Confounding of the association between statins and Parkinson disease: systematic review and meta-analysis

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ABSTRACT
Purpose Both statins and higher serum cholesterol have been reported to reduce the risk of Parkinson Disease (PD). Given the importance of adjusting for cholesterol levels when evaluating the effect of statins, we assessed whether the protective effect of statins would remain when adjustment for cholesterol is performed.

Methods We conducted a systematic review of epidemiologic studies that reported quantitative estimates of the association between statins and incident PD and were published through February 2016. Random-effects meta-analysis was used to assess the effect of statins on PD separately among the studies that adjusted for either cholesterol or hyperlipidemia and studies that did not.

Results Ten eligible studies that evaluated the use of statins and incident PD were identified. Among the six studies that did not adjust for cholesterol, a protective effect of statins was observed (relative risk 0.75; 95% confidence intervals (CI) 0.60 to 0.92). Excluding studies with possible bias because of reverse causation or stratifying on study design did not affect the results. No protective effect was observed among the four studies that adjusted for either cholesterol or hyperlipidemia (relative risk 0.91; 95% CI 0.68 to 1.22). The effect estimate for studies that adjusted for cholesterol was 1.04 (95% CI 0.68 to 1.59) when a study with immortal time bias was excluded.

Conclusions The apparent protective effect of statins on risk of PD is at least partially explained by confounding by statin indication. Immortal time bias and healthy user effects also could have contributed to biased results.

INTRODUCTION
The anti-inflammatory and immunomodulating properties of the cholesterol-lowering medications statins have led to numerous studies assessing their role in diseases other than cardiovascular disease for which they are primarily indicated.1,2 Some authors have suggested that statins may be neuroprotective and therefore play a role in preventing neurodegenerative disorders, including Parkinson’s disease (PD).3–5 A number of animal6,7 and epidemiologic studies support this hypothesis,8,9 although the epidemiologic evidence is not consistent. A recent meta-analysis of epidemiologic studies reported a 23% reduction in PD risk associated with statin use (relative risk (RR) 0.77; 95% confidence interval (CI) 0.64 – 0.92).10

At the same time, cholesterol is involved in many biological functions, including cellular repair,11,12 and there is growing evidence that higher serum cholesterol may be associated with lower occurrence of PD,13–15 although as with the statins, conflicting findings have been reported. It is not known whether a putative protective effect of cholesterol on PD might explain the observed inverse association between statins and PD. Because statins are primarily indicated for high cholesterol, accounting for baseline cholesterol levels in observational studies is necessary to avoid confounding if cholesterol is indeed associated with PD. In the prior meta-analysis, all studies were included regardless of whether they adjusted for cholesterol.
We sought to evaluate the design and analytical methods used in the studies of the association between statins and PD incidence and to conduct a stratified meta-analysis to assess the impact of cholesterol adjustment on the observed association.

METHODS

We followed the Meta-analysis of Observational Studies in Epidemiology guidelines for performing and reporting the present meta-analysis.16

Search strategy

We systematically searched PubMed and Embase for articles published through February 2016 using the following search terms: ((Hydroxymethylglutaryl-CoA Reductase Inhibitors OR statin(s) OR lovastatin OR simvastatin OR atorvastatin OR rosuvastatin OR fluvastatin OR pitavastatin OR pravastatin) AND Parkinson Disease). In addition, reference lists of original articles, reviews and previous meta-analyses were examined to identify potentially relevant studies. No language restrictions were imposed.

Study selection

The titles and abstracts of all identified studies were screened independently by two authors to exclude studies that did not meet the eligibility criteria. Full texts of studies selected for further review were obtained and evaluated. Studies were included in the analysis if they met the following criteria: (i) epidemiologic study in humans; (ii) assessed incident PD as outcome; (iii) defined exposure as use of statins; (iv) described analytical methods used to assess the association; (v) stated the variables used for confounding adjustment; (vi) reported effect estimates with CIs, standard errors or sufficient information to calculate these.

Data extraction

Data extraction was conducted by two authors independently. The following data were extracted for each study: study name, year of publications, type of study, exposure definition, type of controls, outcome definition, methods for confounding adjustment, variables adjusted for and effect estimates with CIs. Each article was critically reviewed and discussed by three pharmacoepidemiologists for the evaluation of methodology. If multiple estimates were reported, maximally adjusted estimates were used in primary analyses; other estimates were included in sensitivity analyses.

