Mediation Analysis in Understanding Mechanism of Alzheimer’s Disease Risk

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Mediation analysis in understanding mechanism of Alzheimer’s disease risk

Gautam Sajeev

A Dissertation Submitted to the Faculty of
The Harvard T.H. Chan School of Public Health
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Science
in the Department of Epidemiology
Harvard University
Boston, Massachusetts.

May, 2015
Mediation analysis in understanding mechanism of Alzheimer’s disease risk

Abstract

Although studies often show reduced risk of dementia with late-life cognitive activity, concerns about residual confounding and reverse causation cast doubt on these findings. In Chapter 1 of this dissertation, we review epidemiologic studies of cognitive activity and incidence of Alzheimer’s disease (AD) and all-cause dementia, and conduct a bias analysis that indicates the observed inverse associations are likely robust to unmeasured confounding, and probably only partially explained by reverse causation. While pursuing enjoyable cognitive activities may reduce dementia risk, better characterization of the type, duration, and timing of activity associated with late-life cognitive benefit is needed to develop recommendations applicable over the lifecourse.

The apolipoprotein episilon4 allele (APOE e4) is the most well established genetic risk factor for AD, and is also a risk factor for cerebrovascular disease (CVD). In Chapter 2, we use the counterfactual approach to mediation analysis to investigate the degree to which the negative effect on cognition of the e4 allele is attributable to its effects on CVD. Using neuroimaging and neuropsychological data from approximately 4,000 participants of the population-based Age, Gene/Environment Susceptibility–Reykjavik Study, we found that 9% of the e4 effect on cognition was jointly mediated by white matter lesion volume and cerebral microbleeds (CMBs). While our finding that the e4 effect largely operates through non-vascular pathways aligns with previous research and present understanding of the action of apoE in AD and CVD pathogenesis, our study is the first to show a small effect specifically via markers of CVD pathology.

In Chapter 3, we investigate the role of CMBs further using the newly developed four-way decomposition approach. We found that when comparing e4 heterozygotes to e4 non-carriers, the e4 effect on memory was independent of CMBs. By contrast, when comparing e4 homozygotes to e4 heterozygotes, the e4 effect on memory was attributable to interaction between the effects of e4 alleles and CMBs, perhaps suggesting a greater vascular contribution for these individuals. Similar analyses in other population-based studies will be needed to confirm these findings and further elucidate the contributions of CMBs and CVD to the e4 effect on cognition.
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Firstly, thank you to my advisor, Deborah Blacker for allowing me the freedom to explore research areas I found interesting, and for connecting me with experts in these areas once I did so. Thank you also to Deborah for sharing her expertise on Alzheimer’s disease, her steady mentorship over the last five years, and for always being accessible when I required advice or guidance. This dissertation has also been importantly shaped by my research committee, Rebecca Betensky, Francine Grodstein, Tyler VanderWeele, and Anand Viswanathan, and I owe them my gratitude for supporting this work from the start, and for the many insightful comments and suggestions that helped to improve it along the way.

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Gautam Sajeev

Boston, Massachusetts

April, 2015
Chapter 1

Late-life cognitive activity and dementia: a systematic review and bias analysis

Gautam Sajeev, Jennifer Weuve, John W. Jackson, Tyler J. VanderWeele, David A. Bennett,
Francine Grodstein, and Deborah Blacker
Abstract

Objective: It is unclear to what extent the available evidence supports a causal effect of late-life cognitive activity in dementia prevention. We systematically review epidemiologic studies of cognitive activity and incidence of AD and all-cause dementia; describe methodological issues and biases relevant to interpretation of these studies; and quantify the degree of bias due to confounding and reverse causation required to nullify the observed associations.

Methods: We conducted a systematic search of PubMed and EMBASE through June 2014 to identify peer-reviewed epidemiologic studies of cognitive activity and incidence of Alzheimer’s disease or all-cause dementia. Eligible articles analyzed data from cohort or nested case-control studies, explicitly defined cognitive activity, evaluated participants for AD or all-cause dementia using clearly defined criteria, and provided effect estimates adjusted for at least age and sex.

Results: We identified and reviewed 13 studies involving a total of 1,852 dementia cases, of which 565 were specifically evaluated as Alzheimer’s disease, and 16,725 participants. Most studies found an association of late-life cognitive activity with a lower incidence of Alzheimer’s disease and/or all-cause dementia. Differences in operationalization of cognitive activity across studies precluded meta-analysis of effect estimates. Our bias analysis indicated that the observed associations are likely robust to bias due to unmeasured confounding, and might only be partially explained by reverse causation.

Interpretation: As engaging in late-life cognitive activity is unlikely to be harmful, pursuing enjoyable cognitive activities seems a safe approach pending further data. Studies focussing on type, timing and dosage of cognitive activity over the lifecourse are needed to build a broader evidence base for more specific guidance on maintaining late-life cognition.
Introduction

An estimated 36 million people worldwide had dementia in 2010, and this number is projected to triple by 2050 (1). Thus, the search for modifiable preventive factors is becoming increasingly urgent. One such potentially modifiable factor is cognitive activity. Cognitive activities are thought to contribute to building and maintaining brain structure and function (2, 3), sometimes viewed as analogous to the role of physical exercise for cardiopulmonary and muscular tissue and function.

A variety of websites and publications encourage older adults to be mentally active (“use it or lose it!”) in order to protect their brain health. Although epidemiologic studies often show reduced risk of dementia with late-life cognitive activity, concerns about residual confounding and reverse causation cast doubt on these findings. Several characteristics and behaviors linked with greater late-life cognitive activity, such as greater formal education, higher socioeconomic status (SES), and better general health also appear to be associated with reduced dementia risk, suggesting that residual or unmeasured confounding may influence study findings, especially given the difficulty of adequately adjusting for these variables (4). Furthermore, falloff in cognitive activity in late life may be a consequence of cognitive losses prior to dementia onset, raising the possibility that reverse causation also contributes to the observed inverse associations.

To investigate these issues, we conducted a systematic review of epidemiological studies of cognitive activity and incidence of Alzheimer’s disease dementia (AD) and other dementias; characterized and quantified the potential bias from confounding and reverse causation; and identified additional methodological issues relevant to the interpretation of these studies.

Methods

Systematic search strategy

We conducted a systematic search of PubMed and EMBASE through June 2014 to identify relevant studies. To build our search strategy, we first obtained lists of index terms relevant to cognitive activity, AD and dementia, and nested case-control and cohort studies using the PubMed Medical Subject Headings (MeSH) database and the EMBASE EMTREE thesaurus. We identified common synonyms for cognitive activity from primary and
review articles and incorporated these terms into our search strategy (provided in full in Supplementary Tables 1.1 and 1.2).

Selection of studies

To be eligible for inclusion in our review, studies had to be published in a peer-reviewed journal; be cohort or nested case-control studies in well-defined cohorts; have a clearly described operationalization of cognitive activity; evaluate cognitive activity prospectively in relation to dementia; evaluate all participants (or a systematically drawn sample) for AD or dementia using established diagnostic criteria; provide effect estimates with confidence intervals or standard errors for the association between cognitive activity and AD or dementia; and adjust for at least age and sex. This review was conducted in part for the online database of AD epidemiology findings, AlzRisk (www.alzrisk.org). These pre-specified criteria are employed site-wide and comply with current standards for systematic review and meta-analyses of observational studies (5, 6).

