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Statin therapy is associated with reduced neuropathologic changes of Alzheimer disease

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ABSTRACT

Background: Treatment with 3-hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitors ("statins") has been associated in some epidemiologic studies with reduced risk of Alzheimer disease (AD). However, direct evidence of statin effects on neuropathologic markers of AD is lacking. We investigated whether antecedent statin exposure is associated with neuritic plaque (NP) or neurofibrillary tangle (NFT) burden in a population-based sample of human subjects.

Methods: Brain autopsies were performed on 110 subjects, ages 65 to 79 years, who were cognitively normal at enrollment into the Adult Changes in Thought Study. Neuropathologic findings were compared between statin users with ≥3 prescriptions of ≥15 pills of simvastatin, pravastatin, lovastatin, or atorvastatin vs nonusers, based on pharmacy dispensing records.

Results: After controlling for age at death, gender, cognitive function at study entry, brain weight, and presence of cerebral microvascular lesions, the odds ratio (OR) for each unit increase in Braak NFT stage in statin users vs nonusers was 0.44 (95% CI: 0.20 to 0.95). The OR for each unit increase in Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) staging of NPs did not deviate significantly from unity (OR 0.69; 95% CI: 0.32 to 1.52). However, the risk for typical AD pathology (Braak stage ≥ IV and CERAD rating ≥ moderate) was reduced in statin users (OR 0.20; 95% CI: 0.05 to 0.86).

Conclusions: These findings demonstrate an association between antecedent statin use and neurofibrillary tangle burden at autopsy. Additional study is needed to examine whether statin use may be causally related to decreased development of Alzheimer disease–related neuropathologic changes. Neurology® 2007;69:878–885

Alzheimer disease (AD) is the most common cause of dementia in late life, affecting 4 million individuals in the United States. Several early epidemiologic studies suggested a protective effect of 3-hydroxy-3-methylglutaryl-coenzyme-A reductase inhibitors ("statins") against development of cognitive impairment, all-cause dementia, and AD. However, recent large prospective cohort studies have not supported these findings. Furthermore, two large randomized placebo-controlled trials of statins for prevention of coronary heart disease failed to provide evidence of a protective effect against cognitive decline. Likewise, our initial analysis of data from the Adult Changes in Thought (ACT) Study did not show a significant protective effect of statins against dementia. However, a later analysis of a larger sample from ACT did suggest a possible protective effect of statins, particularly in subjects younger than age 80 at enrollment.

Growing evidence suggests that the fundamental processes underlying the development of dementia are multiple and heterogeneous, implying that clinical diagnoses may not adequately represent underlying disease pathogenesis. This disjunction may represent a major impediment to the accurate identification of disease risk and/or protective...
factors. To circumvent it, we sought evidence of modified AD risk in statin users by examining associations between statin use and the typical AD-related neuropathologic outcomes, neurofibrillary tangles (NFTs), and neuritic plaques (NPs) in subjects who underwent brain autopsy. Because statins were not widely used before the mid-1990s, most of our older subjects had not been treated with statins in middle or early old age. To minimize potential biases introduced by differential age-related exposure to statins, we restricted our analysis to the 110 brain autopsies of subjects who entered the ACT study before age 80.

METHODS Subjects. The ACT Study was initiated in 1994 at which time it recruited 2,581 cognitively normal individuals age 65 years and older from Group Health Cooperative (GHC) of Puget Sound, a large health maintenance organization. In the years 2000 to 2002, an additional 811 cognitively normal subjects (expansion cohort) were recruited from GHC, resulting in a total of 3,392 participants. Of those, 2,523 (74%) were younger than age 80 at enrollment and are considered here. Detailed ACT study design and methods have been described. \(^\text{13}\) In brief, each subject underwent a baseline neurocognitive examination and was then followed up biennially for cognitive decline as detected by a score less than 86 on the Cognitive Abilities Screening Instrument (CASI). \(^\text{14}\) Those with cognitive decline underwent a complete dementia evaluation with a neuropsychological test battery and a physical/neurologic examination by a geriatrician or neurologist. Relevant laboratory results and brain CT or MRI scans were obtained, after which diagnoses were assigned at a consensus diagnosis conference using Diagnostic and Statistical Manual of Mental Disorders-IV edition \(^\text{15}\) and National Institute of Neurologic and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association criteria for AD. \(^\text{16}\) At each study visit, a detailed medical history was obtained by a trained interviewer. Serum total cholesterol and high-density lipoprotein (HDL) measurements were available from the GHC computerized laboratory database established in 1988. Patients were not routinely expected to be fasting for lipid measurements. Most subjects (94%) had at least one total cholesterol and HDL measurement prior to first prescription of statins or for those who had never received statins before death. There was no difference in number of available lipid measurements between statin users (94%) and nonusers (93%). The protocol for this study was approved by the University of Washington and GHC human subjects review committees. All subjects provided written informed consent prior to enrollment.

