Author's response to reviews

Title: Subregional 6-[18F]fluoro-L-m-tyrosine Uptake in the Striatum in Parkinson’s Disease

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Dr. Eric M Wassermann, Associate Editor
Dr. Amos Korczyn, Section Editor
BMC Neurology

Dear Drs. Wassermann and Korczyn:

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Thank you for your favorable consideration of our manuscript, which has been revised in accordance with your suggestions and those of the reviewers. We have provided a more detailed description of our image analysis methodology, and excluded the data from three patients who took levodopa at 6 hours before PET, although the results did not change significantly.

A point-by-point response to the reviewers’ comments is provided below

Thank you again for your consideration of our manuscript, which we hope might now be considered suitable for publication in BMC Neurology.

Yours sincerely,

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Point-by-point response to the reviewers' comments:

**Reviewer #1**

1. For the symptoms, are there any correlations between them (e.g. between tremor and rigidity, between rigidity and postural instability)?

   There were positive correlations between the severities of major motor symptoms: rigidity vs. axial symptoms \((r = 0.68, p < 0.001)\), rigidity vs. bradykinesia \((r = 0.56, p < 0.001)\), bradykinesia vs. postural instability \((r = 0.54, p < 0.001)\), and tremor vs. bradykinesia \((r = 0.39, p = 0.014)\). However, tremor did not have a significant relation with rigidity \((r = 0.20, p = 0.20)\) or with axial symptoms \((r = 0.12, p = 0.45)\).

   We added these findings to the Results section.

2. Which side is more affected in the patients. In my impression, the right side is more severe usually. Does it happen?

   In our study, the right side was more severely affected in 55 of the 101 patients. We added this finding to the Results section.

3. Why do you use carbidopa?

   Carbidopa is a decarboxylase inhibitor that prevents the peripheral decarboxylation of 6-[\(^{18}\)F]fluoro-L-\(L\)-tyrosine (FMT), thus increasing the availability of tracer to the brain. Carbidopa does not efficiently penetrate the blood brain barrier and generally has no central nervous system effects. The compound is devoid of clinically detectable pharmacologic activity in the dose ranges used in the present study.

4. Is the ROI on the cerebellum located bilaterally?

   Yes. ROIs were placed on the cerebellar cortex on each side.

5. Why do you use ANOVA? Do you mean repeated measures ANOVA? Did you do the correlation analysis separately?

   We used one-way ANOVA to assess the effect of a single categorical explanatory variable (subregions of the striatum) on a single continuous response variable (striatal-to-cerebellum ratio of FMT uptake). When the one-way ANOVA was significant, differences between series of subregional data were determined using Scheffé’s test. The correlations were analyzed separately. We re-worded the Methods to clarify this point.
6. You mention that asymmetry decreased with disease progression; did you do statistical analysis for this?

Because the asymmetry remains significant in the advanced stage ($p < 0.05$), we deleted the last sentence in the Results (Page 7). Deleted: “but shows a decrease with disease progression”.

7. Table 1: Are there any significant difference between groups?

The mean age of the healthy subjects was younger than those with PD ($p = 0.005$). However, there was no significant difference in SCR of FMT uptake between younger (< 59 years old, $n = 10$)) and older (≥ 60 years old, $n = 9$) subjects (putamen, $p = 0.87$; caudate, $p = 0.81$) in the control group. Exclusion of younger subjects in the control group does not change the results of significant difference in SCR between the PD and control groups. Gender ratio did not differ between the control and PD groups ($p = 0.54$).

8. Table 2: May I ask you to add correlation ratio for tremor and ROI data with $p$ value?

As suggested, we added the $r$ and $p$ values for the correlation between tremor and FMT uptake.

9. Figure 1: Please add SCR scale on the figure?

PET/MRI images in Figure 1 represent radioactive counts per voxel rather than ratios. We added color scales for the count levels instead of SCR for comparison between images.

Reviewer #2

Major considerations.

1. Lack of novelty.

To the best of our knowledge, this is the first study in which 6-[18F]fluoro-L-m-tyrosine (FMT) has been applied to Parkinson’s disease (PD) patients in large numbers. The high sensitivity of FMT is suitable for analyzing the various stages of PD. We consider that our findings have implications for understanding the progression pattern of degeneration in PD and may also contribute towards early diagnosis of PD. FMT imaging also has higher spatial resolution than imaging with conventional tracers, and this advantage is essential for assessing the aromatic L-amino acid
decarboxylase (AADC) gene expression in gene therapy trials for PD. Our results provide important baseline data for evaluating the effects of surgical interventions such as gene therapy for PD.

2. The authors describe their methods as 2D ROI method, where striatum per cerebellum ratios are averaged for several ROIs (if I understand correctly their approach). Given the already acquired MRI images why the authors did not try to define VOIs and use VOI based analysis? I would not require them to recalculate their whole dataset, but in a sample size of subjects (~20 subjects) they could demonstrate that their ROI method provide comparable measures with VOI approach.

The ROI in the present study is defined in three dimensions (3-D), and is actually a volume-of-interest (VOI). Subregions in the striatum were delineated on three to five adjacent MRI planes corresponding to the same planes on the PET images. The 3-D ROIs (VOIs) were then extracted automatically by connecting two-dimensional drawings on each plane using a linear interpolation algorithm for VOI outlines. Cerebellar ROIs were also defined in 3-D.

We revised the Methods section to clarify this point.

3. In results when the correlation between the clinical measures and SCR is detailed the authors did not address the potential need for age correction. They should demonstrate that age correction does not change significantly their results. Presynaptic dopamine measures are subject to 3-5% decrease per decade, if the age range is large, this can affect their measurements, and the potential lack of correlation in elderly.

We analyzed the older patients (> 60 years old; n = 25) separately and found similar correlations between major symptoms and FMT uptake. We added these results to Table 3.

Minor considerations.

1. I am not sure that suspending dopaminergic medications only 6 hours before scanning is enough. How could the authors defend this relatively short interval (usually it is at least 12hr).

In fact, all but three patients stopped levodopa for at least 16 hours. These three patients took levodopa early in the morning, 6 hours before PET, before coming to the hospital by themselves. We excluded these three patients from analysis. Accordingly, we revised the clinical characteristics of the patients shown in Figure 2, Figure 3, Table 1, and Table 2. Exclusion of these patients, however, caused no significant changes to the results.
2. The authors should consider to describe their image analysis method with more detail. They state in their paper that they coregistered MR and PET images at one point. I suppose this was done through the CT images which they could achieve from the PET/CT scanner, because in dopaminergic PET/SPECT studies the coregistration of the fully anatomical images with the fully functional PET images is not solved.

We agree that coregistration of PET and MR images is difficult. We used PET/CT and 3T MRI to minimize anatomical dislocation. PET and MRI imaging data were coregistered by a fusion program (Syntegra, Philips) that provided manual and point-based image registration as well as automated methods of gray value-based image registration, including a mutual information algorithm (Maes F, et al., *IEEE Trans Med Imaging* 1996, 15:429-442). In addition, an adaptive level set of segmentation was used for coregistration of the CT and MRI imaging data (Wells WM, et al., *IEEE Trans Med Imaging* 1997, 16:187-198). We added this description to the Methods section.