Data extraction

For each eligible study, we extracted the following: publication year; study cohort; study design; number of participants; age distribution at baseline; follow-up time; method of ascertainment and operationalization of cognitive activity; number of AD and dementia cases; effect estimates, with confidence intervals or standard errors; and model covariates. Due to the heterogeneity of the exposure definitions used, we did not compute meta-analysis summary estimates.

Bias analysis method

While some degree of unmeasured confounding is likely present in all observational studies, more important is the degree to which such confounding biases the estimated effect of cognitive activity on dementia risk. We conducted a bias analysis (7) to evaluate how reported hazard ratios (HR) for the effect on AD of ‘high’ versus ‘low’ levels of cognitive activity—obtained from one representative study (8) —would change under different assumptions about the magnitude of unmeasured confounding.

We used a formal quantitative approach applicable to the case of a binary exposure, a hypothetical unmeasured binary confounder U, and a rare time-to-event outcome (7). It can been shown that the degree of
confounding by U is a function of (a) the HR reflecting the association of U with AD, $HR_{U-AD}$, conditional on exposure and measured covariates; (b) the prevalence of U in the high-activity group, $p_1$, conditional on measured covariates; and (c) the prevalence of U in the low-activity group, $p_0$, conditional on measured covariates. To quantify how an observed estimate reflecting the effect of cognitive activity on AD ($HR_{CA-AD}$) would change due to confounding by U, we calculated a bias-corrected estimate ($HR_{CA-AD|U}$), the estimated effect of cognitive activity on AD that would have been obtained had the hypothetical confounder U also been adjusted for (in addition to the measured covariates). This bias-corrected estimate is a function of the observed estimate of the effect of cognitive activity on AD, and the three parameters defining the degree of confounding and is given by $HR_{CA-AD|U} = \frac{HR_{CA-AD}}{\left[\frac{1}{1 + (HR_{U-AD} - 1)p_1} \right]} \frac{\left[1 + (HR_{U-AD} - 1)p_0\right]}{\left[1 + (HR_{U-AD} - 1)p_0\right]}$. This approach entails an additional simplifying assumption that there be no interaction between the effects of U and cognitive activity on the hazard ratio scale. The impact of unmeasured confounding by U can be assessed in two ways. The first approach compares the observed estimate to the bias-corrected estimate under different specifications of U, and asks “given $HR_{CA-AD}$, and specifying $U$, $p_0$ and $p_1$, what would $HR_{CA-AD|U}$ be?” The second approach calculates how much confounding would be required for the observed association to be eliminated entirely, and asks “given $HR_{CA-AD}$, what parameters of $U$, $p_0$ and $p_1$ would make $HR_{CA-AD|U}$ become 1?”

To use this approach, we needed an effect estimate from a study that reported cognitive activity as a categorical exposure in relation to a time-to-event outcome of AD, and in which AD occurred relatively rarely (in general, this approach provides correct HR estimates when the disease occurs in up to 10% of both exposure groups in the study population). Among the cohort studies in our review, these conditions were most adequately met in Akbaraly et al (8), in which approximately 2% of the study population developed AD over a 4-year follow-up period. AD incidence was in the range of 10-15% over the follow-up periods of the other studies, which would lead to only minor violations of the rare disease assumption. Notably, Akbarly et al. was similar to the other studies with respect to the types of leisure activities assessed (Supplementary Table 1.3), the operationalization of cognitive activity as an exposure, and the estimated magnitude of the association between cognitive activity and dementia.

Akbaraly et al compared AD risks of participants engaging in “high” versus “low” (reference) engagement in both “stimulating” ($HR_{CA-AD} = 0.39$) and “passive” ($HR_{CA-AD} = 0.68$) activity. We estimated how these HRs would change upon adjustment for different levels of confounding.
Application to unmeasured confounding: For our bias analysis, we posited the presence of a binary confounder U, which could represent an unmeasured risk factor, with either a small \((HR_{U-AD} = 1.5)\), moderate \((HR_{U-AD} = 2)\), or large harmful effect on AD \((HR_{U-AD} = 3)\). These three values reflect a range of reported relative risks in studies of individual or multiple cardiovascular risk factors in relation to AD incidence (9, 10). Depression (9), low education (9), and lifecourse socioeconomic position (11, 12), are other potential confounders of the cognitive activity-AD association with relative risk estimates in this range. The studies we reviewed adjusted for confounding by these variables to varying degrees, so the specific unmeasured confounders represented by U, and the degree of residual confounding will differ across individual studies. We used these values of \(HR_{U-AD}\) and allowed \(p_0\) and \(p_1\) to vary between 0 and 1 in the formula above to calculate how the two \(HR_{CA-AD}\) estimates from Akbaraly et al. would change under different amounts of confounding.

Application to reverse causation: As incipient dementia may be a shared cause of reduced cognitive activity and clinical dementia, we used the same approach to determine quantitatively how the observed association between cognitive activity and AD would be influenced by reverse causation. The magnitude of bias due to reverse causation will depend on the degree to which individuals with mild cognitive impairment [MCI] (or potential incipient dementia more broadly, including subjective cognitive concerns), tend to have lower cognitive activity \((p_0)\), and on the magnitude of their increased risk of developing clinical dementia \((HR_{U-AD})\). Individuals with incipient dementia, whether defined as MCI or subjective cognitive concerns (13) are not excluded at baseline in most studies of the effect of cognitive activity on AD, and may account for a quite a large portion of the study population; in three cohorts reviewed here, individuals with MCI made up 19%, 26%, and 39% of all non-demented individuals (8, 14, 15). It has also been established that indications of incipient dementia in cognitively normal individuals are strongly associated with eventual AD or clinical dementia diagnosis, with relative risk of dementia estimates ranging from 4.5 to 6 for subjective cognitive concerns (13, 16), and 2.8 to 6.7 for MCI at baseline (14, 17, 18). On the basis of these reports, we used an HR of 4.5 for the effect of incipient dementia on clinical AD \((HR_{U-AD} = 4.5)\), and again calculated how the two \(HR_{CA-AD}\) estimates from Akbaraly et al. would change due to reverse causation.
Results

*Literature search results*

Using our search strategy, we identified 926 citations. After removing 238 duplicate citations, and reviewing article titles and abstracts against our inclusion criteria, we identified 56 citations for full-text review. Of these, we excluded not published in English articles. Of the remaining English-language articles, 13 met criteria for inclusion in our review (Figure 1.1).

*Figure 1.1: Flow chart showing how studies were selected for inclusion*
**Study design details**

The 13 studies we reviewed included a total of 16,725 participants from 12 cohorts (8, 15, 19-29) (Table 1.1). Two studies analyzed the same participants from the Kungsholmen cohort; we included both in this review, as they examined different aspects of cognitive activity (23, 27). Of these 13 studies, 10 were prospective cohort studies including a total of 13,431 participants (8, 15, 21-24, 26-29), two were nested case-control studies (19, 20), and one was a secondary analysis of a randomized trial of cognitive training (25). The prospective studies assessed cognitive activity at a baseline visit late in life. Almost all studies inquired only about current or habitual participation in leisure activities; one additionally inquired about activity at ages 6, 12, 18 and 40 (15). The two nested case-control studies assessed cognitive activity earlier in life; one inquired about current mid-life activity (at mean age, 45)(19), while the other asked at age 57 about activity before age 40 (20).

In the 10 prospective cohort studies, follow-up for dementia began immediately after the baseline visit. Six of these reported the mean follow-up time (range, 2.5 to 6.1 years). The maximum theoretical follow-up time in any of these cohort studies was 21 years. In the two nested case-control studies with mid-life measures of cognitive activity, dementia follow-up began approximately 20 years after assessment of cognitive activity. All but one of our reviewed studies adjusted for education. Four studies additionally adjusted for SES, or a marker of SES such as occupation or income.

