Imperial College London, UK
3. Department of Neurology, Seoul National University Boramae Hospital, Seoul, South Korea
4. Department of Neurology, UKSH, Christian-Albrechts-University Kiel, Germany
5. Institute of Neuroscience, Newcastle University, UK
6. Department of Nuclear Medicine & PET Centre, Aarhus University, Denmark

Correspondence to:
Professor Nicola Pavese, MD, PhD, FRCP
Newcastle Magnetic Resonance Centre & Positron Emission Tomography Centre
Newcastle University
Campus for Ageing & Vitality
Westgate Road
Newcastle upon Tyne NE4 5PL
Tel: 0191 2081264
Fax: 0191 2081251
E-mail: nicola.pavese@newcastle.ac.uk

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Keywords: Parkinson’s disease; tremor; serotonin; dopamine; levodopa
**Abbreviations:**  
123I-FP-CIT = 123-Ioiflupane-Fluoropropyl-Carbomethoxy-3-beta-4-
Iodophenyltropane; MDS-UPDRS = Movement Disorders Society – Unified Parkinson’s Disease 
Rating Scale; PPMI = Parkinson’s Progressive Markers Initiative; ROI = region of interest; SBR = 
Specific Binding Ratio; SD = standard deviation; SPECT = Single Photon Emission Computed 
Tomography;
Abstract

Rest tremor is one of the cardinal signs of Parkinson’s disease. Kinetic and postural tremors may also occur. The coexistence of these three types of tremor at disease onset and their subsequent progression could have important clinical and therapeutic implications but remain to be fully elucidated. We aimed to: (i) Evaluate prevalence and progression of these three types of tremor in early stages of the disease; (ii) Investigate longitudinally the relationship between dopaminergic and serotonergic terminal dysfunction, rest tremor severity and its response to dopaminergic therapy.

The Parkinson’s Progressive Markers Initiative database provided the baseline and two-year follow-up clinical ratings and 123Ioflupane-Fluoropropyl-Carbomethoxy-3-beta-4-Iodophenyltropane single photon emission computed tomography images for this study. 123Ioflupane-Fluoropropyl-Carbomethoxy-3-beta-4-Iodophenyltropane measured putamen dopamine transporter and median raphe serotonin transporter availability. A Raphe/Putamen uptake ratio was calculated for each patient as an index of relative involvement of these structures.

Clinical analysis of tremor was conducted on three hundred and seventy-eight patients. 87.8% presented with tremor at baseline. Rest tremor occurred in 69.6% of patients at baseline and 67.9% at follow up. Postural and kinetic tremors occurred in about 50% of patients at both baseline and follow up. Over 20% of patients presenting with tremor did not exhibit a rest component at baseline. The number of patients with isolated rest tremor was halved at follow-up.

In tremor predominant patients, rest tremor severity was inversely correlated with raphe serotonin transporter availability both a baseline and follow-up (baseline: constancy $P < 0.05$, tremor index $P < 0.05$; follow-up: amplitude $P < 0.05$, constancy $P < 0.05$, tremor index $P < 0.05$). In the entire cohort, more severe tremor scores correlated with lower Raphe/Putamen uptake ratio values, indicative of more severe raphe dysfunction (baseline: constancy $P < 0.01$, tremor index $P < 0.05$; follow-up: amplitude $P < 0.01$, constancy $P < 0.001$, tremor index $P < 0.001$). The percentage of
improvement in rest tremor amplitude after acute dopaminergic therapy was smaller in patients with lower Raphe/Putamen uptake ratio values ($P<0.01$).

Rest tremor is the most represented type of tremor in early Parkinson’s disease. However, postural and kinetic tremor can affect approximately half of these patients and can occur in absence of resting tremor. As disease progresses, both raphe serotonergic dysfunction and putamen dopamine depletion could contribute to the occurrence of rest tremor. The former is linked to more severe tremor scores and poorer response to dopaminergic therapy. Non-dopaminergic treatments might be beneficial for patients whose tremor is associated with a raphe-predominant dysfunction.
Introduction

Tremor is a rhythmical, involuntary, oscillatory movement of a body part and is clinically classified as rest, postural or kinetic tremor according to the state of activation of the limb in which it occurs (Deuschl et al., 1998). In Parkinson’s disease, rest tremor is a cardinal feature, which supports the diagnosis of the disease. Postural (Jankovic, 2008) and kinetic tremors (Louis and Frucht, 2007) are also common manifestations. However, only a few studies have prospectively evaluated the progression of tremor over the course of the disease (Louis et al., 1999; Vu et al., 2012) and the concomitant prevalence of the three types of tremor in the early stages of the disease has not been fully documented.

The pathophysiology of rest tremor in Parkinson's disease remains poorly understood. Rest tremors are often less responsive to dopamine replacement therapy than rigidity and bradykinesia (Fishman, 2008), suggesting that dopamine deficiency alone does not determine tremor severity, and its response to dopaminergic treatments is influenced by other factors. This clinical observation is supported by a number of in vivo PET and SPECT studies. In these studies, tremor severity did not correlate with levels of nigrostriatal dysfunction (Benamer et al., 2000; Pirker 2003; Rossi et al., 2010) and, generally, tremor predominant patients have been reported to have relatively spared striatal dopaminergic function (Spiegel et al., 2007; Rossi et al., 2010; Helmich et al., 2011; Qamhawi et al., 2015). Conversely, there is growing evidence that rest tremor could be related to degeneration of non-dopaminergic systems. Raphe nuclei serotonergic neuronal dysfunction has been associated with severity of rest tremor. (Doder et al., 2003; Qamhawi et al., 2015), and involvement of the noradrenergic locus coeruleus has also been proposed (Isaias et al., 2011). However, nigro-striatal degeneration is still necessary for a Parkinsonian tremor to occur (Deuschl et al., 2000) and in clinical practice a significant number of tremors respond to dopamine replacement therapy. In one series, dopaminergic depletion in the globus pallidus, rather than in the putamen, was found to be associated with tremor severity (Helmich et al., 2011).
In this study, we evaluated the prevalence and clinical progression of tremor in the early stages of Parkinson's disease. We also examined with $^{123}$I-FP-CIT SPECT the relationship between dopaminergic and serotonergic terminal dysfunction and rest tremor severity both at baseline and after a two-year follow up, and how serotonergic relative to dopaminergic loss influenced the response of rest tremor to dopaminergic therapy. In our previous paper, a cross-sectional baseline analysis of the PPMI cohort data did not show any correlation between serotonergic or dopaminergic dysfunction and severity of postural-kinetic tremor components (Qamhawi et al., 2015; see also Supplementary Table 4); thus, we did not further investigate the association of these two types of tremor and $^{123}$I-FP-CIT findings at the two-year follow up.

