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Brain cholesterol metabolism and Parkinson's disease

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Abstract

Background: Circulating cholesterol levels have been linked to Parkinson's disease (PD), but not directly to brain physiology.

Objective: To assess whether brain cholesterol metabolism is related to PD.

Methods: Sixty PD and 64 controls were recruited from an academic movement disorder clinic (2009–2012). Thirty-five PD and 33 controls returned ca. 36 months later. Fasting plasma (*S*)24-OH-cholesterol (brain-derived cholesterol metabolite) and 27-OH-cholesterol (peripheral cholesterol metabolite) were quantified. Odds ratios (ORs) for PD were derived from logistic regression models, adjusting for potential confounders. Relationships between the oxysterols and clinical measurements were explored using Spearman correlation coefficients.

Results: The mean age of PD subjects was 63.8 ± 8.3 y and disease duration was 5.0 ± 5.4 y. Plasma (*S*)24-OH-cholesterol levels were inversely associated with the odds of having PD, with an OR of 0.92 [95% confidence interval (CI): 0.87–0.97] for each 1 ng/mL increase ($p=0.004$). Compared to the lowest tertile, the OR was 0.34 (0.12–0.98) for the second tertile ($p=0.045$) and 0.08 (0.02–0.31) for the highest tertile ($p<0.001$). Higher (*S*)24-OH-cholesterol levels also were correlated with better sense of smell ($r=0.35$, $p=0.01$). No significant associations were found between clinical measures and 27-OH-cholesterol, a peripheral cholesterol metabolite. Further, (*S*)24-OH-cholesterol levels were stable over time, whereas 27-OH-cholesterol decreased with time in both cases and controls.

Conclusions: The results indicate that plasma (*S*)24-OH-cholesterol (possibly reflecting brain cholesterol metabolism) is inversely linked to PD, is relatively stable over time, and may serve as a new biomarker for PD. Further investigation is necessary to determine the mechanistic and clinical implications.

Keywords

Parkinson's disease; cholesterol; oxysterols; statins; olfaction

Introduction

Epidemiologic studies,^{1–4} including four prospective analyses,^{5–9} have found that higher cholesterol levels are associated with a lower risk of Parkinson's disease (PD). Higher LDL-cholesterol in PD also has been linked to slower loss of motor and executive function^{10, 11} and later age of PD diagnosis.¹² These data suggest that higher LDL-cholesterol status may be favorable for people with, or at risk for, PD. The idea that higher LDL-cholesterol level may be a protective factor for PD runs counter to the common assumption that higher cholesterol generally is detrimental to good health. Another complicating factor is that there is little passage of circulating cholesterol through the BBB.¹³ Although the mature brain contains 25% of total body cholesterol, essentially all brain cholesterol is synthesized *de novo* in astrocytes via the mevalonate pathway, and is then transported to neurons via endocytosis and interaction with the LDL receptor and apolipoprotein E.^{14, 15}

Unlike cholesterol, however, hydroxylated cholesterol metabolites (oxysterols) can cross the BBB,^{16, 17} and their esters are the primary transport forms between brain and peripheral blood. (S)24-OH-cholesterol is the most abundant oxysterol in the brain, and is produced exclusively in the CNS. Thus, plasma (S)24-OH-cholesterol level has been proposed as a possible biomarker of CNS cholesterol metabolism. Because neuronal cell death or regeneration is accompanied by increased cholesterol release, synthesis, and metabolism, the level of plasma (S)24-OH-cholesterol also has been proposed as a possible biomarker of neurodegenerative processes.^{18–21}

In this study, we examined the relationship between circulating (S)24-OH-cholesterol (the brain-derived oxysterol) and PD. The study also included a parallel analysis of 27-OH-cholesterol, the primary cholesterol metabolite that is produced exclusively in the periphery. We hypothesized that (S)24-OH-cholesterol (from brain), but not 27-OH-cholesterol (from the periphery), would be associated with PD. Leveraging the available repeat measurements from some participants, we also investigated potential dynamic changes of the two oxysterols during PD progression.