**Dementia assessment**

Most studies used multi-stage assessments for dementia, consisting of initial cognitive screening, followed by more detailed clinical examinations under a standard protocol. Two studies conducted full annual examinations on all participants (15, 29). Dementia and AD were diagnosed by standard clinical and research criteria (30-33). Diagnoses were available for AD alone in 3 studies (15, 28, 29), for both AD and all-cause dementia in 3 studies (8, 20, 26), and for all-cause dementia alone in 7 studies (19, 21-25, 27).
Table 1.1: Summary of characteristics of studies included in review

<table>
<thead>
<tr>
<th>First Author</th>
<th>Cohort</th>
<th>N</th>
<th>Mean Age at start of follow-up (Range)</th>
<th>Mean (max) follow-up in years</th>
<th>Operationalization of cognitive activity</th>
<th>Dementia diagnosis</th>
<th>AD diagnosis</th>
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<td><strong>Randomized controlled trials</strong></td>
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<td>Unverzagt (25)</td>
<td>Advanced Cognitive Training for Independent and Vital Elderly(34)</td>
<td>2786</td>
<td>73.6 (65+)</td>
<td>NR (5)</td>
<td>Ten group-based sessions over a 6-week period, during which strategies developed to target memory, problem-solving and reasoning were learnt and applied in practice, both on laboratory-tasks and problems related to activities of daily life.</td>
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<td><strong>Prospective cohort studies</strong></td>
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<td>Akbaraly (8)</td>
<td>Three-City Study(35)</td>
<td>5698</td>
<td>74 (65+)</td>
<td>NR (4)</td>
<td>Frequency of participation in: (i) stimulating activity, (ii) passive activity</td>
<td>DSM-IV</td>
<td>NINCDS-ADRDA</td>
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<tr>
<td>Eriksson Sorman (21)</td>
<td>The Betula Project</td>
<td>1475</td>
<td>74 (65+)</td>
<td>NR (15)</td>
<td>Mental activity index</td>
<td>DSM-IV</td>
<td></td>
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<tr>
<td>Hughes (22)</td>
<td>Monongahela Valley Independent Elders Survey</td>
<td>942</td>
<td>76 (65+)</td>
<td>6.1 (10.5)</td>
<td>(i) Number of cognitive activities, (ii) Time commitment to cognitive activities</td>
<td>CDR</td>
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<td>Karp (23)</td>
<td>Kungsholmen Project(36)</td>
<td>732</td>
<td>81 (75+)</td>
<td>NR (NR)</td>
<td>Level of cognitive intensity</td>
<td>DSM-III-R</td>
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<td>Scarmeas (24)</td>
<td>Washington Heights-Inwood Columbia Aging Project</td>
<td>1772</td>
<td>76 (65+)</td>
<td>2.8 (7.2)</td>
<td>Number of cognitive activities (previous month)</td>
<td>DSM-III-R</td>
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<td>Verghese (26)</td>
<td>Bronx Aging Study</td>
<td>469</td>
<td>79 (75+)</td>
<td>5.8 (NR)</td>
<td>Number of activity-days per week</td>
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<td>Wang (27)</td>
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<td>NR (6.4)</td>
<td>Frequency of participation in mental activities</td>
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### Table 1.1: Summary of characteristics of studies included in review (continued)

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<th>Mean (max) follow-up in years</th>
<th>Operationalization of cognitive activity</th>
<th>Dementia diagnosis</th>
<th>AD diagnosis</th>
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<td>76 (65+)</td>
<td>4.1 (NR)</td>
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<td>NINCDS-ADRDA</td>
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<tr>
<td>Wilson (15)</td>
<td>Memory and Aging Project(38)</td>
<td>775</td>
<td>80 (NR)</td>
<td>2.5 (NR)</td>
<td>Participation score</td>
<td>NINCDS-ADRDA</td>
<td>NINCDS-ADRDA</td>
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**Nested case-control studies**

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<th>Cohort</th>
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<th>Mean Age at start of follow-up (Range)</th>
<th>Mean (max) follow-up in years</th>
<th>Operationalization of cognitive activity</th>
<th>Dementia diagnosis</th>
<th>AD diagnosis</th>
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<tr>
<td>Carlson (19)</td>
<td>Duke Twins Study of Memory in Aging(39)</td>
<td>294</td>
<td>45 (NR)</td>
<td>NR (NR)</td>
<td>(i) Number of stimulating activities, (ii) Number of passive activities (during midlife)</td>
<td>DSM-III-R</td>
<td></td>
</tr>
<tr>
<td>Crowe (20)</td>
<td>Swedish Twin Registry(40)</td>
<td>214</td>
<td>57 (NR)</td>
<td>NR (NR)</td>
<td>Intellectual/cultural activity (before age 40)</td>
<td>DSM-III-R</td>
<td>NINCDS-ADRDA</td>
</tr>
</tbody>
</table>


*a Unverzagt dementia diagnosis: As dementia was not one of the primary endpoints of the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) study, for this analysis, the investigators attempted to approximate the clinical diagnosis of dementia used in other epidemiological studies by using ACTIVE study neuropsychological testing and functional status assessments, self- or proxy-reports of AD/dementia diagnosis, self- or proxy-reports of institutionalization, and evidence of refusal of access by the participant's family.
Definition, dimensions and operationalization of cognitive activity

In all studies, popular leisure activities considered to require information seeking and processing were characterized as “cognitive”. Most studies assessed participation in a relatively narrow range of leisure activities, such as reading books, newspapers, or magazines; doing crosswords or playing cards; and watching television and listening to the radio. However, a few studies also inquired about activities such as participation in group discussions and attending cultural and social events (Supplementary Table 1.3).

The ways that studies operationalized cognitive activity varied widely. One study simply categorized activity based on frequency of participation across all cognitive activities, and did not distinguish different frequencies of participation in the activities assessed (27). Most commonly, investigators started with reported participation frequency in each activity, assigned a score corresponding to each frequency, and then combined scores across all activities into a composite score (8, 15, 21, 26, 28, 29). Three studies used the number of reported cognitive activities (19, 22, 24); one study used time devoted to cognitive activities (22); and one used a cognitive factor score derived from an exploratory factor analysis of a mixture of leisure items (20). A few studies also assessed intensity of cognitive activity and further sub-classified activities as stimulating or passive (e.g., reading versus watching television) (8, 19, 23). However, classification of a particular activity as stimulating or passive varied across studies. Finally, one study examined the effect of cognitive training on dementia incidence (25).