In more detail, this study included four main analyses: 1. Estimating the prevalence of rest, postural and kinetic tremors in a large cohort of early Parkinson's disease patients. 2. Evaluating the clinical progression of these tremor components from baseline to the two-year follow up. 3. Investigating how relative levels of serotonin and dopamine dysfunction measured with $^{123}$I-FP-CIT SPECT influence rest tremor severity and whether this relationship changes with disease progression. 4. Determining the association between serotonin and dopamine dysfunctions and the response of rest tremor to dopaminergic therapy.

Methods

Study design and participants
This study included four main analyses: 1. Estimating the prevalence of rest, postural and kinetic tremors in a large cohort of early Parkinson's disease patients. 2. Evaluating the clinical progression of these tremor components from baseline to the two-year follow up. 3. Investigating how relative levels of serotonin and dopamine dysfunction measured with $^{123}$I-FP-CIT SPECT influence rest tremor severity and whether this relationship changes with disease progression. 4. Determining the association between serotonin and dopamine dysfunctions and the response of rest tremor to dopaminergic therapy.
All the clinical data and $^{123}$I-FP-CIT SPECT images used in this study were downloaded from the Parkinson’s Progressive Markers Initiative (PPMI) database. The PPMI is an ongoing longitudinal, international, multicentre, observational clinical study of early Parkinson's disease patients aimed at identifying disease biomarkers (Marek et al., 2011). The study includes ascertainment patients with a clinical disease duration of less than two years who periodically undergo clinical motor evaluation and imaging with $^{123}$I-FP-CIT SPECT. The diagnosis of Parkinson’s disease had to be supported by an *in vivo* evidence of nigrostriatal dopaminergic dysfunction; all patients with tremor and normal scans were followed up as a separate group (Scans Without Evidence of Dopaminergic Deficit). The full list of inclusion and exclusion criteria can be found in the PPMI study protocol, available online at [http://www.ppmi-info.org/study-design/research-documents-and-sops/](http://www.ppmi-info.org/study-design/research-documents-and-sops/).

Our analysis was focused on the period from baseline to the two-year follow up, as not enough patients had completed longer follow ups. We therefore downloaded clinical motor assessments and $^{123}$I-FP-CIT SPECT images at both baseline and two-year follow up from the PPMI database in August 2015. For patients who did not have a two-year assessment the previous or successive one was used.

Baseline clinical assessments were available for 423 patients. Three hundred and ninety-eight of these patients had both baseline and two-year follow up assessments available. Eleven patients at baseline and nine patients at follow up had a total rest tremor amplitude rated > 0 with a constancy rated 0, or vice versa: we assumed that there was an error in data collection and, therefore, these patients were excluded. Thus, 378 patients were eligible for the clinical analysis of tremor.

Two hundred and twenty four patients had both $^{123}$I-FP-CIT SPECT and clinical data available at the two-year follow up allowing correlational analyses between regional tracer binding and tremor component scores. However, the scans of 23 patients did not include the caudal raphe and 28 patients were taking serotonergic agents at the time of SPECT (26 SSRIs, 2 SNRIs). These patients were excluded leaving a total of 173 patients to be included in the correlational analysis.
According to the PPMI study protocol, after starting treatment, patients were asked to attend clinic visits after overnight withdrawal of their anti-parkinsonian medications. MDS-UPDRS was performed in the OFF state and then repeated one hour after receiving medication. Ninety-eight patients had both ON and OFF medication assessments in the same follow-up visit (either the two-year visit or the previous or the successive one) potentially allowing the correlational analyses between regional $^{123}$I-FP-CIT binding and the tremor response to dopaminergic therapy. However, only 38 had a total rest tremor amplitude > 0 and were treated with either levodopa or a dopamine agonist alone (as indicated in the UPDRS III sheet) and were included in this correlational analysis.

**Clinical evaluation**

Clinical features were rated with the Movement Disorder Society’s Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) at baseline and follow up.

For each patient we recorded: rigidity (highest and total score of item 3.3), body bradykinesia (item 3.14), bradykinesia scores (items 3.4, 3.5, 3.6, 3.7, 3.8), postural tremor of the hands (item 3.15), kinetic tremor of the hands (item 3.16), rest tremor amplitude (item 3.17), rest tremor constancy (item 3.18). Then, we calculated:

- The total score for postural tremor (right and left hands; score range: 0-8)
- The total score for kinetic tremor (right and left hands; score range: 0-8)
- The total score for rest tremor amplitude (right and left hands, right and left legs, lip/jaw; score range 0-20)
- The highest score for rest tremor amplitude (score range: 0-4)
- The index of rest tremor, by multiplying rest tremor amplitude total score and rest tremor constancy (score range: 0-80).
In the comparison of tremor scores between patients with unilateral and bilateral rest tremor, the index of rest tremor was calculated using the rest tremor amplitude score of the most affected limb (highest score in item 3.17).

- The percentage improvement in rest tremor amplitude and index of rest tremor scores following treatment, calculated as:

\[
\left( \frac{\text{total rest tremor amplitude in OFF} - \text{total rest tremor amplitude in ON}}{\text{total rest tremor amplitude in OFF}} \right) \times 100
\]

This index was used in order to assess the effect of dopamine replacement therapy.

