D₃ dopamine receptor-preferring [¹¹C]PHNO PET imaging in Parkinson patients with dyskinesia

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ABSTRACT

Objective: To investigate whether levodopa-induced dyskinesias (LID) are associated with D₃ overexpression in levodopa-treated humans with Parkinson disease (PD).

Methods: In this case-control study, we used PET with the D₃-preferring radioligand [¹¹C]-(+)-PHNO to estimate D_{2/3} receptor binding in patients with levodopa-treated PD with LID (n = 12) and without LID (n = 12), and healthy control subjects matched for age, sex, education, and mental status (n = 18).

Results: Compared to nondyskinetic patients, those with LID showed heightened [¹¹C]-(+)-PHNO binding in the D₃-rich globus pallidus. Both PD groups also showed higher binding than controls in the sensorimotor division of the striatum. In contrast, D_{2/3} binding in the ventral striatum was lower in patients with LID than without, possibly reflecting higher dopamine levels.

Conclusions: Dopaminergic abnormalities contributing to LID may include elevated D_{2/3} binding in globus pallidus, perhaps reflecting D₃ receptor upregulation. The findings support therapeutic strategies that target and diminish activity at D₃ to prevent LID. *Neurology*® 2016;86:224-230

GLOSSARY

ANOVA = analysis of variance; **AST** = associative striatum; **DA** = dopamine; **GP** = globus pallidus; **GPI** = internal segment of the globus pallidus; **HC** = healthy control; **LID** = levodopa-induced dyskinesias; **LST** = limbic (ventral) striatum; **PD** = Parkinson disease; **ROI** = region of interest; **SMST** = sensorimotor striatum; **SN** = substantia nigra; **UPDRS** = Unified Parkinson's Disease Rating Scale; **VP** = ventral pallidum.

Motor complications, including levodopa-induced dyskinesias (LID), affect nearly half of patients with Parkinson disease (PD) treated with levodopa in the first 5 years of treatment.¹ However, the mechanisms responsible for LID emergence remain poorly understood, and we cannot predict who will develop complications, or target side effects without compromising treatment.

Proposed mechanisms underlying the development of LID include sensitization to dopamine (DA) replacement medication, likely brought on by excessive DA transmission.² Specifically, rodent and nonhuman primate models of PD with LID point to an increase in D₃ DA receptor expression, but not that of D₁ or D₂ receptors. The increase is particularly evident in ventral striatum and globus pallidus, where D₃ receptors are abundant, but is also observed in dorsal striatum, where D₃ levels are considerably lower.³⁻⁵ Importantly, this levodopa-triggered D₃ overexpression has been related to locomotor sensitization in animal models, which was blocked and reinstated by D₃ antagonists and partial agonists, respectively.⁶ Together, the data suggest that overactivity at D₃ contributes to LID, and that modulating activity at this receptor might help prevent LID development.⁷⁻⁹ However, the status of the D₃ receptor system has never been investigated in living humans.

In humans, D_{2/3} receptor density can be examined with PET imaging, and PD and LID have been addressed using the gold standard D_{2/3} radioligand [¹¹C]raclopride.¹⁰ However, [¹¹C]raclopride cannot distinguish between D₂ and D₃ receptors, and primarily reflects D₂, given

Supplemental data
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the relative abundance of this receptor over D₃; moreover, [¹¹C]raclopride results have been inconclusive regarding PD-LID.^{11–14} A more novel approach involves the D₃-preferring radiotracer [¹¹C]-(+)-PHNO,¹⁵ which has ~20-fold selectivity for D₃ over D₂ and therefore allows for an estimate of D₃ receptor levels in humans.

The aim of the current study was to measure D_{2/3} receptors in patients with levodopa-treated PD with and without LID, using [¹¹C]-(+)-PHNO PET, with the expectation that patients with LID would show elevations in D₃.

METHODS **Standard protocol approvals, registrations, and patient consents.** All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board and complied with the standards set forth by the Declaration of Helsinki. All subjects gave written informed consent before being included in the study.