By July 31, 2006, 608 (24%) of the eligible sample of 2,523 had died, with an overall mortality rate that was lower in statin users than in nonusers (19 vs 26%, \(p < 0.01\)). Among the decedents, 110 (18%) had consented for neurologic postmortem examination and undergone brain autopsy. This autopsied sample was older at death and more likely to be female and Caucasian than other decedents (table 1).

**Table 1** Characteristics of autopsied and nonautopsied subjects

<table>
<thead>
<tr>
<th></th>
<th>Died, without autopsy, n = 498</th>
<th>Autopsied, n = 110</th>
<th>(p) Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry, y</td>
<td>73.6 ± 3.8</td>
<td>74.1 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>79.6 ± 4.9</td>
<td>81.2 ± 4.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Male, %</td>
<td>57</td>
<td>45</td>
<td>0.04</td>
</tr>
<tr>
<td>Race, non-Caucasian, %</td>
<td>11</td>
<td>4</td>
<td>0.04</td>
</tr>
<tr>
<td>Education, y</td>
<td>13.7 ± 3.0</td>
<td>14.2 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Statin users, %</td>
<td>24</td>
<td>33</td>
<td>0.09</td>
</tr>
<tr>
<td>CASI score at baseline</td>
<td>92.5 ± 5.0</td>
<td>93.4 ± 4.7</td>
<td>0.09</td>
</tr>
<tr>
<td>Dementia, %</td>
<td>18</td>
<td>25</td>
<td>0.08</td>
</tr>
<tr>
<td>Probable AD, %</td>
<td>8</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>APOE*4, %</td>
<td>25</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Expansion cohort, n</td>
<td>6</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>BMI at baseline, lb/in²</td>
<td>27.8 ± 5.2</td>
<td>27.4 ± 4.9</td>
<td>NS</td>
</tr>
<tr>
<td>CAD at baseline, n</td>
<td>29</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>DM at baseline, n</td>
<td>19</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>HTN at baseline, n</td>
<td>50</td>
<td>43</td>
<td>NS</td>
</tr>
<tr>
<td>CVA at baseline, n</td>
<td>16</td>
<td>21</td>
<td>NS</td>
</tr>
</tbody>
</table>

*\(t\) test for differences in means or chi-square test or Fisher exact test (for low proportions) for difference in proportions. CASI = Cognitive Abilities Screening Instrument; AD = Alzheimer disease; BMI = body mass index; CAD = coronary artery disease; DM = diabetes mellitus; HTN = hypertension; CVA = cerebrovascular accident.
tin, or atorvastatin, which were available in the GHC formulation during the period of the study. Statin users were defined as those who had received at least three prescriptions (≥15 pills/prescription) for these drugs. There were too few subjects (n = 11) to explore the relationship between neuropathologic outcomes and use of other lipid-lowering agents (LLAs) such as niacin, cholestyramine, colestipol, gemfibrozil, or clofibrate, so use of any non-statin LLA was included as a covariate in the analyses.

**Neuropathologic examination.** Neuropathologic examinations were performed in the UW Division of Neuropathology and the UW AD Research Center (ADRC) Neuropathology Core. All neuropathologic assessments were performed blind to clinical diagnosis and status of risk factors. Brains were immersion-fixed in formalin for at least 2 weeks prior to dissection. Following fixation, all brains were evaluated for any gross lesions, including the extent of atherosclerosis ("mild" when restricted to branch points in the circle of Willis, "moderate" when also in other regions at the base of the brain, and "severe" when present on the convexity of the brain) and the number of gross (macroscopic) cystic infarcts. We limited our evaluation to remote (estimated >1 year old) cystic infarcts, as acute and subacute infarcts were thought unlikely to have contributed to long-standing cognitive decline. Lateral ventricle enlargement was estimated by measuring the maximal cross-section of the lateral ventricles following a coronal section at the temporal tips. Tissue sections were dissected from middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulate gyrus, primary visual cortex, basal ganglia at the level of the anterior commissure, thalamus, hippocampus at the level of the uncus, amygdala, midbrain including substantia nigra, pons at the level of the locus ceruleus, medulla, cerebellar hemisphere, and pituitary gland.