- The total bradykinesia score (sum of items 3.4, 3.5, 3.6, 3.7, 3.8; score range: 0-40)

Patients with an index of rest tremor ≥ 2, a rigidity score ≤ 1 and a bradykinesia score ≤ 1 were classified as “tremulous” or “isolated rest tremor” patients; patients without rest tremor were classified as “without tremor”; patients who did not fall under these conditions were classified as “mixed”. We also used Stebbins’ classification (Stebbins et al., 2013) in order to phenotypically identify patients as “Tremor Dominant (TD)”, “Postural Instability / Gait Difficulty (PIGD)” or “Indeterminate”.

**123I-FP-CIT SPECT protocol and Region of Interest analysis**

A detailed description of the 123I-FP-CIT SPECT protocol and the ROI analysis is available in a previous article from our group (Qamhawi et al., 2015). We repeated a similar imaging analysis with the 123I-FP-CIT SPECT scans available at the two-year follow up.

Briefly, 123I-FP-CIT SPECT was used to assess dopaminergic and serotonergic transporter availability in the putamen and raphe nuclei, respectively. A region of interest analysis was performed using Analyze 11.0 software (Mayo clinic, MN, USA) and regional 123I-FP-CIT specific binding ratios were measured. Regions of interest were manually drawn on a single subject MRI.
template in Montreal Neurological Institute space available in Statistical Parametric Mapping using Analyze 11.0 software (Mayo clinic, MN, USA). Regions of interest sampling the right and left putamen were drawn according to their anatomical borders (average regions of interest volumes 530 mm$^3$ and 478 mm$^3$, respectively). A region of interest sampling the rostral raphe was drawn in the midbrain and rostral pons (average volume 591 mm$^3$) and a region of interest for caudal raphe was drawn in the lower pons and medulla (average volume 264 mm$^3$), according to the anatomical description of Hornung (2003). A region of interest of the left and right occipital cortices was used as reference region.

The MRI template incorporating these regions of interest was loaded onto the SPECT scan of each patient using Analyze 11.0. Regions of interest were then inspected for correct alignment along x, y and z axes and manually adjusted to account for individual variation, without changing their size and shape. Misaligned scans were excluded as stated above. Regions of interest sampling the putamen, the rostral and caudal raphe nuclei, and the occipital cortex were drawn on an MRI template and transferred onto the SPECT images of each patient; these were then inspected for correct alignment along x, y and z axes, and manually adjusted to account for individual variation.

For each region of interest $^{123}$I-FP-CIT specific binding ratio (SBR) was calculated as follows:

$$SBR = \frac{\left( \frac{\text{mean region of interest counts}}{\text{pixel}} \right) - \left( \frac{\text{mean occipital counts}}{\text{pixel}} \right)}{\left( \frac{\text{mean occipital counts}}{\text{pixel}} \right)}$$

This is also defined as the non-displaceable binding potential for tracers at equilibrium (Innis et al., 2007).

The average of right and left Putamen was taken as the total Putamen SBR; we will refer to this as “Putamen SBR”. The total raphe nuclei SBR was calculated by summing rostral and caudal raphe nuclei SBR; this will be referred to as “Raphe SBR”.
As specific binding ratios for these two total regions of interest were not normally distributed, they were normalised logarithmically: \( \ln (SBR + \text{constant}) \).

Raphe / Putamen Ratio

For each patient the specific \(^{123}\text{I}-\text{FP-CIT}\) binding ratios were used to calculate the Raphe / Putamen specific binding ratio.

\[
\frac{\text{Raphe SBR}}{\text{Putamen SBR}} = \frac{\left( \frac{\text{mean raphe counts}}{\text{pixel}} \right) - \left( \frac{\text{mean occipital counts}}{\text{pixel}} \right)}{\left( \frac{\text{mean putamen counts}}{\text{pixel}} \right) - \left( \frac{\text{mean occipital counts}}{\text{pixel}} \right)}
\]

This “Raphe / Putamen” ratio represents raphe serotonin transporter availability normalised to putamen dopamine transporter availability. Thus, it expresses the involvement of Raphe and Putamen relatively to each other in the single patient: low ratios indicate a predominant dysfunction of the Raphe with respect to the Putamen, while higher ratios imply a more profound degeneration of the Putamen relatively to the Raphe. The relationship between Raphe and Putamen binding values and Raphe / Putamen ratios at follow up is described in the relationship between the Raphe / Putamen ratio and Raphe and Putamen specific binding ratios is represented in Supplementary Figure 1.

This ratio allowed us to study the simultaneous contribution of the serotonergic and dopaminergic systems to the occurrence of rest tremor scores and their response to dopaminergic therapy.

The Raphe / Putamen ratio of each patient was calculated at both baseline for a cohort of 345 patients (thanks to the specific binding ratios made available from a previous study from our group, Qamhawi et al., 2015), and at a two-year follow up in the cohort of 173 patients.

Statistical analysis
In the clinical analyses of tremor, rigidity and bradykinesia, the comparisons of features between different groups of patients were assessed with Student’s t-test with a Welch correction in order to address for the different groups sizes, the inequality of variances and mild skewness (Zimmerman 2004; Fagerland & Sandvik 2009; Fagerland et al., 2011).

For the correlational analyses between tremor scores and Raphe binding values and Raphe / Putamen ratios, we used the non-parametric Spearman’s test due to the non-normal distribution of tremor scores.

The association between the percentage improvements in rest tremor amplitude and the Raphe / Putamen binding ratios was explored with a linear regression analysis: in this case the assumptions of linearity, independence of the errors, homoscedasticity and normality of errors distribution were fulfilled.

The relationship between the follow-up Raphe and Putamen FP-CIT binding values and Raphe / Putamen ratios was investigated through a linear regression analysis.

Results

Clinical evaluation of tremor characteristics

A total of 378 patients were eligible for the clinical evaluation of tremor characteristics. All patients were evaluated in the off-state withdrawn from medication. The clinical and demographic characteristics of these patients at the two-year follow up are summarized in Table 1.

