Serotonin transporter in Parkinson’s disease: a meta-analysis of PET studies

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Running head: SERT in Parkinson’s disease

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ABSTRACT

Positron emission tomography (PET) is a powerful analytical tool for the in vivo molecular imaging of the human brain. Over the past years, a number of PET studies imaging the serotonin transporter (SERT) have been employed, and provided evidence for the key role of serotonergic pathology in patients with Parkinson’s disease (PD). Here, we review the role of SERT in the development of motor and non-motor complications in patients with PD, and we performed a meta-analysis to identify the patterns of SERT pathology and the relevance to symptoms. Consistent SERT pathology in raphe nuclei, striatum, thalamus and hypothalamus and associations with ageing, PD progression, development of dyskinesias, and cognitive decline were observed.

Key-words: PET; DASB; Parkinson’s disease

Domain: Neuroscience
INTRODUCTION

Serotonergic dysfunction occurs early in the course of Parkinson’s disease (PD)\(^1\) and is characterized by the presence of Lewy bodies in raphe nuclei\(^2\) and by loss of terminals in serotonin-containing neurons.\(^3\) According to Braak’s staging of PD, the pathological processes in serotonergic median raphe nuclei occur in Stage 2, which is a presymptomatic stage preceding the lesions of dopaminergic midbrain neurons.\(^2\) Biochemical and post-mortem studies have also confirmed that the serotonergic system is affected in PD.\(^4\) – \(^6\)

Positron emission tomography (PET) is a powerful in vivo technique that has been employed for the quantification of serotonin pathology in vivo in patients with PD.\(^7\)\(^,\)\(^8\) This molecular imaging technique allows to measure in vivo metabolic processes and aid in the understanding of aetiology and pathophysiology of diseases, and in the identification of novel pharmacological targets. Several PET ligand have been developed for the evaluation of serotonergic receptors and SERT.\(^9\) \(^[^{11}C]\)-3-Amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile (\[^{11}C\]DASB) is a second generation PET ligand with high selectivity for the SERT,\(^9\) which is superior compared to first generation ligands.\(^10\) Over the past years, a number of PET studies imaging the SERT have been employed, and provided evidence for the key role of serotonergic pathology in patients with PD.\(^3\)\(^,\)\(^4\)

The aims of this study are to systematically review the role of SERT in the development of motor and non-motor symptoms in PD and to examine in vivo SERT changes in PD patients compared to healthy controls (HCs) as measured by \(^[^{11}C]\)DASB PET by conducting a meta-analysis for identifying patterns of SERT pathology and the relevance to symptoms.
METHODS

The study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) and according to the recommendations from the Cochrane Collaboration and Meta-Analysis Of Observational Studies in Epidemiology (MOOSE)\textsuperscript{11} using a methodology as previously described.\textsuperscript{12,13}

**Search Strategy**

MEDLINE, ISI Web of Science, Cochrane Library and Scopus electronic databases were searched for articles in English published before 1\textsuperscript{st} October 2016. Studies were identified, combining the following major Medical Subject Headings: “Parkinson’s disease” and “PET” combined with text and key words. Additional eligible studies were identified screening the reference lists of studies included in our analysis.

**Specific aims for Meta-Analyses**

The current study aims to systematically examine the consistency of \textit{in vivo} SERT changes in PD \textit{vs} HCs as measured by \textsuperscript{[11]}C\textsuperscript{DASB} PET imaging studies. The primary outcome was the mean difference of \textsuperscript{[11]}C\textsuperscript{DASB} binding in striatal and extra-striatal brain areas between PD patients and HCs and in relation to the duration and stage of the disease. The secondary outcome was to explore the pattern of \textsuperscript{[11]}C\textsuperscript{DASB} binding according to motor and non-motor symptoms profile.

**Selection Criteria**

All selected titles and abstracts were independently reviewed by two investigators (GP, FN). Studies were excluded if the title and/or abstract were not appropriate for the aim of the review. Full texts were subsequently obtained for eligible studies or when the relevance of an
article could not be certainty excluded. Selected studies were eligible if they met the following criteria: (a) cross-sectional, case control or longitudinal $[^{11}\text{C}]\text{DASB}$ PET studies including PD patients with confirmed diagnosis based on the UK brain bank criteria, (b) studies published in peer-reviewed journals, (c) $[^{11}\text{C}]\text{DASB}$ PET measures reported as mean ± standard deviation (SD), and (d) Studies not including HCs were included in the meta-analysis only for secondary analyses within PD subgroups. In cases of two or more studies from the same centre, we have carefully checked for overlapping samples by contacting the corresponding authors to verify that there was not a significant overlap in the samples. For studies including overlapping PD samples, we have included only the study with the largest sample in the meta-analysis.