Data analysis

Our primary analysis used random effects meta-analyses with inverse variance weights to obtain pooled estimates separately for studies that adjusted for either baseline cholesterol levels or history or duration of hyperlipidemia and for studies that did not. If only stratified results were presented in individual studies (e.g. for individual statins or separate estimates for current and past use in case-control studies), fixed-effect models were used to summarize the stratified estimates into a single parameter for each study. Presence of heterogeneity in effects was assessed using the Cochran Q test for heterogeneity and the I^2 statistic. The 95% CIs for the I^2 statistic were determined using the test-based methods.17 Publication bias was evaluated with Begg and Egger’s tests, as well as with visual examination of Begg’s funnel plot.18,19 Because time-related biases are common in observational studies of medications and are often responsible for apparent protective effects of drugs,20 we conducted primary analyses both including and excluding studies with immortal time bias (bias because of the inclusion of follow-up time during which events cannot occur)20 and those with potential reverse causation (discontinuing statins because of symptoms of undiagnosed PD).

Sensitivity analyses

Because Huang et al.21 had both unadjusted and adjusted for cholesterol estimates presented and was the only study that reported an increased risk of PD with statin use, we conducted a sensitivity analysis, in which the estimate that was not adjusted for cholesterol was added to the group of studies that did not consider cholesterol adjustment, as well as a sensitivity analysis omitting Huang et al. from the group of studies that adjusted for cholesterol. In addition, Huang et al. presented results adjusting for diabetes, coronary heart disease and either average cholesterol levels evaluated during the same visits that statin exposure was assessed or adjusted for baseline cholesterol levels, in separate models. We considered the former to be the maximally adjusted estimate but also conducted a sensitivity analysis using the latter instead.

Because different observational study designs may have different vulnerabilities to certain biases, we also stratified analyses by study design (i.e., cohort and case–control).
RESULTS

Literature search

The literature search and screen process yielded 14 potentially eligible articles (Figure 1). Upon full-text review, three studies were excluded because prevalent rather than incident PD was assessed, and one study was excluded because it evaluated a different research question (i.e., discontinuation of statins as compared to staying on therapy). Thus, 10 studies met our inclusion criteria and were included in the primary analysis.

Study characteristics

Four of the 10 studies were case–control studies, and six were cohort studies (Table 1). The majority of the studies (9 out of 10) compared the use of statins to no use, while one study compared initiators of statins to initiators of cardiovascular medications. Four studies adjusted for either cholesterol or hyperlipidemia: Becker et al. adjusted for hyperlipidemia measured prior to PD diagnosis, Gao et al. adjusted for duration of hypercholesterolemia, Friedman et al. included baseline low-density lipoprotein (LDL) levels, and Huang et al. adjusted for either baseline total cholesterol (before statin use) or average levels across four visits during which statin exposure was ascertained. Begg’s funnel plot (Figure S1) and Egger’s test suggested possible publication bias; however, the number of studies was small and other factors, such as poor methodological design of individual studies, could be responsible for the asymmetry in the funnel plot.

Association between statin use and PD in studies that did not adjust for cholesterol

The pooled estimate for the six studies that did not adjust for cholesterol was 0.75 (95%CI: 0.60, 0.92) (Figure 2). Significant heterogeneity in results was observed ($I^2=92\%$, 95%CI: 86%, 96%; $p$ for heterogeneity < 0.001). In one study, statin exposure was entered as a time-dependent variable. In that study, individuals using statins could have discontinued the drugs because of subclinical symptoms of PD (e.g., pain because of rigidity in PD could be misconstrued as a statin side effect as these drugs are known to cause muscle cramps and muscle pain) and subsequently presented as statin non-users at the time of PD diagnosis, inducing a form of reverse causation. Excluding this study did not materially change the pooled effect estimate (RR = 0.76; 95%CI: 0.61, 0.94).

Association between statin use and PD in studies that adjusted for cholesterol

The pooled effect estimate for the four studies that adjusted for cholesterol levels or history of hyperlipidemia was 0.91 (95%CI: 0.68, 1.22) (Figure 3). Significant heterogeneity in effects was observed ($I^2=75\%$, 95%CI: 32%, 94%; $p$ for heterogeneity = 0.007). One study was subject to potential immortal time bias as entry into the cohort was defined as the date of the first LDL measurement for everybody, including statin users and, therefore, the time between the entry into the cohort and the subsequent first statin prescription is event free by definition for statin users. Excluding this study increased the estimate adjusted for cholesterol to 1.04 (95%CI: 0.68, 1.59).