Baseline.

At baseline 12.2% of patients (n=46) had no tremor, while 87.8% (n=332) presented at least one feature of tremor.
Rest tremor was present in 69.6% of patients (n=263 of 378). Of these, 88.2% (n=232) had unilateral rest tremor while 10.3% (n=27) had bilateral rest tremor; four patients (1.5%) only had lip / jaw tremor (Table 24). Compared to patients with unilateral tremor, patients with bilateral tremor had a significantly higher amplitude (1.93 ± 0.68 vs 1.64 ± 0.62, P-value < 0.05), higher constancy (2.70 ± 0.91 vs 1.89 ± 0.99, P-value < 0.001), and index (product of amplitude and constancy) of rest tremor (5.44 ± 2.76 vs 3.43 ± 2.62 P-value < 0.01) in the most tremulous limb. The distributions of tremor scores across unilateral and bilateral tremor groups at baseline are reported in Table 32.

Of the 263 patients showing rest tremor at baseline, 68.4% (n=180) presented with tremor in the upper limbs, 6.1% (n=16) in the lower limbs, and 1.5% (n=4) with lip / jaw tremor; 24% (n=63) presented with a combination of all three regions affected (Supplementary Figure 2).

Postural tremor was present in 52.1% of patients (197 of 378); 148 patients had unilateral postural tremor while 49 had bilateral postural tremor; in these 197 patients the mean (± SD) total postural tremor score was 1.42 ± 0.70.

Kinetic tremor was evident in 51.6% of patients (n=195 of 378); 118 patients presented with a unilateral kinetic tremor and 77 with bilateral kinetic tremor; the mean (± SD) total kinetic tremor score in these patients was 1.56 ± 0.70. Results are summarised in Table 24.

Across the 332 patients with tremor, 24.1% (n=80) had rest tremor alone, 6.0% (n=20) had kinetic tremor alone, and 4.5% (n=15) had postural tremor alone. 12.7% (n=42) had rest and postural tremor; 10.6% (n=35) had rest and kinetic tremor; and 10.2% (n=34) had postural and kinetic tremor. 31.9% of patients (n=106) had all three components of tremor (Figure 1).

Compared to patients with postural and/or kinetic tremors, patients presenting only with rest tremor showed significantly lower rigidity (3.24 ± 2.094 vs 4.26 ± 2.565, P-value < 0.01) and bradykinesia (7.28 ± 4.829 vs 9.04 ± 4.480, P-value < 0.05) (Supplementary Table 1).
Phenotypically, 43 patients presented isolated rest tremor (rigidity score ≤ 1, bradykinesia score ≤ 1, index tremor ≥ 2), 115 had no rest tremor (index tremor = 0) and 220 patients had a mixed phenotype. Compared to patients with a mixed phenotype, patients with isolated rest tremor had significantly higher rest tremor amplitude total score (2.77 ± 1.4 vs 1.97 ± 1.13, P-value < 0.01), rest tremor constancy (2.58 ± 0.91 vs 1.83 ± 0.98, P-value < 0.001) and index of rest tremor (7.42 ± 5.45 vs 4.18 ± 4.11, P-value < 0.001).

According to Stebbins' classification 268 patients could be classified as Tremor predominant (TD) 73 as Postural Instability / Gait Difficulty (PIGD) and 37 as Indeterminate.

**Two-year follow up.**

At follow up 16.1% of patients (n=61) had no tremor, while 83.9% (n=317 of 378) had at least one component of tremor.

Rest tremor was present in 67.9% of patients (n=257 of 378). Of these, 75.9% (n=195) had unilateral tremor while 23.7% (n=61) had bilateral tremor; only one patient (0.4%) had isolated lip / jaw tremor. Patients with bilateral tremor had significantly higher amplitude in the most affected limb (2.26 ± 0.73 vs 1.79 ± 0.68, P-value < 0.001), constancy (2.69 ± 1.04 vs 2.09 ± 1.05, P-value < 0.001) and index of rest tremor (6.44 ± 3.59 vs 4.13 ± 3.07, P-value < 0.001) compared to patients with unilateral tremor. The distributions of tremor scores across unilateral and bilateral tremor groups at follow up are reported in **Table 42**.

Postural tremor was present in 49.5% of patients (n=187 of 378); 133 had unilateral postural tremor, while 54 had bilateral postural tremor; the mean (± SD) total postural tremor score in these patients was 1.65 ± 0.92.

Kinetic tremor affected 46.8% patients (n=177 of 378); 96 presented a unilateral kinetic tremor, 81 a bilateral kinetic tremor; the mean (± SD) total kinetic tremor score in these patients was 1.76 ± 0.91. Results are summarised in **Table 24**.
Among the 317 patients with tremor, 22.7% (n=72) presented with rest tremor alone, 7.3% (n=23) had kinetic tremor alone and 5.1% (n=16) had postural tremor alone. 16.4% (n=52) had rest and postural tremor; 11.0% (n=35) had rest and kinetic tremor and 6.6% (n=21) had postural and kinetic tremor. 30.9% of patients (n=98) presented all three types of tremor (Figure 2).

Compared to patients with postural and/or kinetic tremors, patients presenting with rest tremor only showed significantly lower rigidity (3.78 ± 2.663 vs 5.97 ± 3.135, P-value < 0.001) and bradykinesia (7.88 ± 4.487 vs 12.0 ± 5.259, P-value < 0.001) (Supplementary Table 2).

Phenotypically, 23 patients presented an isolated rest tremor, 122 patients had no rest tremor and 233 had a mixed phenotype.

Rest tremor scores in patients with isolated rest tremor were not significantly different compared to rest tremor scores in patients with a mixed phenotype (Supplementary Table 3).

According to Stebbins’ classification 237 patients were Tremor predominant (TD), 105 Postural Instability / Gait Difficulty (PIGD) and 36 Indeterminate.