These tissue sections were embedded in paraffin prior to sectioning and staining. NPs were scored according to the criteria of the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD).23,24 NFTs were scored according to the methods of Braak and Braak.25,26 Based on findings of Knopman et al. regarding maximal burdens of AD-type pathologic findings in cognitively intact, community-dwelling elderly individuals,27 we further categorized subjects into those with typical AD-type neuropathologic findings (Braak stage ≥ IV and CERAD rating ≥ moderate) and others with Braak stage ≤ III and/or CERAD rating of 0 or low. Amyloid angiopathy was scored according to the method of Vonsattel.28 Microvascular lesions (MVLs) were evaluated in bilateral sections of frontal lobe, temporal lobe, parietal lobe, occipital lobe, caudate nucleus, putamen, internal capsule, and thalamus similarly to the protocol published by the Honolulu Asian Aging Study.29 A MVL (microinfarct) was defined as an encephalomalacic lesion, 2 mm or smaller in its greatest dimension, which was not visible on gross inspection of the brain.

**Statistical analyses.** Data are presented as means ± SD. Associations among neuropathologic outcomes were assessed using Spearman rank order correlation. Differences in demographic variables between statin use groups were examined using t tests (for continuous variables) or Fisher exact tests (for categorical variables). Differences in neuropathologic or cerebrovascular disease variables between subject groups were examined using Wilcoxon rank sum test and Fisher exact tests. Relationships between neuropathologic outcomes and statin use were also examined using logistic regression (for presence or absence of AD-type neuropathology) and proportional-odds logistic regression (for Braak stages and CERAD scores). For the proportional-odds models, the estimated odds ratios (ORs) corresponded to the approximate change in odds for each 1-unit increase in Braak stage or CERAD score in statin users divided by the change in odds in nonusers. Models were adjusted for age at death, gender, CASI score at baseline, presence of MVLs, and brain weight. As there were only four subjects who were non-Caucasian, we did not adjust for ethnicity. Analyses were conducted using R 2.4.029 and Stata 9.2.21

**RESULTS** Of the 110 subjects who underwent brain autopsy, 36 (33%) had received at least three prescriptions for statins. Compared with the nonusers, statin users were more often male, had more cardiovascular disease or diabetes mellitus at enrollment, had higher pretreatment total cholesterol levels and lower pretreatment HDL levels, and were more often smokers at enrollment (table 2). Whereas statin users had slightly lower CASI scores at enrollment, the incidence of all-cause dementia during follow-up did not differ between groups (table 2).

On average, participants received their first statin prescription at age 76 (SD = 6, range 64 to 87 years). The mean time of first statin prescription was 2 years after enrollment and 5 years before death. The average number of statin prescriptions filled was 30 (SD = 22, range 4 to 90). Among the statin users, the majority (n = 21, 58%) received simvastatin for ≥50% of their statin prescriptions. Lovastatin was the second most commonly used agent (n = 12, 33%).

**Association between statin use and cerebrovascular pathology.** Compared with nonstatin users, those receiving statins showed nonsignificant trends toward more severe vascular pathologies, being more likely to have cystic infarcts (39 vs 24%, p = 0.20) and MVLs (56 vs 38%, p = 0.13) and higher rates of moderate to severe vs mild or no atherosclerosis (47 vs 28%, p = 0.09). The number of MVLs was correlated with both number of cystic infarcts (Spearman r = 0.36, n = 105, p < 0.01) and severity of atherosclerosis (Spearman r = 0.28, n = 99, p < 0.01). As expected, mean number of MVLs was significantly higher in participants with diabetes mellitus at entry (n = 109, p < 0.01). Total brain weight showed a modest tendency to be lower in statin users (p = 0.20).