Sensitivity analyses

The results of all sensitivity analyses are presented in Table S1. Using the Huang et al. effect estimate adjusted for baseline cholesterol level only did not materially impact the pooled effect estimate (RR = 0.91; 95%CI: 0.68, 1.22). Omitting Huang et al. resulted in a lower pooled estimate adjusted for cholesterol (RR = 0.82; 95%CI: 0.66, 1.01), which increased to RR of 0.88 (95%CI: 0.66, 1.16) after also excluding the study with immortal time bias. Adding the estimate reported by Huang et al. that was not adjusted for cholesterol to the group of studies that did not adjust for cholesterol.

Figure 1. Flow of study identification and inclusion
cholesterol resulted in a pooled estimate of 0.80 (95% CI: 0.65, 0.99).

Three out of four studies that adjusted for cholesterol were cohort studies, and one was a case–control study. Among cohort studies only, the pooled estimate was 0.92 (95% CI: 0.60, 1.40). Three out of six studies that did not adjust for cholesterol were cohort studies and three were case–control studies. The pooled estimates were similar for cohort studies (RR = 0.71; 95% CI: 0.53, 0.94) and case–control studies (RR = 0.78; 95% CI: 0.58, 1.05).

**DISCUSSION**

This meta-analysis of epidemiologic studies shows that, while studies that do not adjust for cholesterol suggest a protective effect of statins on the risk of Parkinson disease, studies that adjust for cholesterol do not show a significant protective effect.
PD, the protective effect is not observed in studies that performed adjustment for cholesterol. After further excluding a study with immortal time bias, the point estimate for studies that adjusted for cholesterol was 1.04 (95%CI 0.68 to 1.59) when comparing patients who were exposed to statins to those who were not. Our study calls into question the findings from previous epidemiological studies that have suggested that statins reduce the risk of PD. While statins may indeed reduce neuroinflammation and have been shown to confer protection against PD in animal and cell models, it is not clear whether the effect is the same in humans. Information on pharmacokinetics and pharmacodynamics of statins in the human brain is quite limited. The only information available on the association between statin use and risk of PD in humans comes from epidemiological studies, which are often prone to confounding, particularly when studying the effects of medications. Cholesterol plays an important role in many biological functions and could be either a precursor or a marker of other potential neuroprotective substrates, such as coenzyme Q10, which has been linked to oxidative stress in the pathogenesis of PD. Four prospective cohort studies have found that higher total serum cholesterol was associated with a lower occurrence of PD while one study yielded a contradictory finding. As with the studies on the association between statin use and PD, these findings could represent a true causal effect, could have arisen by chance, or could be confounded or biased in other ways.

If higher cholesterol is associated with lower risk of PD, use of statins would be crudely inversely associated with PD incidence, even in the absence of the effect of statins on PD incidence. The protective effect of statins that we observed when no adjustment for cholesterol was done (RR = 0.75; 95%CI: 0.60, 0.92) was very similar to the results reported by a previous meta-analysis (RR=0.77; 95%CI: 0.64, 0.92) that included studies published through 2012 but did not stratify based on adjustment for confounding by indication.

Moreover, it is possible that use of statins might not only be just a marker for high cholesterol in observational studies, but their use might actually reverse the protective effect of high cholesterol on PD development. The findings from one prospective cohort study support this hypothesis. After adjusting for age, sex, race, smoking, caffeine intake, total cholesterol, diabetes, and coronary heart disease, Huang et al. found that the use of statins was associated with increased risk of PD (OR = 2.39; 95%CI: 1.11, 5.13). However, given that this was the only study in our analysis that observed a positive association between statins and PD incidence even before adjustment for cholesterol, it could very well be the case that the findings have arisen by chance or that there is something fundamentally different about the study population or study design. Nevertheless, the authors did observe known associations between PD and age, sex and smoking. Further, the adjustment for baseline cholesterol (or cholesterol prior to start of follow-up) was more thorough than the adjustment performed in the other studies. Omitting this study from the meta-analysis, as well as adding the estimate that was not adjusted for cholesterol to the group of studies that did not adjust for cholesterol, did not significantly change the pooled results for either group (Table e-1).

It is important to note that our meta-analysis is limited by methodological shortcomings of the original studies and that there was a high amount of between-study heterogeneity overall and within each stratum of cholesterol adjustment. Heterogeneity...
may arise from studies’ inclusion of different study populations or methodological differences. Given the heterogeneity we observed, it is possible that the differences in estimates stratified by whether or not the studies adjusted for cholesterol could reflect other differences in studies between the two strata and not necessarily the independent effect of adjusting for cholesterol.