Summary of review findings

The 13 reviewed studies included a total of 1852 dementia cases of which 565 were specifically AD. Most studies found inverse associations of late-life cognitive activity with AD and/or all-cause dementia (Table 1.2). In 6 studies that operationalized cognitive activity using composite measures of participation frequency, greater participation was generally associated with lower AD and all-cause dementia incidence (8, 15, 21, 26, 28, 29). Similarly, in 3 studies that examined the number of cognitive activities engaged, higher activity count corresponded to lower dementia incidence (19, 22, 24). In one study, time spent on cognitively engaging hobbies was also related to a lower rate of dementia (22). Of investigations of intensity, two found lower dementia incidence with engagement in stimulating but not passive activity (8, 23), whereas another found the opposite (19). By contrast, the randomized trial of cognitive training found no difference in dementia incidence between training and control arms over 5 years of follow-up (25).
Table 1.2: Summary of results -- Cognitive activity and incidence of Alzheimer’s disease/dementia

<table>
<thead>
<tr>
<th>First Author</th>
<th>N</th>
<th>Cognitive activity</th>
<th>Exposure level or contrast</th>
<th>Alzheimer’s Disease</th>
<th>Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% in level</td>
<td># of Cases</td>
<td>Relative Risk (95% CI)</td>
</tr>
<tr>
<td><strong>Randomized controlled trials</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unverzagt (25)</td>
<td>2786</td>
<td>Cognitive training</td>
<td>Untrained</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trained</td>
<td>75</td>
<td>139</td>
</tr>
<tr>
<td><strong>Prospective cohort studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akbaraly (8)</td>
<td>5698</td>
<td>Stimulating activity</td>
<td>Low</td>
<td>38</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>32</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>30</td>
<td>NR</td>
</tr>
<tr>
<td>Akbaraly (8)</td>
<td>5698</td>
<td>Passive activity</td>
<td>Low</td>
<td>39</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>23</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>38</td>
<td>NR</td>
</tr>
<tr>
<td>Eriksson Sorman (21)</td>
<td>1475</td>
<td>Mental activity index</td>
<td>Per 1 unit increase</td>
<td></td>
<td>357</td>
</tr>
</tbody>
</table>
Table 1.2: Summary of results -- Cognitive activity and incidence of Alzheimer’s disease/dementia (continued)

<table>
<thead>
<tr>
<th>First Author</th>
<th>N</th>
<th>Cognitive activity exposure</th>
<th>Exposure level or contrast</th>
<th>Alzheimer’s Disease</th>
<th>Dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% in level</td>
<td># of Cases</td>
</tr>
<tr>
<td>Hughes (22)</td>
<td>942</td>
<td>Hours/week on cognitive activities</td>
<td>0 - 3 hours</td>
<td>54</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>942</td>
<td></td>
<td>4 - 6 hours</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Hughes (22)</td>
<td>942</td>
<td>Number of cognitive activities</td>
<td>Per 1 activity increase</td>
<td>111</td>
<td>0.86 (0.75, 0.99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>111</td>
<td>0.86 (0.75, 0.99)</td>
</tr>
<tr>
<td>Karp (23)</td>
<td>732</td>
<td>Cognitive intensity</td>
<td>Low</td>
<td>49</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
<td>51</td>
<td>48</td>
</tr>
<tr>
<td>Scarmeas (24)</td>
<td>1772</td>
<td>Number of cognitive activities</td>
<td>Per 1 activity</td>
<td>207</td>
<td>0.76 (0.61, 0.94)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>207</td>
<td>0.76 (0.61, 0.94)</td>
</tr>
<tr>
<td>Verghese (26)</td>
<td>469</td>
<td>Number of activity-days per week</td>
<td>Per 1 activity-day</td>
<td>61</td>
<td>0.93 (0.88, 0.98)</td>
</tr>
</tbody>
</table>
Table 1.2: Summary of results -- Cognitive activity and incidence of Alzheimer’s disease/dementia (continued)

<table>
<thead>
<tr>
<th>First Author</th>
<th>N</th>
<th>Cognitive activity exposure</th>
<th>Exposure level or contrast</th>
<th>% in level</th>
<th># of Cases</th>
<th>Relative Risk (95% CI)</th>
<th># of Cases</th>
<th>Relative Risk (95% CI)</th>
<th>Covariates / Restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verghese</td>
<td>469</td>
<td>Number of activity-days per week</td>
<td>Lowest third</td>
<td>39</td>
<td>NR</td>
<td>1 (Ref.)</td>
<td>NR</td>
<td>0.48 (0.29, 0.74)</td>
<td>Age, education, gender, baseline cognition, chronic disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Middle third</td>
<td>29</td>
<td>NR</td>
<td>0.37 (0.23, 0.61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Highest third</td>
<td>32</td>
<td>NR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wang</td>
<td>732</td>
<td>Frequency of participation in mental activities</td>
<td>None</td>
<td>NR</td>
<td>34</td>
<td>1 (Ref.)</td>
<td>40</td>
<td>0.83 (0.54, 1.30)</td>
<td>Age, education, gender, depression, comorbidity index, physical activity, physical performance, social activity baseline MMSE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Less than daily</td>
<td>NR</td>
<td>49</td>
<td>0.59 (0.37, 0.96)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilson</td>
<td>733</td>
<td>Participation score</td>
<td>Per 1 unit increase</td>
<td>111</td>
<td>0.67 (0.49, 0.92)</td>
<td></td>
<td></td>
<td>Age, education, gender</td>
<td></td>
</tr>
<tr>
<td>Wilson</td>
<td>835</td>
<td>Participation score</td>
<td>Per 1 unit increase</td>
<td>139</td>
<td>0.39 (0.21, 0.73)</td>
<td></td>
<td></td>
<td>Age, education, gender, APOE, occupation, race/ethnicity, follow-up</td>
<td></td>
</tr>
<tr>
<td>First Author</td>
<td>N</td>
<td>Cognitive activity exposure</td>
<td>Exposure level or contrast</td>
<td>% in level</td>
<td>Alzheimer’s Disease</td>
<td>Dementia</td>
<td>Covariates / Restrictions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-----</td>
<td>-----------------------------</td>
<td>---------------------------</td>
<td>------------</td>
<td>---------------------</td>
<td>---------</td>
<td>---------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilson (15)</td>
<td>775</td>
<td>Participation score</td>
<td>Per 1 unit increase</td>
<td>90</td>
<td>0.53 (0.32, 0.86)</td>
<td></td>
<td>Age, education, gender, APOE, income, past cognitive activity, physical activity, social activity, indicators of early-life household and community SES</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Nested case-control studies**

<table>
<thead>
<tr>
<th>Carlson (19)</th>
<th>294</th>
<th>Number of stimulating activities <em>during midlife</em></th>
<th>Per 1 activity increase</th>
<th>147</th>
<th>0.81 (0.57, 1.13)</th>
<th>Age, gender (male twin pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlson (19)</td>
<td>294</td>
<td>Number of passive activities <em>during midlife</em></td>
<td>Per 1 activity increase</td>
<td>147</td>
<td>0.55 (0.33, 0.92)</td>
<td>Age, gender (male twin pairs)</td>
</tr>
<tr>
<td>Crowe (20)</td>
<td>214</td>
<td>Intellectual/cultural activity <em>before age 40</em></td>
<td>Less active</td>
<td>NR</td>
<td>1 (Ref.)</td>
<td>Age (twin study), gender, education</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>More active</td>
<td>NR</td>
<td>0.55 (0.28, 1.08)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>0.80 (0.49, 1.31)</td>
<td></td>
</tr>
</tbody>
</table>


Bias analysis results

Table 1.3 shows how an observed $HR_{CA-AD}$ of 0.39 would change if adjustment were also made for a strong unmeasured confounder, U ($HR_{U-AD} = 3$), calculated under different scenarios for the prevalence of U among the active and inactive groups. If U were more prevalent among the cognitively active, the observed $HR_{CA-AD}$ of 0.39 would actually underestimate the true protective effect ($HR_{CA-AD|U} < 0.39$). Under the more likely scenario that a harmful U is more prevalent among the cognitively inactive, the observed $HR_{CA-AD}$ of 0.39 would overestimate the protective effect ($HR_{CA-AD|U} > 0.39$). In these instances, the estimated protective effect would diminish with adjustment for U, but it would be eliminated entirely or reversed only if there were extreme imbalances in the prevalence of U between activity groups. One such scenario would require the prevalence of U to be 81% in the low-activity group when its prevalence was 1% in the high-activity group. Prevalences of U in the low-activity group greater than 81% would make $HR_{CA-AD}$ exceed 1 in U-adjusted analyses, reversing the observed inverse association. If U were only moderately ($HR_{U-AD} = 2$) or weakly ($HR_{U-AD} = 1.5$) associated with AD, an observed $HR_{CA-AD}$ of 0.39 would be weakened under U-adjustment but would not be nullified or reversed under any combination of U prevalences in the high- and low-activity groups.