**Risk of bias in included studies**

The quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS)\textsuperscript{14} that includes selection, comparability, and exposure (case-control studies) or outcome (cohort studies). The scale ranged from zero to six stars with the highest degree representing the greatest methodological quality. Disagreement was resolved by consensus and by opinion of a third reviewer (MP). The presence of publication bias was explored by performing the test for asymmetry of the funnel plot by Egger.\textsuperscript{15}

**Data extraction**

Two reviewers (GP, FN) independently completed the data extraction and variables extracted were study year, author first name, gender, mean age of participants, number of participants, disease duration (years), Hoehn and Yahr (H&Y) scale, Unified Parkinson's Disease Rating Scale (UPDRS) total score and UPDRS Part-III motor score, MMSE and $[^{11}\text{C}]\text{DASB}$ binding data.
**Statistical Analysis**

Data were analysed using the Comprehensive Meta-Analysis software, version 3 (Biostat, Englewood, NJ, USA). Considering we have included studies using only $^{[11]}\text{C}$DASB PET, comparisons between patients with PD and HC and those within PD patients have been calculated as difference in means (MD). A negative MD indicates a decreased $^{[11]}\text{C}$DASB binding in PD patients compared to HC. The results were pooled using the inverse variance method. Heterogeneity was assessed using the $I^2$ index statistic, which accounts for between-study (or inter-study) variability as opposed to within-study (or intra-study) variability. Heterogeneity was considered substantial if the $I^2$ value was greater than 40%. Due to the heterogeneity of samples, a random effect model was used to summarise data instead of a fixed effect model, independently of statistical evidence for heterogeneity.

To investigate the impact of individual studies on the results, we undertook one study-removed analysis by omitting one study in each turn and recalculating the pooled estimates on remaining studies. To explore the influence of potential effect modifiers on $^{[11]}\text{C}$DASB binding, meta-regression analyses were performed to test age, gender (male %), disease duration, H&Y scale, UPDRS total score and UPDRS Part-III motor score, MMSE and quality of the study (NOS), when at least 10 studies were available for each modifier. For all meta-regression analyses, a random effect model was used to take into account the mean of a distribution of effects across studies. To estimate the additive (between-study) component of variance $\tau^2$, the restricted maximum likelihood method was used to take into account the occurrence of residual heterogeneity, not explained by the potential effect modifiers. All reported test results were two-tailed and statistical significance was set at $P<0.05$. 


SYSTEMATIC REVIEW

SERT pathology in PD

Over the past years $[^{11}\text{C}]$DASB PET studies have demonstrated SERT changes in patients with PD,\textsuperscript{1,20-22}. Staging of SERT dysfunction in PD patients has shown reductions of $[^{11}\text{C}]$DASB binding in the caudate, thalamus, hypothalamus, and anterior cingulate cortex in early PD patients followed by additional $[^{11}\text{C}]$DASB binding reductions in the putamen, insula, posterior cingulate cortex, and prefrontal cortex in patients with established PD. Advanced PD patients showed further $[^{11}\text{C}]$DASB binding reductions in the ventral striatum, raphe nuclei and amygdala.\textsuperscript{1}

Other studies have reported modest widespread reductions of $[^{11}\text{C}]$DASB binding in caudate, putamen, midbrain and orbitofrontal cortex in advanced PD patients compared to controls.\textsuperscript{20} $[^{11}\text{C}]$DASB binding in early PD patients has been reported both reduced\textsuperscript{1,21} and within normal levels.\textsuperscript{22} In Strecker and colleagues study,\textsuperscript{22} however, only nine \textit{de novo} non-demented and non-depressed PD patients were compared to nine controls, and the small sample size may explain the lack of statistical significance.