Methods

Study population

Sixty PD and 64 control participants (hereafter referred to as controls) were recruited between 2009 and 2012 at the movement disorder clinics of Penn State Hersey Medical Center as part of an NIH-funded R01 study (NS060722, PI: X. Huang).²² Controls were spouses and local residents, free of any major neurological disorders. All participants also were free of major and acute medical issues such as liver, kidney, or thyroid abnormalities, or deficiencies of B₁₂ or folate, or signs of dementia (MMSE>24). Upon receiving funding by the NIH-sponsored PD Biomarker Program (PDBP) in 2012 (NS082151),²³ we invited all participants from the original R01 study to participate in the biomarker-focused study. Of those who participated in the original study, 35 PD and 33 controls returned approximately 36 months after the original baseline study. After an 8–12 hour overnight fast, blood samples were obtained from all participants at both visits. The tubes that were used were BD Vacutainer™ Glass Blood Collection Tubes with K₃EDTA (lavender top; Fisher catalog

#02–685-2B). The plasma was made on the day of collection by centrifugation at 1,700 g for 10 min, followed by aspiration, insuring that none of the buffy coat contaminated the plasma. Plasma was either analyzed immediately or banked at Penn State in an –80° C freezer with CO₂ backup and 24/7/365 monitoring.

Written informed consent was obtained from all participants, and the experiment was conducted in accordance with the Declaration of Helsinki. The research study protocol and procedures were reviewed and approved by the Penn State Hershey Institutional Review Board.

Clinical evaluation related to PD and potential confounders for the study

PD diagnosis was confirmed by movement disorders specialists at the Penn State Hershey Movement Disorder clinic according to published criteria.²⁴ Disease duration was defined as years between the date of first PD diagnosis by a medical professional and the study visit date. Unified PD Rating Scale (UPDRS) scores were obtained for PD participants in the “on-medication” state using the revised UPDRS.²⁵ Levodopa-equivalent daily dosage (LEDD) was calculated according to published criteria.²⁶

At the clinical visit, we also obtained information on age, gender, statin usage (yes/no), and cigarette smoking. Participants were considered smokers if they had ever smoked one cigarette per day for six months or more.²⁷ Participants were considered users of statins if they were using statins at the time of blood sampling. Participants also completed the Montreal Cognitive Assessment (MoCA), Hamilton Depression Rating Scale (HAM-D), and University of Pennsylvania Smell Identification Test (UPSIT) as part of the NINDS Common Data Elements (CDE) for PD research at baseline and return visits. We included all of these clinical parameters in our exploratory analyses because non-motor symptoms are common in PD. We suspect that brain cholesterol metabolism, if etiologically linked to PD, may not only influence motor pathways, but also non-motor measures such as loss of sense of smell that occur prior to clinical diagnosis and are not affected by current pharmacological treatments.

Plasma cholesterol levels

Triglyceride,²⁸ total-, and HDL-cholesterol^{29, 30} levels were measured using fresh plasma collected from participants at study visits. Our clinical laboratory enzymatically quantified cholesterol levels by reflectance spectrophotometry using the Vitros 4600/5600 Chemistry Systems (Ortho Clinical Diagnostics, Raritan, NJ). The Friedewald equation was used to calculate LDL-cholesterol ($LDL_C = \text{Cholesterol}_{\text{Total}} - [\text{Triglycerides}/5 + HDL_C]$).

Assay for concentrations of free hydroxylated cholesterol metabolites in plasma

Materials: Unlabeled (*S*)-24-OH- and 27-OH-cholesterol were obtained from Research Plus Inc. (Barnegat, NJ); (*R/S*)-24-OH-cholesterol-d6 [cholest-5-ene-3 β ,24(*R/S*)-hydroxycholesterol (d6)] from Avanti Polar Lipids, Inc. (Alabaster, AL); ammonium formate from J. T. Baker (Philipsburg, NJ); and Optima LC-MS grade water, methanol, and other reagents from Fisher Scientific (Mullica Hill, NJ).

Plasma sample preparation: Plasma samples were thawed and prepared as follows. (*S*)-24-OH-cholesterol and 27-OH-cholesterol stock solutions (2.5 mg/mL) were prepared in 5 mM ammonium formate, and serially diluted in acetonitrile, resulting in concentrations of 49–12,500 ng/mL. The stock solution of the internal standard 24(R/S)-OH-cholesterol-d6 solution (52 µg/mL in methanol) was diluted with 5 mM ammonium formate to yield a concentration of 5,200 ng/mL. Calibration standards were prepared by adding 5 µL standard working solution and 5 µL internal standard working solution into 40 µL of 5 mM ammonium formate to make standards of concentrations 4.9 to 1,250 ng/mL, whereas the internal standard concentration was kept at 520 ng/mL. The stock solutions and working solutions were kept at –20° C before use.