**Associations between cerebrovascular and AD-related pathologies.** A greater number of MVLs was correlated with higher Braak stage (Spearman r = 0.26, n = 109, p < 0.01), but not with CERAD score (r = 0.06, p > 0.05). Lower brain...
weight was similarly correlated with higher Braak stage ($r = -0.21$, $p = 0.03$) but not with CERAD score ($r = -0.01$, $p > 0.05$).

Association between statin use and AD-related pathologies. Statin users exhibited a lower burden of both NFTs (assessed by Braak staging) and NPs (assessed by CERAD grading) compared with nonusers (figure 1). After controlling for age at death, gender, CASI score at entry, brain weight, and presence of cerebral microinfarcts, statin users had significantly decreased odds of higher Braak stages (table 3), showing a more than two-fold reduction in the risk for each increase of 1 unit on the Braak staging scale. The association between statin use and CERAD plaque scores did not reach statistical significance. However, after controlling for age at death, gender, CASI score at entry, brain weight, and presence of MVL, statin users also had a significantly reduced risk for typical AD-type neuropathology (Braak stage $\geq IV$ and CERAD $\geq$ moderate; table 3).

The more complex relationship among statin use, MVLs, and AD-related neuropathologies is presented graphically in figure 2. Without adjustment for presence vs absence of MVLs, Braak staging appears similar in statin users and nonusers (figure 2A). However, after stratification by presence of MVLs, it becomes evident that Braak stages are lower in statin users than nonusers (figure 2B).

**DISCUSSION** In this community-based study, we found that statin use was significantly associated with reduced NFT burden at autopsy. In addition, typical AD-type neuropathology (i.e., Braak stage $\geq IV$ and CERAD $\geq$ moderate) was signifi-
cantly less common in statin users than in nonusers. A similar association between statin use and amyloid plaque burden assessed by CERAD grading failed to reach statistical significance. To our knowledge, this is the first study demonstrating an association between statin exposure and neuropathologic changes associated with AD.

The strengths of the study are the representative sample of community-dwelling elderly subjects from whom its autopsied subjects were drawn, its prospective study design with high-quality data on statin use, and its relatively large number of brain autopsies. Our neuropathologic examinations were also performed blind to status of clinical diagnoses or risk factors. However, the study has several shortcomings. As with all observational studies, there are sources of potential bias or confounding. A relatively obvious concern relates to the study’s reliance on a select sample of individuals who died and gave permission to be autopsied. The overall lower mortality rate in the statin users (19%) compared with the nonusers (26%) could reflect either a statin-induced reduction in mortality or differential mortality due to treatment indication (for example, statin treatments may have been withheld from more medically ill subjects). Because statin use may thus be related in multiple ways to mortality, and because the assessment of AD neuropathy can occur only after death, the possibility of confounding is substantial. Thus, our findings should be extrapolated to living populations with the greatest caution, if at all. Furthermore, differences in age at death, gender, and ethnicity of autopsied and nonautopsied subjects further restrict the generalizability of our findings, which cannot be generalized to non-Caucasian populations without strong (and probably unjustified) assumptions. However, because there was no strong association between statin use and consent for autopsy, and because our regression models could adjust for age at death and gender (both easy to measure accurately), it is unlikely that our findings are confounded by any relationship of these variables to permission for autopsy.

Another potential shortcoming is that the crude, semiquantitative CERAD scale may have offered limited power to detect effects of statin use on NP burden. Additional research using more sensitive measures of amyloid pathology, such as amyloid load, and a larger sample of autopsies might show a significant relationship with NP burden where our study failed to do so.

There is increasing epidemiologic evidence of association between cardiovascular risk factors (such as high blood pressure and high cholesterol) and risk of AD.26,27 Some neuropathologic studies of demented subjects have demonstrated a relatively lower grade of AD pathologies in the presence of comorbid cerebrovascular pathologies, suggesting that comorbid vascular disease reduces the threshold for clinical expression of dementia with a given level of AD-related pathology.12,28 By contrast, the current study of mixed clinically

| Braak stage (0–VI) | 0.76 (0.38, 1.53) | 0.57 (0.27, 1.22) | 0.44 (0.20, 0.95) |
| CERAD score (0, A–C) | 0.78 (0.38, 1.58) | 0.72 (0.34, 1.56) | 0.69 (0.32, 1.52) |
| AD pathology, absent vs present (0, 1) | 0.33 (0.09, 1.22) | 0.24 (0.06, 1.01) | 0.21 (0.05, 0.88) |

*Adjusted for age at death, gender, and CASI score at entry.
†Adjusted for age at death, gender, CASI score at entry, presence of MVLs (0 vs ≥1 MVL), and brain weight.
‡AD-type pathology: present = Braak stage ≥IV and CERAD grade B or C; absent = otherwise.