Clinical Correlations

The baseline correlations between rest tremor component scores and regional raphe \(^{123}\text{I}-\text{FP-CIT}\) binding in the PPMI cohort have been previously reported by our group and were not repeated in this paper. Briefly, we observed that in patients with isolated rest tremor a significant inverse association was present between raphe transporter binding and constancy of rest tremor (\(\beta = -0.380, P\)-value < 0.05) and the index of rest tremor (\(\beta = -0.322, P\)-value < 0.05). Moreover, significant inverse correlations between raphe transporter binding and rest tremor scores were observed in the entire cohort of 345 patients (Qamhawi et al., 2015). In our previous cross sectional baseline study on the PPMI cohort we did not find any correlations between severity of kinetic and
postural tremor components and serotonergic and dopaminergic neuronal dysfunction (Supplementary Table 4).

At the two-year follow up, we studied the 173 patients who had both clinical and neuroimaging data available and were not taking serotonergic drugs. Only 14 of these patients presented with isolated rest tremor; in these patients, a significant inverse correlation was found between raphe transporter binding and rest tremor amplitude scores (Spearman’s ρ = −0.640, P-value < 0.05). We did not find any significant correlation between raphe serotonin transporter availability and rest tremor constancy (Spearman’s ρ = −0.149, P-value = 0.612) or the index of rest tremor severity (Spearman’s ρ = −0.359, P-value = 0.208).

Due to the paucity of patients with isolated rest tremor, we used Stebbins’ method (Stebbins et al., 2013) in order to classify patients’ phenotype. In 112 Tremor predominant (TD) patients, we again found significant inverse correlations between raphe serotonin transporter availability and rest tremor amplitude (Spearman’s ρ = −0.212, P-value < 0.05), rest tremor constancy (Spearman’s ρ = −0.195, P < 0.05) and the index of rest tremor severity (Spearman’s ρ = −0.192, P-value < 0.05).

At follow up, however, across the entire Parkinson’s disease cohort the correlations between raphe serotonin transporter and rest tremor scores were no longer significant (rest tremor amplitude: Spearman ρ = -0.077; P-value = 0.312; rest tremor constancy: Spearman ρ = -0.081; P-value = 0.291; index of rest tremor: Spearman ρ = -0.088; P-value = 0.248).

In order to better understand the combined contribution of serotonergic and dopaminergic dysfunction to the occurrence of rest tremor scores in the entire cohort, we interrogated the Raphe / Putamen ^123^I-FP-CIT binding ratio. At baseline, in the same cohort of 345 patients previously investigated, we found a significant inverse correlation between this ratio and rest tremor constancy (Spearman’s ρ = −0.141, P-value < 0.01) and the index of rest tremor severity (Spearman’s ρ =
−0.130, P-value < 0.05), while rest tremor amplitude showed an inverse but non-significant trend (Spearman’s ρ = −0.093, P-value = 0.083).

In the cohort of 173 patients at the two-year follow up, the Raphe / Putamen $^{123}$I-FP-CIT binding ratio was significantly and inversely associated with rest tremor amplitude (Spearman’s ρ = −0.244, P-value < 0.01), rest tremor constancy (Spearman’s ρ = −0.264, P-value < 0.001) and the index of rest tremor severity (Spearman’s ρ = −0.252, P-value < 0.001); these results show that low ratios, expressing low raphe serotonin transporter availability coupled with relatively less severe putaminal dopamine terminals degeneration, are associated with higher tremor scores, while higher ratios, suggestive of more profound putaminal involvement and relatively higher serotonin transporter availability, are associated with lower tremor scores.

The correlations between Raphe / Putamen ratio and tremor scores at baseline and follow up are summarised in table 54.

**Tremor response to dopaminergic therapy**

In the 38 patients with rest tremor amplitude > 0 who had both ON and OFF medication assessments at follow-up and were treated with either levodopa or a dopamine agonist alone, we found a significant direct association between the Raphe / Putamen $^{123}$I-FP-CIT binding ratio and the percentage improvement in rest tremor amplitude ($β = 0.457$, P-value < 0.01). Higher ratios, suggestive of more severe putaminal degeneration compared to raphe involvement, correlate with bigger improvements of tremor following acute dopamine replacement therapy, while lower ratios, suggestive of relatively greater raphe involvement associated with less severe putaminal degeneration, correlate with smaller therapeutic responses (Figure 3).

**Discussion**
To our knowledge this is the first study to comprehensively evaluate the clinical progression of tremor components together with neuroimaging correlates in a large cohort of well-characterised patients with early stage Parkinson's disease. One additional strength of the PPMI dataset is that the new MDS-UPDRS was used to separately score the amplitude and constancy of rest tremor, and postural and kinetic tremors as independent features.

At baseline, tremor was present in the majority of patients (87.6%); most of them (79.3%) presented with rest tremor, either alone or associated with another component of tremor. Interestingly, more than 20% (20.7%) of early Parkinson's disease patients showing tremor did not have a classical rest tremor but presented with postural and/or kinetic tremor alone. Among the patients presenting with rest tremor, the majority (68.4%) had tremor in the upper limbs and only a small proportion (6.1%) had lower limb tremor.

At the two-year follow up, tremor affected 84.1% of the cohort; again, the majority of these patients (67.9%) had rest tremor, alone or associated with another component of tremor, while 18.6% had postural and/or kinetic tremor alone.

Postural and kinetic tremor were present in approximately half of the patients at both baseline and follow up. Interestingly, patients presenting with postural and/or kinetic tremors showed more severe rigidity and bradykinesia compared to patients with rest tremor only, both at baseline and follow up. This finding is likely to be related to the fact that patients with an isolated rest tremor component have a less severe putaminal dopaminergic loss of function, which is in turn associated with lower bradykinesia and rigidity compared to patients who do not exhibit rest tremor.

We were unable to find previous studies that longitudinally assessed the prevalence of postural and kinetic tremors in a large cohort of idiopathic Parkinson's disease patients.