Internal standard (5 µL of 5,200 ng/mL) was added to 250 µL plasma, and after addition of 750 µL ethanol, the sample was vortexed. After centrifuging for 10 min at 8,765 g at 4° C, the supernatant was removed and the pellet was re-extracted with 750 µL ethanol. The combined supernatant was evaporated (Speed Vac) to half-volume, and solid phase extraction (SPE) done using a Phenomenex Strata-X cartridge (30 mg packing; 1 mL volume). The SPE cartridge had been preconditioned with 1 mL methanol, and then equilibrated with 1 mL water. After loading the samples, the SPE cartridge was washed with 1 mL of water followed by 1 mL of 75:25 methanol:water. The cartridge was dried and then analytes were eluted with 1 mL of 100% methanol and 0.5 mL n-heptane/isopropanol (50/50). The eluent was dried by Speed Vac followed by reconstitution with 50 µL of 5 mM ammonium formate. The reconstituted sample was centrifuged at 4° C for 10 min at 4,000 rpm before loading onto the UPLC/MS/MS system.

UPLC-MS/MS analysis: The detection method was modified from that of Bandaru and Haughey.³¹ Analyses of (*S*)-24-OH-cholesterol and 27-OH-cholesterol levels used a Waters Acquity UPLC with a 1.7 µm 2.1 × 100 mm Acquity UPLC BEH C18 analytical column for separation and an ABSciex 4000 Q Trap mass spectrometer for detection. Elution began with 0.3 mL/min 85% mobile phase B (10 mM ammonium formate in methanol) and 15% solvent A (10 mM ammonium formate in water) for 0.5 min. This was followed by a linear gradient to 92% mobile phase B for 4 min, followed by 92% mobile phase B for 1.5 min to flush the column. The autosampler was kept at 4° C and the column temperature was maintained at 25° C.

The ABSciex 4000 Q Trap mass spectrometer was equipped with an electrospray ionization probe operated in positive mode. The conditions used were: decluster potential (DP) 100 V; entrance potential (EP) 10 V; collision energy (CE) 13 V for (*S*)-24-OH-cholesterol and 27-OH-cholesterol, and 20 V for the internal standard; collision cell exit potential (CXP) 5 V for (*S*)-24-OH-cholesterol and 27-OH-cholesterol, and 10 V for the internal standard; curtain gas (CUR) 40 L/h; collision gas (CAD) 12 L/h; ionSpray voltage 5,500 V; temperature 0° C; ion source gas 1 70 L/h; and ion source gas 2 50 L/h. The multiple reaction monitoring mode (MRM) was used for quantification using transitions of *m/z* 420.5 > 385.3 for (*S*)-24-OH-cholesterol, 420.5 > 385.3 for 27-OH-cholesterol, and 426.5 > 373.3 for (*S*)-24-OH-cholesterol-d6. All peaks were integrated and quantified by ABSciex Multiquan 2.1 software.0020.

Data analysis

Group differences in categorical variables (e.g. gender, statin use, smoking status) were tested using Chi-square tests. Group comparisons of all other clinical characteristics (e.g. age, UPSIT), plasma cholesterol levels, and brain [(*S*)24-OH-cholesterol] and peripheral (27-OH-cholesterol) oxysterol levels, were performed using Student's t-test. Additionally, group comparisons were performed with adjustments for age and gender using logistic regression and analysis of covariance (ANCOVA) as appropriate. Potential changes in clinical features and cholesterol levels from baseline to follow-up visits were investigated using paired t-tests in both cases and controls.

In the primary analyses, baseline plasma cholesterol metabolites [(*S*)24-OH- and 27-OH-cholesterol] were categorized into tertiles based on the distribution among controls. We further tested the statistical significance for linear trend by including them as continuous variables in our models. Odds ratios (ORs) and 95% confidence intervals (CI) for PD, along with corresponding p values, were derived from logistic regression models with adjustment for age, gender, smoking status, and statin usage (Model A). To understand the influence of the other oxysterol and total cholesterol on the primary oxysterol-PD link, we additionally adjusted for the other oxysterol (Model B) and total cholesterol (Model C). Lastly, we obtained ORs further adjusted for HAM-D and UPSIT scores (Model D) because these measures were significantly different between PD and control groups (Table 1). The relationships between oxysterols and clinical measurements of PD subjects were explored using Spearman correlation coefficients adjusted for age, gender, smoking status, disease duration, and statin usage (Analysis A), and additionally adjusted for the other oxysterol (Analysis B). Spearman's correlation analysis is more robust to outliers, and more powerful to detect monotonic relationships. All statistical analyses were performed using SAS software (version 9.4, The SAS Institute, Cary, NC) and statistical tests were considered significant with a two-sided $\alpha=0.05$.