Subjects. Patients with PD were recruited by referral from a collaborating movement disorders clinic. For study inclusion, patients had to meet UK Brain Bank Criteria for idiopathic PD and receive a stable regimen of levodopa (no dose adjustment for at least 2 months). Exclusion criteria included current (within past month) treatment with DA agonist medication, Axis I psychiatric disorders independent from PD, cognitive impairment (Mini-Mental State Examination score <26), and severe medical conditions or history of head trauma. Patients with PD were classified into those without LID at a levodopa dose necessary to relieve PD symptoms and a stable response to the medication (PD-stable) and those with peak-dose levodopa-induced dyskinesia (PD-LID) by 2 of the authors who are experts in movement disorders (M.G., J.R.A.). Disease and dyskinesia severities were assessed at study intake using the Unified Parkinson's Disease Rating Scale (UPDRS) and Unified Dyskinesia Rating Scale, and we additionally collected measures assessing mood (Beck Depression Inventory) and motor dexterity (Purdue Pegboard Task). Age-, sex-, and education-matched healthy control (HC) subjects were recruited through newspaper and Internet ads, and were subject to the same (non-PD) exclusion criteria. In total, 18 HC, 12 PD-stable, and 12 PD-LID subjects participated in the study (table 1). The present sample partially overlaps with a previously described sample of HC and patients with PD with and without impulse control disorders.¹⁶

Imaging procedures. Patients with PD abstained from levodopa overnight (minimum 8 hours), a standard procedure to avoid interference with the PET signal, before completing a PET scan with the radiotracer [¹¹C]-(+)-PHNO. Synthesis of the radiotracer and image acquisition protocols on the CPS-HRRT neuro-PET camera system (Siemens Medical Imaging, Knoxville, TN) are described in detail elsewhere.¹⁷ PET imaging was initiated following bolus injection of the radiotracer (see scan parameters, table 1) and lasted 90 minutes. Raw data were reconstructed by filtered back-projection. In addition to the PET image, we obtained a spin echo proton density-weighted image on a Signa 1.5T MRI scanner (General Electric Medical Systems, Milwaukee, WI) for spatial normalization of PET images and region of interest (ROI) delineation.

Analyses. ROIs were delineated using in-house software (ROMI),¹⁸ including bilateral limbic (ventral) striatum (LST), associative striatum (AST; anterior putamen and dorsal caudate), sensorimotor striatum (SMST; posterior putamen), globus pallidus (GP), ventral pallidum (VP), and substantia nigra (SN) (see reference 17 for details). [¹¹C]-(+)-PHNO time activity curves within these ROIs (and cerebellum as the reference region) were obtained from dynamic data, and specific binding (BP_{ND}) values estimated in each ROI using the simplified reference tissue method¹⁹ implemented in PMOD (Zurich, Switzerland). To compare BP_{ND} between groups, we performed analyses of variance (ANOVAs) (covarying for age and sex) in SPSS (Chicago, IL), followed up with Helmert contrasts and least significant difference *t* tests. Interpretation of [¹¹C]-(+)-PHNO signal was region-dependent, as in healthy subjects signal in D₃-rich SN, VP, and GP is assumed to reflect D₃ availability (100%, 75%, 65% of [¹¹C]-(+)-PHNO signal, respectively), while signal from AST and SMST, where D₃ is not expressed, is assumed to reflect D₂.²⁰

RESULTS Subject characteristics and scan parameters.

Demographic data are reported in table 1. HC matched the PD groups on age, sex, education, and mental status, and the 2 PD groups matched on disease duration and Purdue Pegboard performance, as well as depressive symptoms. However, as expected, PD-LID were more advanced in severity of neurologic symptoms (UPDRS) and received a higher levodopa daily dose, likely reflective of their greater disease progression. PET scan parameters were similar between groups (the slightly lower [¹¹C]-(+)-PHNO dose in HC did not affect results).

Imaging outcomes. A repeated-measures ANOVA investigating [¹¹C]-(+)-PHNO BP_{ND} across ROIs revealed a group × ROI interaction ($F_{10,195} = 5.73$; $p < 0.001$). Follow-up ANOVAs testing for group differences in D₃-rich ROIs (SN, VP, GP) showed a group effect in GP ($F_{2,37} = 3.33$, $p = 0.047$, Cohen *d* = 0.85), with 21% higher [¹¹C]-(+)-PHNO BP_{ND} in PD than HC ($p = 0.022$). Pairwise comparisons revealed that the difference between PD-LID and HC (+27%, $p = 0.016$) was greater than that between PD-stable and HC (+15%, $p = 0.146$), and the difference between PD-LID and PD-stable was significant ($p = 0.032$, Cohen *d* = 1.07) when controlling for differences in levodopa dose. No group effects were found in the other D₃-rich regions (all $p > 0.43$).