Table 1.3: Bias-corrected AD HR (comparing high versus low participation in cognitive activity), adjusted for confounding by U, given that HR unadjusted for U = 0.39 and $HR_{U-AD} = 3$.

<table>
<thead>
<tr>
<th>Prevalence of U among low-activity participants, $p_0^a$</th>
<th>1%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>90%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.39</td>
<td>0.46</td>
<td>0.57</td>
<td>0.76</td>
<td>0.96</td>
<td>1.07</td>
<td>1.14</td>
</tr>
<tr>
<td>10%</td>
<td>0.33</td>
<td>0.39</td>
<td>0.49</td>
<td>0.65</td>
<td>0.81</td>
<td>0.91</td>
<td>0.97</td>
</tr>
<tr>
<td>25%</td>
<td>0.27</td>
<td>0.31</td>
<td>0.39</td>
<td>0.52</td>
<td>0.65</td>
<td>0.73</td>
<td>0.77</td>
</tr>
<tr>
<td>50% Prevalence of U among high-activity participants, $p_1^a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>0.20</td>
<td>0.23</td>
<td>0.29</td>
<td>0.39</td>
<td>0.49</td>
<td>0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>75%</td>
<td>0.16</td>
<td>0.19</td>
<td>0.23</td>
<td>0.31</td>
<td>0.39</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>90%</td>
<td>0.14</td>
<td>0.17</td>
<td>0.21</td>
<td>0.28</td>
<td>0.35</td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td>99%</td>
<td>0.13</td>
<td>0.16</td>
<td>0.20</td>
<td>0.26</td>
<td>0.33</td>
<td>0.37</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*conditional on other covariates.

When $HR_{U-AD} = 3$, the bias-corrected AD HR will be exactly 1 when $p_1 = 1\%$ and $p_0 = 81\%$. 
Similarly, Supplementary Table 1.4 shows how a weaker observed $HR_{CA-AD}$ of 0.68 would change if adjustment were made for a strong confounder U ($HR_{U-AD} = 3$). Smaller, but still substantial imbalances in the prevalences of U across activity groups would be required to entirely eliminate an observed effect of this magnitude. For example, when the prevalence of U in the high-activity group is 1%, a prevalence of U in the low-activity group of 25% would be required to eliminate the observed association. For U prevalences in the high-activity group of 10%, 25% and 50%, the corresponding prevalences required in the low-activity group to eliminate the observed association would be 39%, 61% and 98% respectively. If U were more weakly associated with AD (e.g., $HR_{U-AD} = 2$ or $HR_{U-AD} = 1.5$), larger prevalence differences than these would be required to eliminate an observed $HR_{CA-AD}$ of 0.68 entirely.

Bias due to reverse causation: Table 1.4 and Supplementary Table 1.5 show how estimated $HR_{CA-AD}$ of 0.39 and 0.68 could reflect the presence of incipient dementia as specified ($HR_{U-AD} = 4.5$), under a range of incipient dementia prevalences in the active and inactive groups. If the incipient dementia prevalence was only 1% in the active group, then a prevalence of 48% in the inactive group would be required for an observed $HR_{CA-AD}$ of 0.39 to increase to 1 upon adjustment for incipient dementia. An incipient dementia prevalence of 10% in the high-activity group would require an incipient dementia prevalence of 71% in the low-activity group for an $HR_{CA-AD}$ of 0.39 to increase to 1 (Table 1.4).

Table 1.4: Bias-corrected AD HR (comparing high versus low participation in cognitive activity), adjusted for U, given that HR unadjusted for U = 0.39 and $HR_{U-AD} = 4.5$.

<table>
<thead>
<tr>
<th>Prevalence of U among low-activity participants, $p_0^a$</th>
<th>1%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>90%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.39</td>
<td>0.51</td>
<td>0.71</td>
<td>1.04</td>
<td>1.37</td>
<td>1.56</td>
<td>1.68</td>
</tr>
<tr>
<td>10%</td>
<td>0.30</td>
<td>0.39</td>
<td>0.54</td>
<td>0.79</td>
<td>1.05</td>
<td>1.20</td>
<td>1.29</td>
</tr>
<tr>
<td>25%</td>
<td>0.22</td>
<td>0.28</td>
<td>0.39</td>
<td>0.57</td>
<td>0.75</td>
<td>0.86</td>
<td>0.93</td>
</tr>
<tr>
<td>50%</td>
<td>0.15</td>
<td>0.19</td>
<td>0.27</td>
<td>0.39</td>
<td>0.51</td>
<td>0.59</td>
<td>0.63</td>
</tr>
<tr>
<td>75%</td>
<td>0.11</td>
<td>0.15</td>
<td>0.20</td>
<td>0.30</td>
<td>0.39</td>
<td>0.45</td>
<td>0.48</td>
</tr>
<tr>
<td>90%</td>
<td>0.10</td>
<td>0.13</td>
<td>0.18</td>
<td>0.26</td>
<td>0.34</td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td>99%</td>
<td>0.09</td>
<td>0.12</td>
<td>0.16</td>
<td>0.24</td>
<td>0.32</td>
<td>0.36</td>
<td>0.39</td>
</tr>
</tbody>
</table>

$^a$conditional on other covariates.

When $HR_{U-AD} = 4.5$, the bias-corrected AD HR will be exactly 1 when $p_1 = 1\%$ and $p_0 = 48\%$, and when $p_1 = 10\%$ and $p_0 = 71\%$. 

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Smaller differences in the prevalence of incipient dementia are required to fully account for an observed $HR_{CA-AD}$ of 0.68. If the prevalence of incipient dementia in the high-activity group was 1%, the corresponding prevalence in the low-activity group would only need to be about 15% for an $HR_{CA-AD}$ of 0.68 to increase to 1 upon adjustment for incipient dementia. Calculating similarly, for incipient dementia prevalences in the high-activity group of 10%, 25% or 50%, the corresponding prevalences required in the low-activity group to eliminate the observed association, upon adjustment, would be 29%, 50% and 87% respectively (Supplementary Table 1.5).

**Discussion**

The reviewed epidemiologic studies generally reported associations of greater participation in late-life cognitive activities with lower risk of both AD and all-cause dementia. In this section, we consider the implications of our bias analysis to understand the extent to which these findings might result from confounding or reverse causation. We also briefly discuss issues relevant to the definition, ascertainment and operationalization of cognitive activity that might also have led to biased results.

**Bias due to confounding and reverse causation**

*Confounding by shared causes of cognitive activity and dementia:* Characteristics associated with higher cognitive activity such as formal education and higher socioeconomic status have also been consistently associated with reduced dementia risk (41, 42). Almost all studies adjusted their estimates for formal education, and a few additionally adjusted for occupational history (8), or income and indicators of early-life SES (15), with generally unchanged results. However, some concern about residual confounding remains warranted, given the strong associations between different dimensions of SES and dementia risk (42, 43), and because education and SES are often measured and modeled inadequately (4). Given the correlation between higher SES and lower cardiovascular risk, adjustment for cardiovascular risk factors may also help reduce residual confounding by SES. Another potential source of confounding is longstanding intellectual ability, which is likely correlated with cognitive activity (44), education and a variety of dementia risk factors; in one study, adjustment for intelligence quotient at age 11 mostly eliminated the positive associations between late-life cognitive activity and late-life cognitive performance (44). Additionally, as some cognitive activities also involve social engagement or the ability to travel, failure to adjust for
general health status (or a proxy such as physical or social activity), which is also related to dementia (45), may lead to an overestimated influence of cognitive activity on dementia risk.