Results

Study subjects

PD participants were older than controls by approximately four years ($p=0.005$), but they were not statistically different from controls in gender, statin usage, or smoking status (Table 1). For cases, the average age at PD diagnosis was 58.8 ± 8.3 y and the average disease duration was 5.0 ± 5.4 y. PD participants had significantly higher HAM-D and lower UPSIT scores than controls. All plasma cholesterol (total-, LDL-, HDL-) and oxysterol measurements [(*S*)24-OH, and 27-OH] were lower in PD compared to controls, however, only (*S*)24-OH-cholesterol levels reached statistical significance. Demographic and clinical measures of the participants who did and did not follow-up were not statistically different (Supplemental Table 1).

Associations between plasma oxysterols and PD

Prior to commencing this study, we evaluated the literature and made the decision to focus on the free oxysterol metabolites rather than their esterified derivatives that account for a majority of the oxysterols in the blood. Not only is the free (*S*)24-OH the biologically active

form in the brain,^{2, 3} but Bandaru and Haighey¹ reported that this was a better biomarker for function than was total (*S*)24-OH.

As seen in Table 2, higher (*S*)24-OH-cholesterol levels were associated with lower odds of having PD, with an adjusted OR of 0.92 (95% confidence interval (CI): 0.87–0.97) for each 1 ng/mL increase ($p=0.004$) in (*S*)24-OH-cholesterol. When analyzed as a categorical variable using the lowest tertile as reference, the adjusted OR was 0.34 (0.12–0.98) for the second tertile ($p=0.045$), and 0.08 (0.02–0.31) for the highest tertile ($p<0.001$). Additional adjustment for 27-OH-cholesterol (Model B), total cholesterol (Model C), or HAM-D and UPSIT scores (Model D) did not materially change the results. In contrast, 27-OH-cholesterol levels were not statistically associated with PD in any of the analyses.

Because statin usage may influence brain and peripheral lipid synthesis, we also performed a sensitivity analysis by repeating the analyses after excluding all participants who were using statins at study enrollment. As seen in the Supplemental Table 2, the key associations between (*S*)24-OH-cholesterol and PD remain statistically significant.

Associations between plasma oxysterols and clinical measurements at baseline

Higher baseline (*S*)24-OH-cholesterol levels were associated with higher UPSIT scores ($r=0.36$, $p=0.01$), but not with other clinical measures (Table 3). No association was found for 27-OH-cholesterol levels and clinical measurements ($p>0.2$).

Longitudinal changes in plasma oxysterols and clinical measurements

For those who returned for the second visit, PD and control compositions were similar to those at enrollment (Table 4). PD subjects had a significant increase in LEDD levels ($p<0.001$) and a trend for higher UPDRS scores ($p=0.06$). HAM-D improved in controls ($p=0.006$) but remained stable in PD participants, whereas UPSIT scores remained stable in controls but declined significantly in PD participants ($p<0.001$).

(*S*)24-OH-cholesterol levels were stable over time in both PD subjects and controls. There was a tendency for higher baseline (*S*)24-OH-cholesterol levels being associated with a smaller decrease in UPSIT scores from baseline, and negatively with annual change in UPDRS-total scores, but none of these were statistically significant. Conversely, 27-OH-cholesterol levels declined significantly over time in both PD subjects and controls. Baseline 27-OH-cholesterol levels were associated negatively with annual change in UPDRS-total scores ($r=-0.468$, $p=0.01$), but unrelated to annual change in UPSIT scores ($r=-0.031$, $p=0.20$).

Discussion

Although the evidence connecting circulating cholesterol and PD has grown substantially in the past decade,^{1–12} a brain mechanism linking these measures has not been identified because cholesterol synthesis in the brain is largely *de novo*.^{13–15} The present study provides robust evidence that higher levels of (*S*)24-OH-cholesterol, a brain oxysterol and downstream product of cholesterol metabolism, was inversely associated with PD risk. The study also provides the first hint that higher (*S*)24-OH-cholesterol may be related to better

olfactory function, a known deficit in prodromal PD. In contrast, 27-OH-cholesterol (a peripherally generated oxysterol) showed no such association with PD. In addition, (S)24-OH-cholesterol remained stable during the three-year study period, whereas 27-OH-cholesterol levels declined in both PD cases and controls. Together, these data suggest that rather than being an epiphenomenon, cholesterol and/or its metabolism may have an important role in the pathoetiology of PD. Future studies are needed to investigate the role of circulating (S)24-OH-cholesterol as a biomarker for PD risk and progression.