In striatum ROIs (AST, LST, SMST), ANOVAs testing for group differences in [¹¹C]-(+)-PHNO BP_{ND} showed a group effect in SMST ($F_{2,37} = 24.08$, $p < 0.001$) and LST ($F_{2,37} = 5.92$, $p = 0.006$). In SMST, patients with PD had significantly higher BP_{ND} than HC (+70%, $p < 0.001$), and pairwise comparisons revealed that both PD-LID and PD-stable had higher BP_{ND} than HC (PD-LID +74%, PD-stable +66%; both $p < 0.001$), while the difference between PD groups was not significant ($p > 0.2$). In contrast, BP_{ND} in LST was significantly lower in PD than HC (−14%,

Table 1 Clinical and demographic characteristics of patients with Parkinson disease

	HC (n = 18), mean ± SD	PD-stable (n = 12), mean ± SD	PD-LID (n = 12), mean ± SD	p Value for PD group comparison
Demographic and PD characteristics				
Male/female	11/7	8/4	6/6	0.7
Age, y	58.7 ± 8.9	62.4 ± 7.5	60.9 ± 9.1	0.67
Education, y	14.7 ± 2.2	14.9 ± 2.0	15.6 ± 2.4	0.46
Mini-Mental State Examination	29.3 ± 1.1	28.6 ± 2.2	29.3 ± 0.9	0.32
Beck Depression Inventory	2.1 ± 2.2 ^a	9.1 ± 2.8	9.2 ± 5.3	0.92
Purdue Pegboard total	12.89 ± 1.2 ^a	9.8 ± 1.8	8.7 ± 1.3	0.59
UPDRS total	NA	36.6 ± 14.1	56.8 ± 7.8	0.001 ^b
UPDRS-III (motor)	NA	23.7 ± 9.0	40.1 ± 4.6	0.001 ^b
UDysRS (n = 8)	NA	0 ± 0	8.5 ± 7.2	0.001 ^b
PD duration, y	NA	7.8 ± 5.2	10.8 ± 4.5	0.13
Levodopa dose	NA	412.5 ± 126	775.0 ± 390	0.01 ^b
Patients on other medications, n				
Amantadine	NA	7	8	0.67
MAO inhibitors	NA	0	2	0.14
COMT inhibitors	NA	1	4	0.13
Benzodiazepine	NA	2	2	1.0
Scan parameters				
Body mass index	25.1 ± 7.0	23.9 ± 2.7	24.2 ± 3.8	0.89
[¹¹ C]-(+)-PHNO dose, mCi	8.44 ± 1.6 ^a	9.66 ± 0.9	8.93 ± 0.8	0.14
[¹¹ C]-(+)-PHNO SA, mCi/μmol	1059.0 ± 607	1226.8 ± 305	1126.0 ± 400	0.61
[¹¹ C]-(+)-PHNO mass, μg	2.2 ± 0.5	2.0 ± 0.3	2.2 ± 0.5	0.44

Abbreviations: COMT = catechol-O-methyltransferase; HC = healthy controls; LID = levodopa-induced dyskinesia; MAO = monoamine oxidase; NA = not applicable; PD = Parkinson disease; SA = specific activity; UDysRS = Unified Dyskinesia Rating Scale; UPDRS = Unified Parkinson's Disease Rating Scale.

^aSignificant difference between the HC and patients.

^b $p < 0.05$.

$p = 0.009$), with PD-LID showing lower BP_{ND} than both PD-stable (-13% , $p = 0.05$) and HC (-21% , $p = 0.001$). PD-stable subjects did not differ from HC ($p = 0.245$) (figure 1). Taking into account differences in symptom severity did not change the LST [¹¹C]-(+)-PHNO effect (PD-LID < PD-stable; $p = 0.049$), but the effect was lost after controlling for differences in levodopa dose ($p = 0.46$).

No relationships were found between severity of disease symptomatology, mood, or motor performance and [¹¹C]-(+)-PHNO BP_{ND}, even when taking levodopa dose into account.