The proportion of patients presenting with any component of tremor in this cohort at the early stages of the disease is slightly higher than previously reported in other studies: Hughes et al., reported a prevalence of tremor of 69% at disease onset and 75% during the course of the disease (Hughes et al., 1993); Rajput et al., reported that in 30 patients with pathologically
confirmed cases of idiopathic Parkinson's disease tremor had been present in all patients at some point of the disease (Rajput et al., 1991). The higher proportion of patients with tremor in our cohort may reflect the fact that the MDS-UPDRS emphasises and rates the three different components of tremor separately. However, as study participants in the PPMI study were asked not to take anti-parkinsonian medication for at least six months, it is likely that tremor predominant subjects, who generally have less severe disability, were more ready to enter our study.

A small reduction in the prevalence of all three components of tremor was observed from baseline to the two-year follow up. This reduction could simply reflect daily tremor variability or a residual effect of medications in the practically defined OFF state (see limitations); however, a reduction of the prevalence of tremor during the disease has been reported (Hughes et al., 1993; Toth et al., 2004), possibly due to worsening in rigidity. A longer follow up might help confirm the same trend in this cohort. Interestingly, while the overall prevalence of tremor at follow up was slightly reduced, the proportion of patients with bilateral rest tremor more than doubled (9.8% vs 23.7%) over the two years, suggesting a rapid progression of rest tremor in these patients. Also, at both baseline and follow up, patients with bilateral rest tremor presented higher rest tremor scores compared to patients with unilateral tremor. The proportion of patients with bilateral postural or kinetic tremors also showed a milder increasing trend.

Phenotypically, we observed a reduction of patients presenting with “isolated rest tremor” (tremor with absent or very mild bradykinesia and rigidity) from baseline to the two-year follow up. Initially, tremulous patients showed significantly higher tremor scores compared to patients with a mixed phenotype; however, at the two year follow up, mean tremor scores were similar. This was due to the stability of rest tremor scores in tremulous patients and to increased scores in patients with a mixed phenotype. Again, it will be interesting to evaluate the trend of tremor scores at later follow-ups, even though a further reduction in the number of patients with isolated tremor might be expected, caused by increasing scores in rigidity and bradykinesia.
A previous study from our group on the PPMI cohort baseline data highlighted a significant inverse correlation between raphe transporter availability and tremor scores in a cohort of 345 Parkinson's disease patients, and in a subgroup of patients with a tremulous phenotype (Qamhawi et al., 2015).

In the present study we analysed the two-year follow-up data of these patients: we found an analogous association in a group of 14 patients with a tremulous phenotype, and in 112 Tremor predominant patients. However, across the entire cohort such a correlation was no longer present: this could be due to the fact that as disease progresses a more severe dopaminergic deficit or other factors such as the degeneration of other neurotransmitter systems, influences the manifestation of tremor.

In order to understand the combined contribution of the serotonergic and dopaminergic systems to the generation of tremor, raphe serotonin transporter binding values were normalised to putamen dopamine transporter binding values for each patient. This $^{123}$I-FP-CIT binding ratio (“Raphe / Putamen”) represents raphe serotonin transporter availability adjusted for nigro-putaminal degeneration in the single patient. We found that this Raphe / Putamen $^{123}$I-FP-CIT binding ratio was significantly associated with measures of tremor severity both at baseline and follow up. In particular, lower Raphe / Putamen $^{123}$I-FP-CIT binding ratios were associated with higher tremor scores, while higher ratios were associated with lower tremor scores. Therefore, higher tremor scores seem to be simultaneously associated with low raphe serotonin transporter availability coupled with a less severe dopaminergic dysfunction; conversely, lower tremor scores are associated with a relatively higher serotonin than dopamine transporter availability, due to more severe dopaminergic involvement.

Post-mortem studies have highlighted that tremulous patients have a milder degeneration of substantia nigra pars compacta compared to rigid-akinetic patients (Paulus & Jellinger 1991; Selikhova et al., 2009). Several in vivo imaging studies have confirmed this finding (Spiegel et al., 2007; Rossi et al., 2010; Helmich et al., 2011) and have reported the absence of a correlation
between putamen dopamine transporter availability and tremor scores (Benamer et al., 2000; Pirker 2003; Rossi et al., 2010). In line with these studies, tremulous patients in the PPMI cohort had higher putamen dopamine transporter availability and lower raphe serotonin transporter availability compared to non-tremulous patients at baseline (Qamhawi et al., 2015). These studies demonstrate that tremulous patients have a less profound involvement of dopaminergic neurons in the substantia nigra compared to non-tremulous ones, and while nigro-striatal degeneration is necessary, it is not a sufficient factor in the generation of rest tremor (Deuschl et al., 2000).

These and our findings, however, do not imply the cause of tremor, rather they illustrate that the dysfunction and/or degeneration of different neurochemical systems have an important modulating role on the circuits responsible for facilitating tremor, as suggested by Dovzhenok and Rubchinsky (Dovzhenok and Rubchinsky, 2012).

One of the most striking characteristics of Parkinsonian tremor is its variable response to dopaminergic therapy, as opposed to rigidity and bradykinesia (Fishman, 2008). We explored the response of tremor scores in 38 patients treated with levodopa or a dopamine agonist alone. We found a significant direct correlation between the Raphe / Putamen \( ^{125}\text{I}-\text{FP-CIT} \) binding ratio and the percentage improvement of rest tremor amplitude scores (Figure 3). This means that patients with higher ratios, indicative of a relatively greater involvement of nigro-striatal dopaminergic system compared to the raphe serotonergic system, tend to respond better to dopaminergic therapy; in contrast, patients with lower ratios, representative of a greater dysfunction of the raphe coupled with a less severe involvement of dopaminergic terminals, tend to respond less to dopaminergic therapy. Therefore, from our analysis, it emerges that patients presenting tremor associated with a relatively large putaminal dopaminergic degeneration and less severe involvement of the raphe nuclei, respond better to dopaminergic treatment; this could be due to the fact that dopamine deficiency is, in these patients, the primary neurochemical cause of tremor. On the other hand, patients with a relatively bigger involvement of the raphe compared to the putamen, respond less;
this, instead, could be due the fact that tremor in this subgroup of patients is driven not only by dopamine deficiency, but also by serotonergic dysfunction, and possibly by other neurotransmitter systems. Overall, these findings suggest that the concomitant neuroimaging evaluation of putamen dopaminergic terminals and raphe serotonergic nuclei could be important in the clinical setting in order to detect factors that might influence the response of tremor to dopaminergic medication. This might be particularly important in the subgroup of tremulous patients, in which tremor is the most disabling symptom. We can also hypothesize that patients presenting with a raphe-predominant degeneration might benefit from different treatments with respect to those with a putamen-predominant degeneration. Further investigation in a more controlled setting is needed to better evaluate this relationship.