The association between plasma oxysterols and PD

There have been three previous studies investigating the relationship between (S)24-OH-cholesterol levels and PD, each with fewer than 26 PD subjects. Lee et al.³² and Di Natale et al.³³ reported lower plasma levels in PD compared to controls, whereas Björkhem et al.³⁴ found no difference. The latter authors³⁴ suggested that the analysis in the Lee et al. study³² was confounded by the experimental choice of deuterated internal standard. The d6-deuterated standard we used is not subject to the problems noted by Björkhem et al.³⁴ because the fragmentation pattern of our internal standard will not generate a species with the same mass as that from unlabeled 24-hydroxycholesterol. Moreover, the sample size in the current study is much larger than the three previous studies, and our analyses also were controlled for potential confounders such as age, gender, smoking status, and statin use.^{35–39} Our study, consistent with Di Natale et al.,³³ provides the most robust evidence to date supporting the hypothesis that higher levels of (S)24-OH-cholesterol are inversely associated with PD.

The potential mechanisms that link lipids to PD processes

Despite the accumulating epidemiological evidence for the association of plasma cholesterol with PD, understanding the nature of this association has been challenging. There are reasons to speculate that the observation may not be causal. For example, patients with a PD diagnosis (or in the prodromal stage of PD) may adopt a “healthier” lifestyle, thereby leading to lower cholesterol, or the association could be confounded by the use of statins, or by other diet and lifestyle factors. Alternatively, lower plasma cholesterol may reflect metabolic or non-motor (gastrointestinal and nutritional status) changes that are associated with PD and/or advanced aging. Consistent with the latter hypothesis, circulating cholesterol levels steadily decline during advanced aging in both genders (males after ~60 and females ~70 y).^{40, 41} In our current study (Table 4), plasma total- and LDL-cholesterol levels decreased in PD subjects at the three-year follow-up visit, whereas these cholesterol levels were stable in controls. In addition, we also noted a significant decline of 27-OH-cholesterol levels, a peripheral cholesterol metabolite, in both PD and controls, although the decline appeared more robust in PD compared to controls (Table 4). Together, these data are consistent with the idea that PD may have accelerated the aging process.

Our group has explored the potential causality of the cholesterol-PD relationship by studying a number of factors (such as APOE polymorphisms and LDL receptor (LDLR), low-density lipoprotein receptor-related protein 1 (LRP1), and low-density lipoprotein receptor-related protein associated protein (LRPAP1) SNPs) that may affect both circulating cholesterol levels and PD risk. These data suggest that circulating total- and LDL-

cholesterol levels are related to PD risk,⁴² although this notion has been challenged by the fact that there is thought to be negligible communication between peripheral and central cholesterol pools.^{13, 15}

Several lines of evidence are consistent with the hypothesis that brain cholesterol metabolism, marked by (*S*)24-OH-cholesterol levels, could be one of the factors mediating the observed plasma cholesterol-PD relationship. First, in the literature^{16,43} and in the current study (data in Supplemental Figure 1), (*S*)24-OH-cholesterol is associated with circulating total- and LDL-cholesterol. Second, in our analysis, (*S*)24-OH-cholesterol, but not total-, LDL-, or the 27-OH metabolite, robustly differentiates PD from controls (Table 1). Finally, (*S*)24-OH-cholesterol levels, in contrast to total-/LDL-cholesterol and/or 27-OH-cholesterol levels, are stable in both controls and PD cases over an average of three years, arguing against the possibility of reverse causation (i.e., that lower (*S*)24-OH-cholesterol levels are secondary to PD progression and/or the aging process). These data do not directly test the hypothesis that medication may affect (*S*)24-OH-cholesterol levels. On the other hand, adjusting either plasma total- (Model C in Table 2) or LDL-cholesterol (data not shown) did not significantly alter the (*S*)24-OH-cholesterol-PD association. Further investigations on this brain metabolite of cholesterol may elucidate the role of cholesterol in PD pathogenesis and potentially a novel biomarker for PD development.

Potential mechanisms of a brain cholesterol metabolism-PD link

Cholesterol is essential for synaptogenesis.¹⁴ It is therefore possible that higher brain oxysterols may be the result of higher cholesterol turnover in compensatory repair of injured neuronal pathways in PD pathogenesis. Consistent with this possibility, previous studies reported higher levels of cholesterol metabolites in the cortices of postmortem PD patients and more cholesterol breakdown metabolites in cerebrospinal fluid.^{32, 34} Furthermore, (*S*)24-OH-cholesterol seems to be increased in the initial stages of Alzheimer's disease (AD) and multiple sclerosis (MS) when the diseases are relatively mild and brain compensatory mechanisms are robust, yet the compensatory capacity decreases as the disease progresses.¹⁹ Because poor sense of smell probably develops many years before PD clinical diagnosis,⁴⁴ the positive association with UPSIT score is consistent with the possibility that (*S*)24-OH-cholesterol changes may be an early process of PD pathogenesis.