Confounding by indication is a threat to the validity to all the studies reviewed. Even among the studies that attempted to control for measures of high cholesterol, residual confounding by cholesterol likely remains when adjustment involved a diagnosis or history of lipid-related disorders, such as hypercholesterolemia or hyperlipidemia, and not actual baseline cholesterol levels. Because this was the case in two out of four studies that adjusted for either cholesterol or hyperlipidemia, the pooled estimate for this group might still be confounded and might underestimate the true effect of statin use on PD incidence. Confounding by indication can be less of an issue in studies that compare active treatments. Among the studies reviewed, only one study considered this approach. Wolozin et al. used initiators of other cardiovascular medications as a comparator group; however, cardiovascular medications include a broad range of therapeutic classes and most cardiovascular drugs are not used to treat high cholesterol. Thus, we did not consider this comparison as sufficient for control of confounding by indication. Other confounders, such as smoking or caffeine intake, might also have affected the estimates, if differential between the exposure groups. Most of the studies adjusted for either smoking or chronic obstructive pulmonary disease as a proxy for smoking in their analysis. While two studies did not present information on or adjust for smoking, there were no major difference in smoking patterns between statin users and non-users in the studies that did present this information, suggesting that smoking is not likely to be a strong confounder.

Immortal time bias, which can occur when follow-up time during which no outcomes can possibly occur is included in the analysis, is a concern in studies of medications that compare users to non-users. As it often involves the inclusion of event-free unexposed time in the exposed group, immortal time bias has been responsible for apparent protective effects of drugs. We identified one study that incurred immortal time bias. As expected, exclusion of that study moved the pooled effect estimate in the upward direction. The healthy user and healthy adherer effects can also be a potential source of bias, particularly in the context of preventive medications. Both statin initiation and statin adherence have been found to be associated with reduced rates of clinical outcomes that are unlikely to be biologically related to either the need for or the use of a statin, particularly when comparison group includes patients not on statin therapy. This suggests that patients on statins might have a better prognosis or be more health conscious and thus more likely to stay on therapy. While it is plausible that both the need for statins (high cholesterol) and statins themselves could have independent effects on PD, it is possible that some of the protective effect of statins detected in observational studies could be attributed to healthy user effect, particularly when prevalent users are compared to patients who are not using statins. Such effects can be minimized through the use of an active comparator group and a new user design.

In addition to methodological limitations, several issues related to the etiology of PD could have affected the validity of the studies included in our analysis. As PD is a slowly progressing disease with an insidious onset and a latency period of 10–20 years, the window of opportunity for disease prevention or modification might be many years before the clinical diagnosis is made. Many of the included studies evaluated periods that might not be relevant to PD development. In particular, of the four case–control studies included in our analysis, two assessed exposure to statins immediately prior to PD diagnosis while two considered a lag period, but only of two or five years.

In conclusion, previous findings of a protective effect of statins on occurrence of PD observed in epidemiological studies are likely confounded by cholesterol levels. Because of the methodological limitations of the studies, as outlined above, our stratified meta-analysis may not reflect the true causal effect of statins on PD. However, our findings suggest that, upon adjustment for cholesterol, the inverse association between statins and PD is no longer present. Future studies should be conducted to confirm our findings and to better estimate the effect of statins on PD independent of the impact of cholesterol on PD. These studies should more fully address confounding by indication as well as the other methodological challenges identified in this review. Appropriate induction periods with lag times before start of follow-up and latency periods after drug discontinuation and sufficiently long follow-up periods should be used. Furthermore, new user design and the use of an active comparator group can reduce the potential for immortal time bias and healthy user effect.
CONFLICT OF INTEREST

The authors declare no conflict of interest.

KEY POINTS

- The apparent protective effect of statins on the risk of Parkinson Disease could be confounded by statin indication
- Most of the prior observational studies on the association between statin use and Parkinson Disease did not adjust for cholesterol levels
- Our stratified meta-analysis showed that while studies that did not adjust for cholesterol suggest a protective effect of statins, that effect is not observed in studies that performed the adjustment for cholesterol
- Other biases, such as immortal time bias and healthy user effect, could also have contributed to biased results

ACKNOWLEDGEMENTS

Dr. Yoshida receives tuition support jointly from Japan Student Services Organization (JASSO) and Harvard T. H. Chan School of Public Health (partially supported by training grants from Pfizer, Takeda, Bayer and PhRMA). Dr. Bykov is supported by a training grant from Takeda through Harvard T. H. Chan School of Public Health

REFERENCES


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