We acknowledge that our study design presents a number of limitations that need to be discussed.

PPMI defines the OFF state as more than 6 hours from the last antiparkinsonian medication dose. It is possible that some of the longer acting dopamine agonist medications, and even levodopa, might still be affecting to a small extent the clinical ratings in this practically defined OFF state. It is, therefore, possible that the decrease in the percentage of patients presenting with tremor at follow up was in part a residual effect of medication; studies that allow for a longer medication wash-out time are needed to elucidate this point. As previously discussed, literature suggests otherwise and a longer follow up is needed to elucidate this point. As for the evaluation of the response of rest tremor to dopaminergic therapy, the use of the percentage improvement between the OFF and the ON states should have eliminated this possible confounding factor.

70% of the patients recruited in the PPMI exhibited a Tremor Dominant phenotype at baseline. Such high percentage might have produced an overestimation of the proportion of patients
presenting with tremor at both baseline and follow up. However, our results seem to fall in line with the data retrieved in the literature.

At the time of the analysis only 173 patients had a valid $^{123}$I-FP-CIT SPECT scan for clinical correlation, as opposed to 345 patients at baseline. However, 173 patients are a reasonable number to trust the correlations we found.

At the two-year follow up, only 38 patients had simultaneously a valid $^{123}$I-FP-CIT SPECT scan, rest tremor, and OFF and ON assessment during the same visit. Further investigation in a more controlled setting is needed (i.e. bigger number of patients, rest tremor instrumentally recorded, PET scans with a selective tracer for serotonergic neurons, etc.) in order to better characterize the relationship between imaging biomarkers and the variable response of tremor to different types of medications.

We were unable to assess the test / retest variability of the Raphe / Putamen ratio, since patients underwent $^{123}$I-FP-CIT SPECT at screening, and at 12- and 24-months follow ups. However, we correlated individual patients’ baseline and follow up Raphe / Putamen ratios, and we found a strong positive correlation ($\beta = 0.768, P$-Value < 0.001) (Supplementary Figure 3). This finding suggests that the Raphe / Putamen ratio is a stable and reliable biomarker, at least at the initial stages of Parkinson’s disease.

**Conclusion**

Overall a high percentage of patients from the PPMI cohort presented with tremor at disease onset and it was still present after a follow up of two years. Rest tremor is the most represented component across the cohort, while postural and kinetic tremors affect approximately half of the patients.
Raphe serotonergic dysfunction appears to be a driver of the rest tremor clinically observed at baseline and at follow up, especially in patients with a predominantly tremulous phenotype. However, in order to better understand the pathophysiology of rest tremor, it is important to consider the concurrent role of putaminal dopamine deficiency.

Last, we found that better responses of tremor amplitude to acute dopaminergic therapy were associated simultaneously with higher serotonin transporter availability and relatively more severe putaminal dopaminergic dysfunction (high Raphe / Putamen $^{125}$I-FP-CIT binding ratio). Therefore, tremor responsivity to dopaminergic therapy seems to be determined by an interaction between the serotonergic and dopaminergic systems, and non-dopaminergic treatments might be useful for those patients with a raphe-predominant degeneration.

**Acknowledgements**

Data used in the preparation of this article were obtained from the Parkinson’s Progressive Markers Initiative (PPMI) database (www.ppmi-info.org/data); for up to date information on the study, visit www.ppmi-info.org.

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Figure Legend

Figure 1.

A) Venn diagram of the distribution of the three types of tremor at baseline. Percentages are based on the 332 patients with tremor.

B) Pie chart representing the subgroups of patients divided according to the types of tremor they present at baseline. Percentages are based on the whole cohort of 378 patients.
Figure 2.

A) Venn diagram of the distribution of the three types of tremor at the two-year follow up. Percentages are based on the 317 patients with tremor.

B) Pie chart representing the subgroups of patients divided according to the types of tremor they present at the two-year follow up. Percentages are based on the whole cohort of 378 patients.
Figure 3 Raphe / Putamen ratio association with the rest tremor amplitude percentage improvement

Scatter plot showing Raphe / Putamen $^{123}$I-FP-CIT uptake ratio of 38 patients plotted against their respective percentage rest tremor amplitude improvement. Line of best fit is shown. Patients taking levodopa only are represented by a green dot; patients taking a dopamine agonist only are represented by a blue square. The Raphe / Putamen ratio is associated with the percentage improvement of rest tremor amplitude ($\beta = 0.457$, $P$-value < 0.01).
## Tables

**Table 1.** Two-year follow up clinical and demographic characteristics of the 378 Parkinson’s disease patients included in our analysis.