The obvious question is whether we report an epiphenomenon or, conversely, a finding of mechanistic importance. The production of (*S*)24-OH appears to be catalyzed solely by the enzyme cholesterol-24-hydroxylase, known now to be CYP46A1, a member of the cytochrome P450 superfamily. Originally assumed to be a pathway for the removal of excess cholesterol, there now are data that it affects neuronal function in many ways. For example, (*S*)24-OH is reported to be a positive allosteric modulator of the NMDA receptor,⁴⁵ and L-glutamate, among other neuroactive compounds, may activate CYP46A1 allosterically, thereby increasing the production of (*S*)24-OH.⁴⁶ Moreover, down-regulated CYP46A1 leads to a compensatory decrease in cholesterol synthesis and consequent decreases in geranylgeraniol, a key metabolite in synaptic plasticity/learning.^{47, 48} In addition, CYP46A1 knockout mice have reduced cholesterol biosynthesis, significant impairments in cognitive function, along with effects on a variety of signaling pathways.⁴⁹ This knockout is a

profound insult to development, yet these data indicate that there are plausible mechanisms that might explain how lower cholesterol may be a risk factor for PD (see Introduction), and how cholesterol metabolites may be critically involved. This speculation warrants further investigation in future clinical and experimental studies.

Potential clinical relevance of a (S)24-OH-cholesterol-PD link

If cholesterol metabolism truly contributes to PD development one way or another, it inevitably will lead to the controversial speculation as to whether statins should be used in PD patients or individuals at risk of PD, mainly because of the well-known benefits of statins on cardiovascular health. Our finding that cholesterol mechanisms in the brain, but not in the periphery, are specifically linked to PD provides a possible resolution. If our findings are proven causal, one may advise PD patients or individuals at risk for PD to choose statins that would lower peripheral cholesterol, but maintain the integrity of brain cholesterol synthesis and turnover. Several lines of evidence support such a notion. First, only brain permeable lipophilic statins (the ones that could inhibit CNS synthesis and metabolism⁵⁰) facilitate the manifestation of PD.⁵¹ Second, highly lipophilic statins (such as atorvastatin and simvastatin) appear to confer a greater risk for adverse cognitive side effects compared to less lipophilic or hydrophilic properties.⁵² Third and most recently, lipophilic statins also have been associated with higher AD risk compared to hydrophilic statins.⁵³ We recognize that others have viewed the available literature and come to the opposite conclusion,⁵⁴ and cholesterol-lowering drugs that do not penetrate the BBB also may affect brain cholesterol metabolism via complex feedback and modulatory processes.⁵⁵ Thus, further research is needed to resolve these controversies, and guide clinical practice in the choice of cholesterol lowering strategies in people who may be at risk for PD and other related neurodegenerative processes.

Limitation and future directions

Although our study is larger than previous reports, the sample size still is relatively small. For this reason, we were unable to investigate associations of statin use or the use of individual statins with PD risk. The (S)24-OH-cholesterol-PD links, however, persisted even after excluding the small minority of statin users. In addition, although we accounted for age and other potential confounders in our statistical analyses, residual confounding still may exist. Furthermore, although controversial, BMI has been suspected to influence plasma LDL/cholesterol levels and the PD process,^{56–58} but its potential etiological role in PD development is far from established with the majority of cohort studies identifying a null association.⁵⁹ Nonetheless, it is possible that our observed lower (S)24-OH-cholesterol levels are due to lower BMI in PD patients. In the current study, we asked self-reported body weight and height at enrollment, and objectively measured it at the return visits. On both occasions, the mean BMIs were almost identical between PD cases and controls (see Supplementary Table 3). Thus, it is unlikely that low BMI contributed to the observed lower (S)24-OH-cholesterol in PD. For these reasons, we did not adjust for BMI in the current analysis. Nonetheless, it is essential to confirm our study results in larger studies, preferably prospective cohorts of at-risk populations or individuals with prodromal PD. These will help elucidate the nature of our observations and potentially provide mechanistic insights.