Motor symptoms and complications

Serotonergic pathology has been associated with the development of motor symptoms\textsuperscript{23,24} and complications.\textsuperscript{25-30} The development of tremor in PD has been speculated to be underlined by non-dopaminergic mechanisms due to the fluctuating response of this symptom to dopaminergic supplementation and the lack of correlations with dopaminergic molecular imaging markers.\textsuperscript{31-33} $[^{11}\text{C}]$DASB PET studies have shown reduced SERT binding in the caudate, putamen, raphe nuclei and thalamus of tremor-dominant PD patients with compared with those who had akinetic-rigid phenotype. Moreover, caudate, putamen and raphe nuclei
SERT levels correlated with postural and action tremor. These findings complement earlier results which demonstrated a correlation between reduced raphe 5-HT$_{1A}$ binding and severity of resting tremor in patients with PD.

The role of serotonergic mechanisms in the development of levodopa-induced dyskinesias (LIDs) in PD patient have been evaluated in four PET studies. First, by using [$^{11}$C]DASB and [$^{11}$C]raclopride (to evaluate striatal dopamine release) PET imaging, we found a relative preservation of SERT binding in putamen in PD patients with LIDs and demonstrated that identical levodopa doses induced markedly higher striatal synaptic dopamine concentrations in PD patients with LIDs compared with PD patients with stable responses to levodopa. Moreover, oral administration of the serotonin receptor type 1A agonist buspirone prior to levodopa reduced levodopa-evoked striatal synaptic dopamine increases and attenuated LIDs.

Lee and colleagues found no difference in striatal dopaminergic pathology (assessed by [$^{18}$F]FP-CIT PET) between dyskinetic and non-dyskinetic PD patients, however, [$^{11}$C]DASB to [$^{18}$F]FP-CIT binding ratio (indicating serotoninergic to dopaminergic terminal availability) was highest in the putamen of PD patients with LIDs. These findings were confirmed later by Roussakis et colleagues which demonstrated that SERT to DAT ratio increases as PD progresses and patients experience LIDs. Overall these findings suggest that when the dopaminergic innervation in the striatum is critically low, the serotonergic system plays an important role in development of LIDs. Smith et colleagues used [$^{11}$C]DASB PET and found relative preservation of SERT binding in the globus pallidus in PD patients with LIDs that was associated with a significant rise in pallidal synaptic dopamine levels, detected by
[\textsuperscript{11}C]raclopride PET, and suggesting that pallidal serotonergic terminals are also important in the development of LIDs in PD.

Serotonergic mechanisms such as excessive striatal innervation and high serotonin to dopamine striatal terminal ratio have been also associated with the development of graft-induced dyskinesias in PD patients who underwent striatal transplantation with foetal ventral mesencephalic tissue.\textsuperscript{29,30}

\textit{Non-motor symptoms}

The pathophysiology of non-motor symptoms in PD remains unclear, and serotonergic pathology has been hypothesized as one of the main mechanisms underlying depression,\textsuperscript{22,34-36} fatigue,\textsuperscript{37} apathy,\textsuperscript{38,39} weight changes,\textsuperscript{40} sleep problems,\textsuperscript{41,42} olfactory dysfunction\textsuperscript{43} and visual hallucinations in PD.\textsuperscript{44} An [\textsuperscript{11}C]DASB PET study in transplanted PD patients with dopamine-rich foetal ventral mesencephalic tissue and successful dopaminergic reinnervation has shown widespread progressive decline of SERT binding up to three decades following diagnosis that could be associated with the development of non-motor symptoms in PD.\textsuperscript{45}