However, our analysis suggests that the bias due to residual confounding by these variables is unlikely to be large enough to completely explain inverse associations of the magnitude observed in the reviewed studies. Our calculations indicate that to eliminate protective HRs of 0.68 and 0.39, a strong harmful confounder would need to be at minimum 24 and 80 percentage points more prevalent, respectively, among inactive individuals. Empirically, such imbalances appear unlikely. In Akbaraly et al., the greatest imbalance across activity groups reported for any confounder was 17 percentage points for low education, and imbalances of just 2, 5 and 6 percentage points were reported for diabetes, hypertension and vascular disease, respectively (8). In a different study of cognitive function in which the size of similar confounder-cognitive activity relationships was reported, the prevalence imbalances were similarly modest (46).

Reverse causation: Individuals in the prolonged preclinical stages of dementia, might be more likely to eschew participation in cognitively stimulating activities than their healthy counterparts (15, 47), resulting in an inverse association between activity and dementia risk. In most reviewed studies, sensitivity analyses excluding individuals judged more likely to be in the prodromal stages of dementia (e.g., those with a diagnosis of MCI, or with poorer screening test performance, or who developed dementia early in the follow-up period) returned largely unchanged results. However, such exclusions may be insufficient, considering that average follow-up time in the late-life cognitive activity studies was between 2 and 7 years, while detectable cognitive decline begins as early as 5-8 years prior to dementia diagnosis (48-50), and even more subtle impairments, usually noticeable only to affected individuals themselves, occur still earlier (51). If some of those with incident dementia in these studies were, at baseline, already experiencing enough decline to affect their cognitive activity but not their likelihood of being excluded, it is likely that the observed relationship at least partially reflects reverse causation.

We also analyzed how robust the observed associations might be to reverse causation by incipient dementia, observing that associations of incipient dementia with both late-life activity and AD diagnosis would need to be large to completely explain findings of the magnitude observed. The magnitude of the effect of incipient dementia will differ between studies based on their average duration. We used an HR (4.5) from the middle of the range of reported estimated effects of MCI and subjective cognitive concerns on risk for clinical dementia (13, 14,
16-18). Smaller HRs for the effect of incipient dementia yield results similar to those described for confounding, and would be unlikely to entirely explain the observed associations. However, under larger HRs for the effect of incipient dementia, even smaller prevalence differences would be sufficient to fully explain an \( HR_{CA-AD} \) of 0.68, and attenuate an \( HR_{CA-AD} \) of 0.39. Overall, it is plausible that reverse causation could bias these findings; the degree of bias could be substantial if the disparity in prevalence of incipient dementia between high- and low-activity groups is large.

However, quantifying the degree to which incipient dementia is more prevalent among low- rather than high-activity individuals is difficult. In Akbaraly et al., cognitive impairment at baseline, defined as scoring less than 24 on the Mini-Mental State Examination (MMSE), was more common among those with low (7.8%) rather than high (1.9%) levels of stimulating activity (8). Overall, data on how late-life cognitive activity changes with declining cognition are sparse. In Wilson et al., MCI at baseline was associated with lower cognitive activity at baseline, although not with further decline in activity over a three-year follow-up (15). The same investigators also showed that whereas cognitive activity predicted global cognitive function over the following year, global cognitive function did not predict subsequent degree of cognitive activity. However, performance in two specific cognitive domains, working memory and perceptual speed, was associated with cognitive activity over the following year (52).

Furthermore, in another study, both early- and late-life cognitive activity were associated with cognitive decline in analyses adjusted for post-mortem measures of neuropathology (53), but the timing of both cognitive activity and cognitive change in relation to neuropathological changes is difficult to discern in this setting, as cognitive activity was assessed at study baseline and neuropathology at death. To better understand the influence of reverse causation, longitudinal data on the extent and timing of cognitive activity changes will have to be evaluated in parallel with changes in cognition in longer prospective follow-up studies.

The findings of our bias analysis can also be informative in interpreting other studies in which the conditions required for use of this approach are met. In our estimation, these conditions are unlikely to be severely violated in the cohort studies reviewed here, where AD incidence over the relatively short study follow-up periods remains quite low. However, while the calculations reported here provide some measure of the potential degree of bias for a typical effect estimate, analyses using study-specific parameters will be better able to assess the degree of bias in individual studies.
Methodological considerations in measurement of cognitive activity

Type and context. Although cognitive activity scales are starting to be developed (54) further investigation into valid measurement of the construct is warranted. In an effort to isolate the effect of purely cognitive activities (54), most studies focussed on leisure activities that are primarily or exclusively cognitive. Some of the more frequently assessed leisure activities (e.g., reading, attending theatre or museums) are more likely to be favored by individuals of higher SES or educational attainment. Failure to assess other leisure or non-leisure activities requiring cognitive processing would result in an underestimate of overall late-life cognitive activity for many individuals, and could bias estimates of its effect on dementia risk given the associations of higher SES/education with many of the assessed activities. By contrast, some investigators have taken the approach of multi-modal interventions rather than attempting to isolate an effect of cognitive activity that is distinct from other activities, psychosocial constructs, and risk factors (55, 56).

Self-reporting. Valid measurement of cognitive activity is particularly challenging in populations that may have cognitive deficits. All reviewed studies used self-reported data, but validation against other sources (e.g., diary records, electronic devices) was rare even though individuals with memory loss would be expected to self-report less accurately (57, 58). If individuals with MCI systematically under-report cognitive activity, lower participation frequency would be spuriously linked with greater dementia risk. Alternatively, if participation frequency is overstated because of failure to accurately recognize or recall declines from lifetime activity levels, this would attenuate any protective association between cognitive activity and dementia.

Timing and 'dose'. A critical question from a prevention perspective is whether the inverse association between late-life cognitive activity and dementia can be attributed specifically to greater late-life cognitive activity. Evidence of an effect of late-life activity independent of earlier life activity would suggest that even cognitively 'sedentary' individuals might benefit from increasing their cognitive activity late in life. Two studies in the same cohort concluded that the effect of cognitive activity depends on late-life activity, because even after adjusting for activity earlier in life, late-life activity was strongly associated with lower AD incidence (15, 53). Similarly, more detailed investigation of the ‘dose’ of cognitive activity associated with lower AD risk will be required to inform prevention guidelines. Questions about timing and dose of activity are germane to possible benefits of intervening
throughout the lifecourse, such as recommending engagement in specific types of activities in midlife and possibly even policies and practices that shape childhood intellectual development.

*Cognitive training versus cognitive activity:* These epidemiologic findings, coupled with the hypothesized neuroprotective effects of cognitive activity, have inspired interest in cognitive training as a means to enhance cognitive function in late-life. The largest trial to date, the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) study, compared three group-based behavioral interventions in 2786 cognitively normal, independently living adults (mean age 74). The interventions individually targeted memory, reasoning and processing speed, and were compared with a no-contact regime (34). Post-training improvements were maintained for at least 5 years in all three arms (59-61). Additionally, self-reported difficulty performing instrumental activities of daily living was lower in the training arms than among the controls from year 2 onwards. However, the training interventions had no effects on performance-based measures of daily function at any of the follow-up points (60), and a secondary analysis including 189 incident dementia cases found no difference between trained and untrained groups in the rate of dementia occurrence over 5 years of follow-up (25). Expanding training to simultaneously target multiple cognitive domains, lengthening intervention time, and targeting an older population, have been suggested as modifications that may yield treatment effects on dementia incidence, but such strategies remain untested.