In summary, this study for the first time suggests a role of brain cholesterol metabolism in PD pathogenesis.⁵¹ More importantly, there is a large body of evidence showing a potential relationship between cholesterol metabolism and neurodegeneration in diseases such as AD, MS, and Huntington's,¹⁹ and also data regarding epigenetic regulation of cholesterol homeostasis.⁶⁰ For these reasons, further studies of these phenomena and their underlying mechanism(s) are interesting heuristically as they relate to the pathoetiology of PD, and timely because of the practical implications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Population characteristics of PD patients and controls at study enrollment

Group	PD	Controls	Raw P value	Adjusted P value*
Total number of subjects	60	64		
Gender: Male [N (%)]	35 (58.3%)	32 (50.0%)	0.352	NA
Age (years)	63.8 (8.3)	59.7 (7.8)	0.005	NA
Statin medication [N (%)]	17 (28.3%)	12 (18.8%)	0.208	0.813
Statin type: lipophilic [N (%)]	14 (23.3%)	11 (17.2%)	0.394	0.841
History of Smoking [N (%)]	18 (30.0%)	15 (23.4%)	0.409	0.716
PD-related clinical information				
Disease duration (years)	5.0 (5.4)	NA	NA	NA
LEDD	647 (458)	NA	NA	NA
UPDRS-total	39.6 (21.7)	NA	NA	NA
I	11.7 (7.9)	NA	NA	NA
II	9.5 (7.8)	NA	NA	NA
III	17.8 (11.5)	NA	NA	NA
MoCA	24.8 (3.4)	26.1 (2.5)	0.025	0.180
HAM-D	8.0 (4.7)	4.0 (2.5)	<0.001	<0.001
UPSIT	21.5 (7.4)	34.0 (5.6)	<0.001	<0.001
Plasma lipid measurements				
Total-cholesterol (mg/dL)	195 (35)	207 (44)	0.100	0.222
LDL-cholesterol (mg/dL)	122 (31)	128 (39)	0.396	0.697
HDL-cholesterol (mg/dL)	52 (15)	54 (18)	0.394	0.951
(S)24-OH-cholesterol (ng/mL)	23 (8)	29 (11)	<0.001	0.002
27-OH-cholesterol (ng/mL)	51 (20)	57 (17)	0.073	0.346

Data are presented for PD and controls as mean (standard deviations, SD) unless otherwise noted in the first column. Units, where appropriate, are listed with parentheses after criteria. **Abbreviations:** HAM-D (Hamilton Depression Rating Scale); LEDD (levodopa equivalent daily dosage); MoCA (Montreal Cognitive Assessment); UPDRS (Unified PD Rating Scale); UPSIT (University of Pennsylvania Smell Identification Test); NA (not applicable).

* P values are adjusted for age and gender. Significant group differences are indicated by bold text (p<0.05).

Table 2.

Odds ratios (OR) for PD according to tertiles of plasma oxysterol levels at baseline

	(S)24-OH-cholesterol			27-OH-cholesterol		
	OR	95% CI	P value *	OR	95% CI	P value *
<i>Model A: adjusts for age, gender, smoking status, and statin usage</i>						
Tertile 1 ** (PD/C) ***	1.0 (ref) (39/21)	--	--	1.0 (ref) (30/21)	--	--
Tertile 2 ** (PD/C) ***	0.34 (16/21)	0.12 – 0.98	<i>0.045</i>	0.58 (16/21)	0.23 – 1.46	<i>0.249</i>
Tertile 1 ** (PD/C) ***	0.08 (5/22)	0.02 – 0.31	<i><0.001</i>	0.60 (14/22)	0.23 – 1.53	<i>0.282</i>
Linear	0.92	0.87 – 0.97	<i>0.004</i>	0.99	0.97 – 1.01	<i>0.377</i>
<i>Model B: Model A + adjustment for the other oxysterol level</i>						
Tertile 1	1.0 (ref)	--	--	1.0 (ref)	--	--
Tertile 2	0.34	0.12 – 0.98	<i>0.046</i>	0.73	0.27 – 1.92	<i>0.518</i>
Tertile 3	0.08	0.02 – 0.32	<i><0.001</i>	0.85	0.31 – 2.39	<i>0.762</i>
Linear trend	0.92	0.87 – 0.98	<i>0.006</i>	1.00	0.98 – 1.02	<i>0.892</i>
<i>Model C: Model A + adjustment for total-cholesterol level</i>						
Tertile 1	1.0 (ref)	--	--	1.0 (ref)	--	--
Tertile 2	0.35	0.12–1.02	<i>0.054</i>	0.61	0.24–1.53	<i>0.290</i>
Tertile 3	0.08	0.02–0.33	<i><0.001</i>	0.62	0.24–1.61	<i>0.325</i>
Linear trend	0.92	0.87–0.98	<i>0.006</i>	0.99	0.97–1.01	<i>0.466</i>
<i>Model D: Model A + adjustment for HAMD and UPSIT</i>						
Tertile 1	1.0 (ref)	--	--	1.0 (ref)	--	--
Tertile 2	0.17	0.03–1.06	<i>0.057</i>	0.50	0.10–2.58	<i>0.411</i>
Tertile 3	0.04	0.004–0.48	<i>0.010</i>	1.80	0.39–8.31	<i>0.451</i>
Linear trend	0.94	0.87–1.01	<i>0.076</i>	1.02	0.98–1.06	<i>0.274</i>

* Significant odds ratios are indicated by bold italicized text.