We have found three studies investigating SERT levels in PD depression\textsuperscript{22,34,35}. In two of them\textsuperscript{34,35} an increase of SERT binding in limbic structures\textsuperscript{34} and in raphe nuclei\textsuperscript{35} was found in depressed compared with non-depressed PD patients. Higher SERT levels were correlated with worse depressive symptoms as assessed by Hamilton Depression Rating Scale,\textsuperscript{34,35} or Beck Depression Inventory-II.\textsuperscript{35} A third study\textsuperscript{22} found no correlation between depression and SERT levels in PD. However, this study was not conducted on PD patients with a clinical diagnosis of depression. The prevalence of depression in PD is higher than general population. This might due to the combination of SERT upregulation, which is typical of depression, and loss of serotonergic terminals, which is typical of PD.
We found only one study investigating the relationship between SERT and fatigue. Compared to the PD patients without fatigue, PD patients with fatigue showed about 75% $[^{11}\text{C}]$DASB binding reduction in putamen, caudate, ventral striatum, thalamus, cingulate and amygdala. SERT dysfunction has also been associated to apathy in one recent study. They used $[^{11}\text{C}]$DASB and $[^{11}\text{C}]$PE2I PET to index presynaptic serotonergic and dopaminergic function, respectively, in fifteen apathetic and fifteen non-apathetic untreated patients with Parkinson’s disease. Their findings demonstrate greater serotonergic loss in the basal ganglia in apathetic patients with Parkinson’s disease compared to patients without apathy, while both Parkinson’s disease groups showed reduced dopaminergic uptake compared to controls. Moreover, greater serotonergic loss in caudate and orbitofrontal cortex correlated with the severity of apathy in patients with Parkinson’s disease, whereas apathy was not associated with dopaminergic deficits. One $[^{11}\text{C}]$DASB PET investigated the link between SERT dysfunction and body mass index (BMI). PD patients with abnormal BMI changes over a 12-month period showed significantly increases of $[^{11}\text{C}]$DASB binding in rostral raphe nuclei, hypothalamus, caudate nucleus and ventral striatum compared to subjects with no significant BMI changes.

Only few human studies have been conducted investigating the role of SERT pathology in the development of sleep problems in PD. Kotagal et colleagues found no differences in brainstem and striatal $[^{11}\text{C}]$DASB binding between PD patients with and without RBD. Lelieveld and colleagues found decreased $[^{11}\text{C}]$DASB binding in the brainstem of PD patients with sleep-disordered breathing that was not associated with the severity of sleep-disordered breathing.
Olfactory dysfunction can precede the motor onset of PD symptoms by many years. Only one study investigated the association between smell problems and SERT dysfunction. They found no significant correlations between UPSIT scores and $[^{11}\text{C}]$DASB binding within raphe nucleus, amygdala, hippocampus, striatum, or neocortex. This finding suggests no major role of SERT damage in the deficit of odor identification in PD.

No studies have investigated SERT binding and visual hallucinations using $[^{11}\text{C}]$DASB PET. However, a study using $[^{18}\text{F}]$setoperone PET imaging in PD patients showed that visual hallucinations were associated with increased 5-HT2A binding in ventral visual pathway, dorsolateral prefrontal cortex, medial orbitofrontal cortex, and insula. These findings suggested that abnormalities in serotonin neurotransmission could be involved in the neural mechanisms underlying the development of visual hallucinations that are associated with PD, and support the use of selective 5-HT2A receptor antagonists for their treatment.

RESULTS OF META-ANALYSIS

Of 6380 articles identified by the initial search, 49 were retrieved for more detailed evaluation. We then excluded 29 PET and SPECT studies which used ligands with no specific SERT binding. Finally, 20 $[^{11}\text{C}]$DASB PET studies were included in the meta-analysis. The study cohorts in this meta-analysis included 234 PD patients and 225 HCs (Table 1). The quality of included studies was moderate or good, varying from four to six NOS stars (Table 2).

Primary outcome: $[^{11}\text{C}]$DASB binding in PD patients compared to HCs and in relation to the duration and stage of the disease
PD patients showed a significant reduction of $[^{11}\text{C}]\text{DASB}$ binding compared to HCs in rostral raphe, caudal raphe, putamen, caudate, ventral striatum, thalamus and hypothalamus (Figure 1).