CASI = Cognitive Abilities Screening Instrument; MVL = microvascular lesions.

Figure 2 Boxplots of Braak stage by statin use, combined (A) and separated by presence of microvascular lesions (B).
normal and demented subjects showed increased NFTs in those subjects with MVLs compared with those without. Whether vascular disease causes, exacerbates, or just coexists with AD pathology is not clear and is beyond scope of this study. Regardless of the mechanism, however, we did find that vascular disease (specifically, MVLs) was associated with both statin use (exposure) and NFTs (outcome), thus suggesting a potentially confounded relationship between statin use and AD pathology. It is virtually certain that subjects with vascular risk factors such as diagnosed cardiovascular disease, diabetes, and lower pretreatment HDL cholesterol are more likely to be prescribed statins (“confounding by indication”). Because presence of MVLs was correlated with increased NFT burden in our subjects, this sort of confounding would be expected to result in an increased burden of AD pathology in the statin users and a possible offset to any “real” countervailing protective effect of statins on the development of NFTs. To control for the effect of such confounding by indication, we included cerebral MVLs as an adjustment in our logistic regression models. Although such adjustment rarely accomplishes full control on confounding effects, its use here was sufficient to reveal a significant association between statin use and Braak stage or AD-type pathology where this association had been inconclusive in unadjusted models.

The mechanism(s) by which statins might slow the development of AD-type neuropathologic findings are unclear. Early findings from in vitro and animal studies indicated that high levels of cholesterol in the brain could alter amyloid precursor protein (APP) processing, resulting in accumulation of β-amyloid (Aβ) and formation of NPs29,30 and that statin treatment could reverse these changes.31 However, clinical trials in humans failed to demonstrate a consistent effect of statins in altering CSF APP processing or Aβ levels32,33 or in slowing disease progression in AD patients.32,34 By contrast, other in vitro and animal studies have shown that statins may also inhibit tau phosphorylation via multiple mechanisms including: inhibition of the hypothesized “amyloid cascade,”35,36 anti-inflammatory effects,37 and/or direct inhibition of prenylation-induced activation of one or more mitogen-activated protein kinases.38 Consistent with these findings, we have recently reported that 14 weeks of treatment with simvastatin (a statin with high CNS penetration) reduced levels of tau protein phosphorylated at threonine 181 (p-tau181) in cognitively normal middle-aged and old hypercholesterolemic subjects.39

Our findings are consistent with the results of previous case-control epidemiologic studies suggesting a decreased risk of clinical AD in statin users vs nonusers2 but stand in contrast to more recent prospective epidemiologic studies5,6,9 that found no overall reduction in AD risk in statin users compared with nonusers. However, in the Canadian Study of Health and Aging2 and our recent age-stratified analysis with the expanded ACT cohort,10 a protective effect against AD was seen in those subjects who began statin use before age 80. In contrast, controlled trials have shown no significant effect of statin treatment on the progression of established AD.32 This pattern of results supports the hypothesis that statins slow the progression of one or more neurodegenerative processes leading to the development of clinical AD but may not be able to reverse neuronal degeneration once it has occurred, thus rendering them ineffective in treating AD once clinical signs and symptoms have developed. Similar arguments have been raised to explain null or negative results of estrogen and nonsteroidal anti-inflammatory treatment trials in AD.40,41

In summary, the association between statin therapy and reduced NFTs in the current study and our previous observation of statin-induced reductions in CSF p-tau181 levels provide converging evidence that statins might reduce pathologic phosphorylation of tau in humans. Additional observational studies and controlled trials will be required to determine if there is a causal link between statin treatment and the development of NFTs or other AD-related neuropathologies.

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