In addition to [¹¹C]-(+)-PHNO scans, all subjects also completed PET scans with the radiotracer [¹¹C]raclopride. The results echoed those found with [¹¹C]-(+)-PHNO, and are reported in table e-1 and figure e-1 on the *Neurology*[®] Web site at Neurology.org.

DISCUSSION This study used the D₃-preferring PET radiotracer [¹¹C]-(+)-PHNO to provide novel insights into the in vivo status of the D_{2/3} DA receptor system in PD-LID. Our main finding of

heightened [¹¹C]-(+)-PHNO binding in the D₃-rich GP of patients with PD, particularly those with LID, points to D₃ upregulation as a feature of PD-LID, since one-third of GP DA receptors are D₃²¹ and the majority of GP [¹¹C]-(+)-PHNO signal stems from D₃ in healthy subjects.²⁰

The finding extends previous [¹¹C]-(+)-PHNO evidence showing low GP D₃ levels in de novo patients,¹⁷ and is highly consistent with evidence from nonhuman primate and rodent models, where DA denervation leads to lower D₃ expression that can be reversed with levodopa; however, rather than simply “normalizing” D₃ levels, treatment-induced D₃ upregulation exceeds control levels, i.e., levodopa triggers D₃ overexpression.^{7,22,23} This, in turn, correlates with the occurrence and severity of LID in nonhuman primates,²⁴ supporting our interpretation of D₃ overexpression in our PD-LID sample. The finding that the difference between PD-stable and PD-LID became significant only when taking into account levodopa dose could be explained by the higher doses taken by PD-LID than PD-stable

Figure 1 [^{11}C]-(+)-PHNO binding in healthy controls (HC) and patients with levodopa-treated Parkinson disease (PD) with and without dyskinesias

[^{11}C]-(+)-PHNO binding for HC (open circles), patients with levodopa-treated PD without levodopa-induced dyskinesias (LID) (PD-stable; grey circles), and patients with levodopa-treated PD with LID (PD-LID; black circles). AST = associative striatum; GP = globus pallidus; LST = limbic striatum; SMST = sensorimotor striatum; SN = substantia nigra; VP = ventral pallidum. *Significantly different from HC ($p < 0.05$, corrected). **Significantly different from PD-stable when taking into account levodopa dose. ***Significantly different from PD-stable, effect lost when taking into account levodopa dose.

patients: despite the overnight medication abstinence, PD-LID may have had greater levodopa-induced DA synthesis/higher (residual) DA concentrations on the day of the scan, decreasing [^{11}C]-(+)-PHNO binding—a possibility supported by the fact that D_3 has the highest affinity for endogenous DA²⁵ and that [^{11}C]-(+)-PHNO is highly sensitive to DA fluctuations.²⁶ The present study therefore provides *in vivo* confirmation in humans of what has long been suggested in animal models, and supports development of D_3 antagonist strategies for addressing LID in the clinic.

In terms of potential mechanisms, GP is the primary striatal output structure to the thalamus to coordinate involuntary movement, and a primary target for PD treatment. In a primate model of PD, GP was shown to express D_3 but no D_3 mRNA, suggesting that the receptors are located on incoming afferents.²² In humans, D_3 mRNA is expressed in striatal medium spiny neurons that project to the internal segment of the GP (GPi),²⁷ and it has been suggested that D_3 upregulation occurs on this neuronal population at both the somato-dendritic (putamen) and terminal (GPi) levels.²⁴ This D_3 upregulation could lead to the overinhibition of GP targets and over-reduction of GP firing that has been linked to LID in monkeys and patients with PD,²⁸ which in turn would disinhibit thalamic nuclei and result in over-activation of cortical motor areas. However, it should be noted that it was not possible to distinguish functionally distinct GP divisions (internal/external or dorsal/ventral²⁹) in the present study.

The failure to find any group differences in the other D_3 -rich regions (SN, VP) could mean that GP is selectively affected, a view that is supported by [^{11}C]-(+)-PHNO data in *de novo* patients with PD showing D_3 downregulation in GP but not SN.¹⁷ It could also mean that since the main disease characteristic of PD is loss of SN neurons, a finding of heightened [^{11}C]-(+)-PHNO binding in the SN (presumably reflecting presynaptic D_3 binding sites) was annulled by a reduction in surviving neurons.