In presence of relevant heterogeneity ($I^2>40\%$) and where more than 10 $[^{11}\text{C}]\text{DASB}$ studies were included in the analysis, a meta-regression was carried out for the primary outcome of $[^{11}\text{C}]\text{DASB}$ binding in putamen, caudate, ventral striatum, thalamus and hypothalamus (Figure 2). Longer disease duration was correlated with decreased $[^{11}\text{C}]\text{DASB}$ binding within putamen (Figure 2A; n of studies=19, $\beta=-0.0204$, change of $\tau^2=0.0277$, 95% CI=−0.0339 to −0.0069, $P=0.0031$; $r^2=0.95$). Older age and lower MMSE but not disease duration was correlated with decreased $[^{11}\text{C}]\text{DASB}$ binding in caudate (Figure 2B; n of studies=19, change of $\tau^2=0.0192$; Age: $\beta=-0.0258$, 95% CI=−0.0469 to −0.0048, $P=0.0163$; MMSE: $\beta=0.1193$, 95% CI=0.0154 to 0.2233, $P=0.0244$; $r^2=0.56$). Decreased $[^{11}\text{C}]\text{DASB}$ binding in ventral striatum was not correlated with disease duration or with any other potential modifiers. Decreased $[^{11}\text{C}]\text{DASB}$ binding in thalamus was correlated with longer disease duration (n of studies=17, $\beta=-0.0156$, change of $\tau^2=0.0311$, 95% CI=−0.0246 to −0.0067, $P=0.0006$; $r^2=0.94$) and H&Y stage (n of studies=13, $\beta=-0.1243$, change of $\tau^2=0.0021$, 95% CI=−0.2043 to −0.0443, $P=0.0023$; $r^2=1.00$) at univariate analysis. However, at multivariate meta-regression including disease duration and H&Y stage, there were no correlations. Decreased $[^{11}\text{C}]\text{DASB}$ binding within hypothalamus was not correlated with disease duration but with male gender (n of studies=12, change of $\tau^2=0.0163$; $\beta=-0.0073$, 95% CI=−0.0128 to −0.0018, $P=0.0093$; $r^2=1.0$).

**Secondary outcomes: $[^{11}\text{C}]\text{DASB}$ binding within PD subgroups according to motor and non-motor symptoms**
[\textsuperscript{11}C]DASB binding between dyskinetic PD vs non-dyskinetic PD patients showed no differences in putamen (n of studies=3, PD patients with dyskinesia=51, PD patients without dyskinesia=33; MD=-0.106, 95% CI=-0.284 to 0.072, P=0.243; \textit{I}^2=71.3\%) and globus pallidus (n of studies=2, PD patients with dyskinesia=22, PD patients without dyskinesia=22; MD=-0.091, 95% CI=-0.147 to 0.328, P=0.455; \textit{I}^2=83.9\%). Caudate [\textsuperscript{11}C]DASB binding was reduced in PD dyskinetic compared to non-dyskinetic PD patients (n of studies=3, PD patients with dyskinesia=51, PD patients without dyskinesia=33; MD=-0.178, 95% CI=-0.294 to –0.062, P=0.003; \textit{I}^2=46.4\%).

We found only one study investigating [\textsuperscript{11}C]DASB binding in PD patients with and without tremor\textsuperscript{,23} thus no meta-analysis has been performed for this outcome. Three studies investigated the association between [\textsuperscript{11}C]DASB binding and depression levels in PD.\textsuperscript{22,34,35} However, no meta-analysis has been performed for this outcome because two studies did not report [\textsuperscript{11}C]DASB binding in PD without depression.\textsuperscript{34,35} Only one study was found investigating [\textsuperscript{11}C]DASB binding in PD patients with and without fatigue,\textsuperscript{37} one in PD patients with rapid eye movement sleep behavior disorder,\textsuperscript{41} and one with sleep-disordered breathing\textsuperscript{42} thus, no meta-analyses have been performed for these outcomes.

\textit{Publication Bias and Sensitivity Analysis}

For the primary outcome, the Egger test was not significant in any of the brain areas, with the exception of ventral striatum (P=0.011), indicating a risk of publication bias only for this area. Robustness of meta-analytic findings was confirmed by sequentially removing each study and re-analysing the remaining data set (producing a new analysis for each study removed). The results remained unchanged regarding direction and magnitude (data not shown). Meta-regression revealed no significant effect of the quality of the studies (NOS
scores) on $[^{11}\text{C}]{\text{DASB}}$ binding in any brain area, with the exception of hypothalamus (n of studies=12, $\beta=0.2145$, change of $\tau^2=0.0163$, 95% CI=0.0369 to 0.3922, $P=0.0018$; $\tau^2=0.39$) in which lower study quality was associated with lower $[^{11}\text{C}]{\text{DASB}}$ binding.