** Range for (S)24-OH-cholesterol tertiles: 1=10–24; 2=24–31; 3=31–68 ng/mL; Range for 27-OH-cholesterol tertiles: 1=21–48; 2=48–61; 3=61–145 ng/mL; Linear trend is based on a 1 ng/mL increase in oxysterol level.

*** : The numbers of Parkinson's disease (PD) and Control (C) subjects in each of the (S)24-cholesterol tertiles are reported in parenthesis in rows under Model A, and are the same for Models B, C, and D.

Abbreviations: CI: Confidence interval; OR: Odds ratio.

Table 3.

Spearman correlations^{*} between plasma oxysterols and clinical features of PD subjects at enrollment

		Model A^{**}		Model B^{***}	
Oxysterols		(S)24-OH-	27-OH-	(S)24-OH-	27-OH-
UPDRS-total	r	0.06	−0.11	0.06	−0.10
	p-value	<i>0.69</i>	<i>0.45</i>	<i>0.67</i>	<i>0.47</i>
MoCA	r	−0.18	0.06	−0.18	0.07
	p-value	<i>0.20</i>	<i>0.66</i>	<i>0.20</i>	<i>0.61</i>
HAM-D	r	0.06	−0.07	0.10	0.02
	p-value	<i>0.69</i>	<i>0.62</i>	<i>0.47</i>	<i>0.91</i>
UPSIT	r	0.36	0.18	0.35	0.15
	p-value	<i>0.01</i>	<i>0.21</i>	<i>0.01</i>	<i>0.30</i>

^{*} P values for these explorative analyses were not corrected for multiple comparisons. Values ≤ 0.05 are in bold.

^{**} Model A: Base model that adjusts for age, gender, smoking, disease duration, and statin use

^{***} Model B: Model A + adjustment for the other oxysterol

Abbreviations: HAM-D: Hamilton Depression Rating Scale; LEDD; levodopa equivalent daily dosage; MoCA: Montreal Cognitive Assessment; UPDRS: Unified PD Rating Scale; UPSIT: University of Pennsylvania Smell Identification Test.

Table 4.

Longitudinal data in PD cases and controls

		PD (n=35)	Controls (n=33)				
Baseline characteristics							
Gender: Male [N (%)]		17 (48.6%)	14 (42.4%)				
Age [Mean (SD)]		62.4 (8.8)	59.4 (8.0)				
Statin usage [N (%)]		8 (22.9%)	6 (18.2%)				
History of Smoking [N (%)]		10 (28.6%)	8 (24.2%)				
Longitudinal PD-related clinical information and plasma lipid measurements							
	Baseline	Follow-up	P-values [*]	Baseline	Follow-up	P-values [*]	
	LEDD	613 (467)	956 (537)	0.001	--	--	--
	UPDRS-total	38.1 (20.5)	43.4 (28.0)	0.060	--	--	--
	MoCA	25.2 (2.8)	24.4 (3.3)	0.169	25.9 (2.7)	25.8 (2.5)	0.699
	HAM-D	7.3 (4.2)	7.6 (6.4)	0.901	3.6 (1.9)	2.0 (3.7)	0.006
	UPSIT	21.6 (7.3)	18.5 (7.3)	<0.001	34.1 (5.2)	33.3 (6.2)	0.085
	Total-cholesterol (mg/dL)	201 (34)	189 (36)	0.010	207 (39)	212 (37)	0.296
	LDL-cholesterol (mg/dL)	128 (30)	113 (30)	<0.001	127 (39)	131 (35)	0.317
	HDL-cholesterol (mg/dL)	52 (16)	54 (16)	0.073	55 (18)	56 (17)	0.674
	(S)24-OH-cholesterol (ng/mL)	23 (7)	23 (7)	0.812	29 (10)	29 (9)	0.842
	27-OH-cholesterol (ng/mL)	54 (22)	42 (13)	<0.001	56 (17)	47 (14)	0.013

* Analyses limited to enrollees who had a second clinical visit on average 3 years later. Baseline and follow up measures within each group were compared using a paired t-test. Significant differences are indicated by bold text (p<0.05).

Abbreviations: HAM-D: Hamilton Depression Rating Scale; LEDD: levodopa equivalent daily dosage; MoCA: Montreal Cognitive Assessment; UPDRS: Unified PD Rating Scale; UPSIT: University of Pennsylvania Smell Identification Test.