In the striatum, we observed elevations in dorsal striatal (SMST) $\text{D}_{2/3}$ receptor levels in PD (see also supplementary materials for corroboration with [^{11}C] raclopride); importantly, some of the findings suggest that heightened SMST $\text{D}_{2/3}$ availability may also contribute to LID, as the difference in [^{11}C]-(+)-PHNO BP_{ND} between PD and HC was greater in PD-LID than PD-stable, and [^{11}C]raclopride BP_{ND} (see supplementary materials) was higher in PD-LID than PD-stable. The finding could reflect D_2 upregulation (e.g., greater disease progression in PD-LID could have driven a compensatory increase in D_2 ³⁰), although this interpretation is at odds with the literature suggesting normalization of denervation supersensitivity (adaptively heightened D_2 receptor levels) with long-term levodopa treatment.¹³ It could be that previous studies included patients on longer-acting DA agonist medications (e.g., pergolide³¹), introducing competitive interaction at radioligand binding sites and masking upregulated D_2 receptors. However, an alternative (or additional) explanation is that D_3 receptors in our PD-LID sample may have been ectopically upregulated, in line with our hypothesis. This view is supported by preclinical findings,^{4,22} including that putamen D_3 expression was 154% higher (and GP 425% higher) in parkinsonian monkeys with LID than without, and that putamen D_3 overexpression correlated with LID occurrence and severity.²⁴ It could also be that levodopa treatment did lead to some degree of reduction in D_2 receptor levels, without completely normalizing them.

In contrast to SMST, LST [^{11}C]-(+)-PHNO (and [^{11}C]raclopride, see supplementary materials) BP_{ND} were consistently lower in PD than HC. This could suggest either that $\text{D}_{2/3}$ expression is lower, as previously observed in *de novo* patients,¹⁷ or that DA tone is greater, displacing more radiotracer. This, too, may contribute to LID, as PD-LID had significantly lower LST [^{11}C]-(+)-PHNO BP_{ND} than PD-stable. It is possible that the lowered LST $\text{D}_{2/3}$ levels observed in medication-naïve patients¹⁷ recover with treatment in PD-stable but not PD-LID patients; however, our loss of a group difference when taking levodopa dose (i.e., residual DA synthesis) into account favors the interpretation that it reflects synaptic DA. This is consistent with findings that levodopa administration

results in greater striatal DA release and changes in DA reuptake in PD-LID,^{32,33} and is supported to some degree by the related finding of greater LST DA levels in patients with PD with impulse control disorders (another levodopa-induced complication).^{34,35} The mechanism leading to excessive DA levels is not clear, but may stem from the pattern of DA denervation in PD, which affects nigrostriatal before mesolimbic projections,³⁶ causing levodopa to treat healthy neurons with excessive DA. However, it should be noted that rather than reflecting synaptic DA, the disappearance of the effect when accounting for levodopa dose could also reflect the group difference in disease severity, as levodopa dose is often associated with greater disease progression.

Several other limitations of the study should be noted. For one, the PD-stable and PD-LID groups differed in levodopa dose and disease severity, and although control analyses were conducted to better understand the potential effects of these factors (e.g., entering them as covariates or correlating with

imaging outcomes), we cannot exclude the possibility that these differences accounted for the present findings. Moreover, we cannot exclude the possibility that our findings reflect features of disease progression/levodopa dose needed, rather than LID per se. While the results, therefore, contribute novel technology and insights to the study of LID, they should be interpreted cautiously with these factors in mind, and future research will need to disentangle these alternative possibilities. In addition, the use of amantadine by patients with PD in both groups could represent a confound, as amantadine reduces the severity of LID and could have suppressed the onset of LID in the PD-stable group. This effect has not been studied, but the fact that both groups were matched with respect to use of amantadine reduces the possibility that our main findings are explained by use of this drug. Finally, the study used small samples of subjects, resulting in high variability of the data. However, the variability is within the range of other [¹¹C]-(+)-PHNO studies, and analyses of distributions suggest no outliers that influenced the findings.