**DISCUSSION**

To the best of our knowledge, this is the first comprehensive systematic review and meta-analysis investigating SERT changes in PD. Compared to HCs, $[^{11}\text{C}]{\text{DASB}}$ binding has been generally reported decreased in PD. Only studies in PD depression demonstrated relative increased $[^{11}\text{C}]{\text{DASB}}$ binding in patients with PD depression compared to non-depressed PD patients.\textsuperscript{34,35} $[^{11}\text{C}]{\text{DASB}}$ binding in early PD patients has been reported either reduced\textsuperscript{1,21} or within normal levels.\textsuperscript{22}

With regards to motor symptoms, we found in our systematic review that SERT dysfunction is associated with the development of tremor and dyskinesias in PD. With regards to non-motor symptoms, we found in our systematic review that SERT dysfunction is associated with depression, fatigue, apathy and weight changes. Associations with sleep problems and olfactory dysfunction need further investigations while visual hallucinations seems to be related with impairment of serotonergic receptors more than SERT levels. However, due to the small number of studies and relatively small sample sizes, the conclusions regarding the relationship between serotonergic dysfunction and clinical features of PD need further evaluations.

Our meta-analysis demonstrates that SERT binding is reduced in patients with PD in rostral and caudal raphe, putamen, caudate, ventral striatum, thalamus and hypothalamus. Reduced SERT binding in putamen is associated with longer duration of the disease. PD patients with
dyskinesias showed preserved SERT binding in putamen compared to non-dyskinetic PD patients. Lower \(^{11}\)C]DASB binding in caudate is associated with worse cognitive function and older age.

The widespread decrease of SERT binding in patients with PD confirms the important role of serotonergic system dysfunction in the pathophysiology of PD. The highest decreases in SERT binding were observed in the rostral raphe and caudate followed by putamen, thalamus, ventral striatum, caudal raphe, and hypothalamus. According to Braak’s staging, raphe nuclei are one of the first brain regions affected by Lewy body and neurite deposition and could be preceding dopaminergic pathology and the development of motor symptoms.\(^2\) Later stages involve pathology in the hypothalamus, the striatum and the thalamus. Also, previous molecular imaging and post-mortem studies have reported a preferential loss of SERT binding in the caudate compared to the putamen.\(^1\),\(^4\),\(^6\) Our meta-analytical findings are in line with these observations.

Our findings demonstrate that loss of SERT binding in caudate and putamen have important clinical relevance. The risk of cognitive impairment increases with ageing and our findings show that SERT binding in caudate is reduced with advanced age and is associated with worse cognitive scores. Several pathological processes have been implicated in the development of cognitive impairment in PD and these include degeneration of subcortical cholinergic and dopaminergic projections, microglial activation, and neocortical pathology associated with misfolded protein depositions or vascular pathology.\(^49\) A recent study has demonstrated that the combined presence of striatal and cortical \(\beta\)-amyloidopathy is associated with greater cognitive impairment than cortical \(\beta\)-amyloidopathy alone in PD,\(^50\) indicating an important role of striatal \(\beta\)-amyloid pathology in the development of cognitive
impairment in PD. Additional studies have shown that β-amyloid levels could affect serotonin signalling\textsuperscript{51,52} and that striatal serotoninergic degeneration may promote the development of cerebral amyloidopathy in patients with PD.\textsuperscript{48}

Our findings confirm that SERT binding in putamen plays a crucial role in the pathophysiology of dyskinesias in patients with PD. Our data show relative preservation of SERT binding within putamen in dyskinetic PD compared to non-dyskinetic PD patients. Previous experimental studies have demonstrated that abnormal involuntary movements in rats are critically dependent on the integrity of serotonergic projections and that removal of striatal serotonergic afferents or the dampening serotonergic activity with serotonin receptor agonists attenuated abnormal involuntary movements.\textsuperscript{53–55} Clinical and molecular imaging studies in humans have shown that striatal serotonergic terminals contribute to the abnormal levodopa- or graft-induced increases in synaptic dopamine levels in PD patients and in the development of dyskinesias.\textsuperscript{25–30,56} Although SERT binding in putamen decreases with advanced disease, it remains unclear why PD patients with dyskinesias have relatively preserved putaminal serotonergic function. It is possible that SERT upregulation or interactions with other systems may play a role. Future studies will be needed to elucidate this mechanism.