<table>
<thead>
<tr>
<th>Feature</th>
<th>378 PD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD, (range) at baseline</td>
<td>63.1 ± 12.2 (41-86)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>248/130</td>
</tr>
<tr>
<td>Disease duration, months ± SD (range) at baseline</td>
<td>30.79 ± 6.72 (25-60)</td>
</tr>
<tr>
<td>MDS-UPDRS III, mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td>- Baseline</td>
<td>20.70 ± 8.80 (4-51)</td>
</tr>
<tr>
<td>- 24 months</td>
<td>25.9 ± 11.4 (3-68)</td>
</tr>
<tr>
<td>MDS-UPDRS total, mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td>- Baseline</td>
<td>31.9 ± 13.1 (7-70)</td>
</tr>
<tr>
<td>- 24 months</td>
<td>42.2 ± 17.4 (7-103)</td>
</tr>
<tr>
<td>Hohen and Yahr, mean ± SD, range (1-3)</td>
<td></td>
</tr>
<tr>
<td>- Baseline</td>
<td>1.55 ± 0.51 (1-3)</td>
</tr>
<tr>
<td>- 24 months</td>
<td>1.78 ± 0.54 (1-4)</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td></td>
</tr>
<tr>
<td>- Baseline</td>
<td>No medication</td>
</tr>
<tr>
<td>- 24 months</td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>64 (16.9%)</td>
</tr>
<tr>
<td>- Levodopa</td>
<td>109 (28.8%)</td>
</tr>
<tr>
<td>- DA</td>
<td>69 (18.3%)</td>
</tr>
<tr>
<td>- Other PD medication</td>
<td>61 (16.1%)</td>
</tr>
<tr>
<td>- Combination of two or more types of PD medication</td>
<td>75 (19.9%)</td>
</tr>
</tbody>
</table>

PD = Parkinson’s disease; MDS-UPDRS = Movement Disorders Society – Unified Parkinson’s Disease Rating Scale.
Table 2. Prevalence of rest, postural and kinetic tremor in 378 patients at baseline and follow up.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest tremor, %</td>
<td>69.6</td>
<td>67.9</td>
</tr>
<tr>
<td>Rest tremor – Unilateral vs Bilateral, %</td>
<td>88.2 vs 10.3</td>
<td>75.9 vs 23.7</td>
</tr>
<tr>
<td>Postural tremor, %</td>
<td>52.1</td>
<td>49.5</td>
</tr>
<tr>
<td>Postural tremor – Unilateral vs Bilateral, %</td>
<td>75.1 vs 24.9</td>
<td>71.1 vs 28.9</td>
</tr>
<tr>
<td>Kinetic tremor, %</td>
<td>51.6</td>
<td>46.8</td>
</tr>
<tr>
<td>Kinetic tremor – Unilateral vs Bilateral, %</td>
<td>60.5 vs 39.5</td>
<td>54.2 vs 45.8</td>
</tr>
</tbody>
</table>
Table 3
Distributions of the highest rest tremor amplitude score (highest score in item 3.17) and rest tremor constancy (item 3.18 score) across groups with unilateral and bilateral tremor at baseline.
For each of the two MDS-UPDRS items considered, the distribution of each score (from 1 to 4) is compared between patients with unilateral tremor and patients with bilateral tremor.

<table>
<thead>
<tr>
<th>MDS-UPDRS Item</th>
<th>Group / Score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest rest tremor amplitude score (item 3.17)</td>
<td>Unilateral tremor (232 patients)</td>
<td>43%</td>
<td>50%</td>
<td>7%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Bilateral tremor (27 patients)</td>
<td>26%</td>
<td>56%</td>
<td>18%</td>
<td>0%</td>
</tr>
<tr>
<td>Rest tremor constancy (item 3.18)</td>
<td>Unilateral tremor (232 patients)</td>
<td>47%</td>
<td>25%</td>
<td>20%</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Bilateral tremor (27 patients)</td>
<td>11%</td>
<td>26%</td>
<td>44%</td>
<td>19%</td>
</tr>
</tbody>
</table>

Table 4
Distributions of the highest rest tremor amplitude score (highest score in MDS-UPDRS item 3.17) and rest tremor constancy (MDS-UPDRS item 3.18 score) across groups with unilateral and bilateral tremor at follow up.
For each of the two MDS-UPDRS items considered, the distribution of each score (from 1 to 4) is compared between patients with unilateral tremor and patients with bilateral tremor.

<table>
<thead>
<tr>
<th>MDS-UPDRS Item</th>
<th>Group / Score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest rest tremor amplitude score (3.17)</td>
<td>Unilateral tremor (195 patients)</td>
<td>36%</td>
<td>49%</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Bilateral tremor (61 patients)</td>
<td>15%</td>
<td>46%</td>
<td>38%</td>
<td>1%</td>
</tr>
<tr>
<td>Rest tremor constancy (3.18)</td>
<td>Unilateral tremor (195 patients)</td>
<td>37%</td>
<td>29%</td>
<td>21%</td>
<td>13%</td>
</tr>
<tr>
<td></td>
<td>Bilateral tremor (61 patients)</td>
<td>16%</td>
<td>25%</td>
<td>33%</td>
<td>26%</td>
</tr>
</tbody>
</table>
Table 5

Association between the Raphe / Putamen ratio and rest tremor scores at baseline and two-year follow up (Spearman ρ, asymptotic 2-tailed significance).

<table>
<thead>
<tr>
<th></th>
<th>Raphe / Putamen ratio vs</th>
<th>Spearman ρ</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest tremor amplitude</td>
<td>-0.093</td>
<td></td>
<td>0.083</td>
</tr>
<tr>
<td>Rest tremor constancy</td>
<td>-0.141</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Index of rest tremor</td>
<td>-0.130</td>
<td></td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td><strong>Follow up</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rest tremor amplitude</td>
<td>-0.244</td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Rest tremor constancy</td>
<td>-0.264</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Index of rest tremor</td>
<td>-0.252</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Baseline: number of patients = 345
Follow up: number of patients = 173
A) Venn diagram showing the distribution of tremors among different types:
- Kinetic: 23 (7.3%)
- Postural: 16 (5.1%)
- Rest: 72 (22.7%)
- Kinetic and Postural: 98 (30.9%)
- Kinetic and Rest: 35 (11.0%)
- Postural and Rest: 52 (16.4%)

B) Pie chart showing the distribution of patients across different tremor types:
- Rest, Postural, and Kinetic: 26%
- Rest Only: 19%
- Rest and Postural: 14%
- Rest and Kinetic: 9%
- Kinetic and Postural: 6%
- Kinetic Only: 6%
- Postural Only: 4%
- No Tremor: 16%

Total = 378 patients