Also important to note are the limitations intrinsic to [¹¹C]-(+)-PHNO (see references 17 and 26). These include scanning at nontracer doses of the radioligand and cerebellar binding, which may lead to lower tracer binding and underestimation of the signal.²⁹ However, our PD groups were matched with respect to [¹¹C]-(+)-PHNO dose injected (table 1), and repeating all [¹¹C]-(+)-PHNO analyses with [¹¹C]-(+)-PHNO mass as an additional covariate did not change the results (data not shown). Another caveat of [¹¹C]-(+)-PHNO is its lack of absolute specificity for D₃, so that [¹¹C]-(+)-PHNO signal can reflect both D₂ and D₃ receptor binding. It is therefore not possible to disentangle whether our findings of heightened [¹¹C]-(+)-PHNO BP_{ND} in GP and SMST reflect the same or different phenomena, and whether these relate to D₂ or D₃. Further, the region-dependent interpretation of [¹¹C]-(+)-PHNO binding (based on D₃ vs D₂ fractions calculated in healthy human subjects²⁰) may not translate to patients with PD. However, an appropriate D₃ blocking study that could address this potential confound has not been conducted. Finally, the ROI template used in PET analyses does not distinguish between GPi and external GP, limiting mechanistic explanations of our findings.

These considerations notwithstanding, our findings reveal new insights about the status of the DA system in PD with and without LID. As a group, findings in patients with levodopa-treated PD challenge the notion of normalizing denervation supersensitivity. Importantly, we also provide novel human evidence suggesting D₃ upregulation in GP, which is exaggerated in PD-LID and could present new avenues for intervening in LID development.

Comment: Increased D₃ binding— A substrate for levodopa-induced dyskinesias?

Levodopa remains the mainstay of treatment for Parkinson disease (PD), but its use is associated with dyskinesias (LID) in many patients. LID likely reflect dysregulated release of dopamine combined with altered postsynaptic mechanisms due to pulsatile dopamine receptor stimulation. Dopamine D₃ receptors are increased in animal models of LID, and experimental LID is attenuated by suppression of D₃ signaling, but the relevance to LID in humans is uncertain.¹ Payer et al.² used PET with the D₃-preferring dopamine receptor agonist [¹¹C]PHNO. Compared to patients with PD without LID, those with LID had increased binding in the globus pallidus and decreased binding in the ventral striatum. In the dorsal striatum, PHNO binding was increased in both PD groups, but the difference between PD-stable and PD-LID was not significant. However, [¹¹C]raclopride binding to D₂ and D₃ receptors was increased in patients with LID when controlling for levodopa dose.

The findings support the importance of D₃ receptor upregulation in pallidum and possibly dorsal striatum in the pathogenesis of LID in humans. At least 4 issues prompt caution: (1) PHNO binds preferentially but not selectively to D₃ receptors; (2) the proportion of PHNO binding to D₃ receptors varies from one region to another, but could be affected by disease and treatment; (3) PHNO binding is susceptible to competition from endogenous (levodopa-derived) dopamine; and (4) the low doses of PHNO injected are not trace, further complicating the interpretation of the imaging data. In ventral striatum, patients with PD-LID had more severe disease and higher daily levodopa requirements than those without LID; thus reduced PHNO binding may reflect more severe dopamine denervation. Finally, there was considerable variability in pallidal PHNO binding, suggesting that the effect seen could have been driven by outliers or that D₃ receptor upregulation alone may be neither necessary nor sufficient to result in LID.

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AUTHOR CONTRIBUTIONS

Doris Payer: analysis and interpretation of data, drafting/revising the manuscript for content, study coordination, acquisition of data. Mark Guttman: revising the manuscript for content, referral of patients, acquisition of data. Stephen Kish: study concept and design, revising the manuscript for content, interpretation of data. Junchao Tong: study concept and design, revising the manuscript for content, interpretation of data. John Adams: revising the manuscript for content, referral of patients, acquisition of data. Pablo Rusjan: statistical analysis of data, revising the manuscript for content. Sylvain Houle: study concept and design, study medical supervision, director of PET facilities. Yoshiaki Furukawa: study concept and design. Alan Wilson: study concept and design, contribution of vital tools (PHNO). Isabelle Boileau: drafting/revising the manuscript for content, study concept and design, analysis and interpretation of data, acquisition of data, study supervision and coordination, obtaining funding.

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