Although evidence from randomized trials so far does not support the efficacy of specific cognitive training interventions in preventing dementia, this does not rule out potential benefits of cognitively active lifestyles. The short-term influence of prescribed training interventions may differ from the cumulative stimulation attained via habitual performance of personally selected cognitive leisure activities. Interventions in line with a way of life that more fully incorporates cognitive activity as practiced in observational studies are difficult to define and operationalize in randomized trials. Evidence for any salutary effects of this broader notion of “engagement” (social, cognitive and otherwise) on late-life cognition may be better assessed by trials investigating more holistic interventions (55).
**Conclusion**

Our systematic review and bias analyses suggest that late-life cognitive activity may offer some reduction in risk of AD and all-cause dementia. Our bias analyses put these epidemiologic findings in greater context by quantifying of the potential degree of bias for a typical effect estimate. While the observed inverse associations are unlikely to be explained entirely by unmeasured confounding, reverse causation remains a more plausible but still little understood source of bias. Although a number of limitations in the measurement of cognitive activity might have led to bias, they do not appear sufficient to account for the observed findings. It remains possible, however, that confounding, reverse causation and measurement limitations could combine to substantially bias estimates. Over time, better characterization of the type, duration, intensity and timing of activity (62, 63) associated with late-life cognitive benefit will be required to develop more specific recommendations that are applicable over the lifecourse. Prospective observational studies will likely be better suited to answer these questions than randomized trials because they can better capture long-term patterns in activity—particularly habitual self-selected cognitive activity. While we await research informing more specific guidelines, it makes sense to recommend late-life engagement in enjoyable cognitive activities, as they are unlikely to cause harm, can enhance quality of life, and might also reduce dementia risk.
**Supplementary Tables**

**Supplementary Table 1.1: PubMed electronic search strategy for cognitive Activity**

<table>
<thead>
<tr>
<th>DATABASE</th>
<th>PUBMED</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DATES</strong></td>
<td>Search was conducted on 07/07/2014, and captures citations included in the databases until 06/30/2014.</td>
</tr>
<tr>
<td><strong>STRATEGY</strong></td>
<td>#1 AND #2 AND #3 AND #4 AND #5</td>
</tr>
<tr>
<td>#1</td>
<td>&quot;dementia&quot;[mesh:noexp] OR &quot;alzheimer Disease&quot;[mesh] OR (&quot;AD&quot;[tw] OR &quot;dementia&quot;[tw] OR &quot;alzheimer&quot;[tw] or &quot;alzheimers&quot;[tw] or &quot;alzheimer's&quot;[tw])</td>
</tr>
<tr>
<td>#5</td>
<td>0000/00/00:2014/06/30[EDAT]</td>
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</tbody>
</table>
Supplementary Table 1.2: EMBASE electronic search strategy for cognitive activity

<table>
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<tr>
<th>DATABASE</th>
<th>EMBASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DATES</td>
<td>Search was conducted on 07/07/2014, and captures citations included in the databases until 06/30/2014.</td>
</tr>
<tr>
<td>STRATEGY</td>
<td>(#1 AND #2 AND #3 AND #4 ) NOT #5</td>
</tr>
</tbody>
</table>

#1

('dementia' OR 'alzheimer disease')/de OR (ad OR dementia OR alzheimer*):ti,ab

#2

('risk' OR 'risk factor' OR 'population risk' OR 'attributable risk')/de OR (risk OR inciden* OR onset OR prevent* OR caus*):ti,ab

#3

'clinical trial'/exp OR ('intervention study' OR 'cohort analysis' OR 'longitudinal study' OR 'prospective study' OR 'evaluation and follow up' OR 'follow up' OR 'case control study' OR 'population based case control study' OR 'controlled study' OR 'major clinical study')/de OR (longitudinal* OR prospective* OR follow* OR follow-up OR 'follow up' OR cohort OR later OR 'case control' OR 'case-control' OR 'clinical trial' OR 'controlled trial' OR 'intervention study' OR 'intervention studies'):ti,ab

#4

'cognitive reserve'/de OR 'brain reserve'/de OR 'cognitive reserve':ab,ti OR 'brain reserve':ab,ti OR 'cognitive activity':ab,ti OR 'cognitive activities':ab,ti OR 'cognitive engagement':ab,ti OR 'cognitive stimulation':ab,ti OR 'leisure activity':ab,ti OR 'leisure activities':ab,ti OR 'leisure engagement':ab,ti OR 'mental activity':ab,ti OR 'mental activities':ab,ti OR 'mental engagement':ab,ti OR 'mental stimulation':ab,ti OR 'intellectual activity':ab,ti OR 'intellectual activities':ab,ti OR 'intellectual engagement':ab,ti OR 'intellectual stimulation':ab,ti OR 'cognitive lifestyle':ab,ti OR 'cognitive inactivity':ab,ti OR 'mentalt inactivity':ab,ti OR 'sudoku':ab,ti OR 'crossword':ab,ti OR 'crosswords':ab,ti OR 'puzzles':ab,ti OR 'bridge':ab,ti OR 'cognitive exercise':ab,ti OR 'cognitive exercises':ab,ti OR 'cognitive training':ab,ti OR 'cognitive intervention':ab,ti OR 'cognitive interventions':ab,ti OR 'brain exercise':ab,ti OR 'brain exercises':ab,ti OR 'brain training':ab,ti OR 'brain intervention':ab,ti OR 'brain interventions':ab,ti

#5

[1-7-2014]/sd
Supplementary Table 1.3: Leisure activities deemed to be cognitive in studies of cognitive activity and Alzheimer’s disease/dementia

<table>
<thead>
<tr>
<th>First Author</th>
<th>Activities considered cognitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unverzagt (25)</td>
<td><strong>Cognitive training interventions</strong>: group-based sessions in which strategies developed to target memory, problem-solving and reasoning were learnt and applied to laboratory- and daily life tasks.</td>
</tr>
</tbody>
</table>
| Akbaraly (8)       | **Stimulating cognitive activities**: crossword puzzles, playing cards, attending organizations, attending the cinema/theatre, practising an artistic activity  
                      **Passive cognitive activities**: watching television, listening to the radio, listening to music, knitting/sewing                                                                                                                                 |
| Eriksson Sorman (21) | reading books, reading magazines, attending movies/concerts/theatre, playing musical instruments, needlework, hunting or fishing                                                                                                         |
| Hughes (22)        | reading books, reading magazines, reading newspapers, playing board games, doing crafts, jigsaw puzzles, playing musical instruments, playing bridge, playing other card games                                                              |
| Karp (23)          | **High mental intensity**: reading literature, crossword puzzles, political/cultural interests, cards/chess, attending courses, attending theatres/concerts, attending exhibitions/museums, travelling, painting/drawing/photography, collecting stamps or other items, writing, following the stock market  
                      **Moderate mental intensity**: handicraft, visiting the summerhouse, watching TV, meeting friends/participating in groups, listening to radio, engaging in family/charity, outdoor activities, cooking, attending church activities, playing music, playing solitaire, playing bingo, singing  
                      **Low mental intensity**: playing sport, housekeeping                                                                                                                                 |
| Wang (27)          | reading books/newspapers, writing, studying, working crossword puzzles, painting, or drawing                                                                                                                                 |
| Scarmeas (24)      | reading newspapers or magazines, playing cards/games/bingo, going to classes.                                                                                                                                                       |
| Verghese (26)      | reading books or newspapers, writing for pleasure, doing crosswords, playing board games or cards, participating in organized group discussions, playing musical instruments                                                                                     |
| Wilson (29), Wilson (28) | watching television, listening to the radio, reading newspapers, reading magazines, reading books, playing cards, checkers, crosswords, or puzzle games, attending museums                                                                                                               |
| Wilson (15)        | visiting library, attending museums, attending concerts, time reading, reading newspapers, reading magazines, reading books, writing letters, playing games                                                                                                                                 |
| Carlson (19)       | **Stimulating cognitive activities**: reading, studying for courses and extra work  
                      **Passive cognitive activities**: watching television or listening to the radio and going to movies, theater, art or music                                                                                                                                 |
| Crowe (20)         | reading, listening to the radio or watching television, social visits, cultural activities                                                                                                                                              |
Supplementary Table 1.4: Bias-corrected AD HR (comparing high versus low participation in cognitive activity), adjusted for confounding by U, given that HR unadjusted for U = 0.68 and HR_{U-AD} = 3.