In this meta-analysis, data showed that reduced SERT binding in the hypothalamus was associated with male gender. Previous studies on gender differences in PD have shown a more benign phenotype in women with PD, which could be the result of higher physiological striatal dopamine levels, possibly due to the activity of oestrogens.\textsuperscript{57} Oestrogens also regulate gene expression of serotonin in midbrain and hypothalamus and their effect in SERT binding cannot be excluded.\textsuperscript{58}
The main strength of our meta-analysis was the systematic strategy and the inclusion of PET studies with high selectivity for SERT. The main limitations are that the meta-analysis was not carried out on individual patient data stratified for different PD symptoms and was limited to non-cortical regions. We have decided to exclude cortical areas due to the lack of data and the heterogeneity of reporting results among the studies. Additional limitations included the small sample sizes and low quality of some studies that represent a potential risk of bias (e.g. for the hypothalamus). Another possible limitation is the presence of publication bias for ventral striatum. The risk of bias evidenced by the funnel plot asymmetry needs to be interpreted considering the small number of studies included.

**Future directions**

Serotonergic pathology plays a key role in the progressive neurodegenerative process in the course of PD and PET imaging such as $^{[11]}$C]DASB PET provides the means for direct visualisation and quantification *in vivo* in patients with PD. Although several studies have been performed using serotonergic PET ligands, the vast majority of associations between serotonergic pathology and PD symptoms requires further validation. Future $^{[11]}$C]DASB PET studies in subjects at higher risk for developing PD and in familial forms of PD may shed light for the premanifest stages and pathology. Currently all studies have been cross-sectional and new longitudinal designs, perhaps employing various PET ligands for the quantification of serotonergic markers, are needed to better understand the progression of serotonergic pathology and the relation to symptoms and complications in patients with PD.

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**Authorship**

G.P. collected the data and performed the statistical analysis. F.N., P.F.P. and M.P. validated the extracted data, and contributed to the analysis and the interpretation of the results. G.P. and M.P. drafted the manuscript whereas all authors gave input.

**Potential Conflicts of Interest**

The authors report no conflict of interest.
REFERENCES


FIGURE LEGENDS

**Figure 1** Pooled analysis for $[^{11}\text{C}]$DASB binding in PD patients compared with HCs.

PD = Parkinson’s disease; HCs = Healthy controls.

**Figure 2** Meta-regression analysis for primary outcome exploring effect modifiers and $[^{11}\text{C}]$DASB binding within putamen (A) and caudate (B). Differences in means are displayed on a logarithmic scale. Circles represent studies included in the meta-analysis. The size of circles represents the weight of each study on the results of the meta-analysis.

MMSE = Mini-Mental State Examination; PD = Parkinson’s disease; HCs = Healthy controls.
Table 1 [14C]DA51B PET studies included in the meta-analysis.

<table>
<thead>
<tr>
<th>Study and Group</th>
<th>Age (yrs)</th>
<th>Male (%)</th>
<th>Disease duration (years)</th>
<th>H&amp;Y stage</th>
<th>OFF MDS-UPDRS total</th>
<th>OFF MDS-UPDRS part 3</th>
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<td>83.3%</td>
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<td>PD non-depressed (n=24)³⁵</td>
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<td>60%</td>
<td>10.3</td>
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<td>81.9</td>
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<td>PD Early (n=10)¹</td>
<td>62.7</td>
<td>80%</td>
<td>3.4</td>
<td>1.9</td>
<td>30.9</td>
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<td>28.8</td>
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<td>PD Established (n=10)¹</td>
<td>62.4</td>
<td>80%</td>
<td>7.5</td>
<td>3.4</td>
<td>69.4</td>
<td>N/A</td>
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<tr>
<td>PD Advanced (n=10)¹</td>
<td>67.2</td>
<td>80%</td>
<td>14.1</td>
<td>3.4</td>
<td>78.7</td>
<td>N/A</td>
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<tr>
<td>PD without fatigue (n=8)³⁷</td>
<td>64.3</td>
<td>62.5%</td>
<td>4.25</td>
<td>N/A</td>
<td>N/A</td>
<td>33.9</td>
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<tr>
<td>PD with fatigue (n=7)³⁷</td>
<td>64.8</td>
<td>62.5%</td>
<td>5.75</td>
<td>N/A</td>
<td>34.1</td>
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<td>PD Normal BMI (n=17)²⁶</td>
<td>64.1</td>
<td>82.4%</td>
<td>9.6</td>
<td>2.6</td>
<td>63.8</td>
<td>N/A</td>
<td>28.7</td>
</tr>
<tr>
<td>PD Abnormal BMI (n=17)²⁶</td>
<td>65.3</td>
<td>82.4%</td>
<td>8</td>
<td>2.5</td>
<td>63.9</td>
<td>N/A</td>
<td>28.8</td>
</tr>
<tr>
<td>PD (n=12)²⁴</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>PD (n=9)²²</td>
<td>59.2</td>
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<td>15.3</td>
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<tr>
<td>PD (n=13)⁴⁶</td>
<td>68.4</td>
<td>92.3%</td>
<td>4.77</td>
<td>2.65</td>
<td>N/A</td>
<td>33.7</td>
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</tr>
<tr>
<td>PD (n=51)²⁴</td>
<td>64</td>
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<td>6</td>
<td>2.2</td>
<td>32</td>
<td>N/A</td>
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<tr>
<td>PD (n=12)²⁴</td>
<td>62.9</td>
<td>83.3%</td>
<td>8.1</td>
<td>N/A</td>
<td>72.3</td>
<td>43.2</td>
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<tr>
<td>PD transplanted (n=3)³⁷</td>
<td>62</td>
<td>100%</td>
<td>28.3</td>
<td>N/A</td>
<td>29.7</td>
<td>12.3</td>
<td>29.3</td>
</tr>
<tr>
<td>PD without RBD (n=24)³¹</td>
<td>65.3</td>
<td>66.1%</td>
<td>5.8</td>
<td>2.3</td>
<td>N/A</td>
<td>27.6</td>
<td>(26)</td>
</tr>
<tr>
<td>PD with RBD (n=11)³¹</td>
<td>63.4</td>
<td>92.6%</td>
<td>6.4</td>
<td>2.3</td>
<td>N/A</td>
<td>25.1</td>
<td>(26.3)</td>
</tr>
<tr>
<td>PD (n=18)³¹</td>
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<td>66.7%</td>
<td>6.2</td>
<td>N/A</td>
<td>32.3</td>
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<td>PD without tremor (n=12)²³</td>
<td>62.8</td>
<td>66.7%</td>
<td>8.1</td>
<td>N/A</td>
<td>39.7</td>
<td>29.6</td>
<td>N/A</td>
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<td>PD with tremor (n=12)²³</td>
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<td>91.7%</td>
<td>9.4</td>
<td>N/A</td>
<td>39.7</td>
<td>27.8</td>
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<tr>
<td>PD without dyskinesia (n=12)²³</td>
<td>66.6</td>
<td>83.3%</td>
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<td>2.4</td>
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<tr>
<td>PD without dyskinesia (n=24)²³</td>
<td>65.2</td>
<td>79.2%</td>
<td>11.8</td>
<td>3.2</td>
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<td>40%</td>
<td>1.1</td>
<td>1.6</td>
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<tr>
<td>PD without dyskinesia (n=10)³⁸</td>
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<td>30%</td>
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<td>20.4</td>
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<td>PD with dyskinesia (n=10)³⁸</td>
<td>63.9</td>
<td>40%</td>
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<td>2.1</td>
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<td>PD without dyskinesia (n=11)³²</td>
<td>69.3</td>
<td>90.9%</td>
<td>7.8</td>
<td>2.3</td>
<td>40.6</td>
<td>26.7</td>
<td>28</td>
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<tr>
<td>PD with dyskinesia (n=11)³²</td>
<td>61.7</td>
<td>52.9%</td>
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<td>47.8</td>
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<td>28.4</td>
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<td>75%</td>
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<td>65.2</td>
<td>83.3%</td>
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<td>AVERAGE</td>
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<td>28.4</td>
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HC = healthy controls; PD = Parkinson’s disease; RBD = REM Behaviour Disorder; N/A = Data not available; *ON medication.

Table 2 Quality score (Newcastle-Ottawa Scale)

<table>
<thead>
<tr>
<th>Study</th>
<th>Case Definition</th>
<th>Selection of Control</th>
<th>Comparable PET Method</th>
<th>Incomplete PET data</th>
<th>Validated diagnosis of PD</th>
<th>Disease characteristic</th>
<th>Total score</th>
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*Based on UK Brain Bank criteria. *=1 point; X=0 point.