<table>
<thead>
<tr>
<th>Prevalence of U among low-activity participants, p_0^\text{a}</th>
<th>1%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>90%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.68</td>
<td>0.80</td>
<td>1.00</td>
<td>1.33</td>
<td>1.67</td>
<td>1.87</td>
<td>1.99</td>
</tr>
<tr>
<td>10%</td>
<td>0.58</td>
<td>0.68</td>
<td>0.85</td>
<td>1.13</td>
<td>1.42</td>
<td>1.59</td>
<td>1.69</td>
</tr>
<tr>
<td>25%</td>
<td>0.46</td>
<td>0.54</td>
<td>0.68</td>
<td>0.91</td>
<td>1.13</td>
<td>1.27</td>
<td>1.35</td>
</tr>
<tr>
<td>Prevalence of U among high-activity participants, p_1^\text{a}</td>
<td>50%</td>
<td>0.35</td>
<td>0.41</td>
<td>0.51</td>
<td>0.68</td>
<td>0.85</td>
<td>0.95</td>
</tr>
<tr>
<td>75%</td>
<td>0.28</td>
<td>0.33</td>
<td>0.41</td>
<td>0.54</td>
<td>0.68</td>
<td>0.76</td>
<td>0.81</td>
</tr>
<tr>
<td>90%</td>
<td>0.25</td>
<td>0.29</td>
<td>0.36</td>
<td>0.49</td>
<td>0.61</td>
<td>0.68</td>
<td>0.72</td>
</tr>
<tr>
<td>99%</td>
<td>0.23</td>
<td>0.27</td>
<td>0.34</td>
<td>0.46</td>
<td>0.57</td>
<td>0.64</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a}conditional on other covariates.

When HR_{U-AD} = 3, the bias-corrected AD HR will be exactly 1 at the following combinations of \( p_1 \) and \( p_0 \): \( p_1 = 1\% \) and \( p_0 = 25\% \); \( p_1 = 10\% \) and \( p_0 = 29\% \); \( p_1 = 25\% \) and \( p_0 = 50\% \); and \( p_1 = 50\% \) and \( p_0 = 89\% \).

Supplementary Table 1.5: Bias-corrected AD HR (comparing high versus low participation in cognitive activity), adjusted for U, given that HR unadjusted for U = 0.68 and HR_{U-AD} = 4.5.

<table>
<thead>
<tr>
<th>Prevalence of U among low-activity participants, p_0^\text{a}</th>
<th>1%</th>
<th>10%</th>
<th>25%</th>
<th>50%</th>
<th>75%</th>
<th>90%</th>
<th>99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>0.68</td>
<td>0.89</td>
<td>1.23</td>
<td>1.81</td>
<td>2.38</td>
<td>2.73</td>
<td>2.93</td>
</tr>
<tr>
<td>10%</td>
<td>0.52</td>
<td>0.68</td>
<td>0.94</td>
<td>1.39</td>
<td>1.83</td>
<td>2.09</td>
<td>2.25</td>
</tr>
<tr>
<td>Prevalence of U among high-activity participants, p_1^\text{a}</td>
<td>25%</td>
<td>0.38</td>
<td>0.49</td>
<td>0.68</td>
<td>1.00</td>
<td>1.31</td>
<td>1.51</td>
</tr>
<tr>
<td>50%</td>
<td>0.26</td>
<td>0.33</td>
<td>0.46</td>
<td>0.68</td>
<td>0.90</td>
<td>1.03</td>
<td>1.10</td>
</tr>
<tr>
<td>75%</td>
<td>0.19</td>
<td>0.25</td>
<td>0.35</td>
<td>0.52</td>
<td>0.68</td>
<td>0.78</td>
<td>0.84</td>
</tr>
<tr>
<td>90%</td>
<td>0.17</td>
<td>0.22</td>
<td>0.31</td>
<td>0.45</td>
<td>0.59</td>
<td>0.68</td>
<td>0.73</td>
</tr>
<tr>
<td>99%</td>
<td>0.16</td>
<td>0.21</td>
<td>0.29</td>
<td>0.42</td>
<td>0.55</td>
<td>0.63</td>
<td>0.68</td>
</tr>
</tbody>
</table>

\textsuperscript{a}conditional on other covariates.

When HR_{U-AD} = 4.5, the bias-corrected AD HR will be exactly 1 at the following combinations of \( p_1 \) and \( p_0 \): \( p_1 = 1\% \) and \( p_0 = 15\% \); \( p_1 = 10\% \) and \( p_0 = 29\% \); \( p_1 = 25\% \) and \( p_0 = 50\% \); and \( p_1 = 50\% \) and \( p_0 = 89\% \).
References


63. Bielak AA (2010) How can we not 'lose it' if we still don't understand how to 'use it'? Unanswered questions about the influence of activity participation on cognitive performance in older age--a mini-review. *Gerontology* 56(5):507-519.
Chapter 2

Mediation of the effect of the apolipoprotein E e4 allele on cognition by MRI markers of brain pathology in the Age, Gene/Environment Susceptibility–Reykjavik Study

Gautam Sajeev, Tyler J. VanderWeele, Anand Viswanathan, Sigurdur Sigurdsson, Gudny Eiriksdottir, Thor Aspelund, Rebecca A. Betensky, Francine Grodstein, Vilmundur Gudnason, Lenore J. Launer, and Deborah Blacker
Abstract

Objective: The apolipoprotein episilon-4 allele (APOE-e4) is the major genetic risk factor for Alzheimer’s disease (AD), but it is also a risk factor for cardiovascular disease. The extent to which its negative effect on cognition is due to its effects on cerebrovascular disease is unclear. We investigated the extent to which the effect of e4 carrierson late-life cognition is statistically mediated by magnetic resonance imaging markers of cerebrovascular disease.

Methods: This study included approximately 4,000 participants of the population-based Age, Gene/Environment Susceptibility–Reykjavik Study. Neuroimaging markers of cerebrovascular disease (white matter lesion volume, cerebral microbleeds, subcortical infarcts and cortical infarcts) as well as a non-specific marker of neurodegeneration (total brain parenchymal volume), were obtained from magnetic resonance images. Composite z-scores for global cognition, memory, executive function and information processing speed were derived from a battery of neuropsychological tests. We used a regression-based approach to examine mediation of the e4 effect on cognition by these five markers.

Results: About 9% of the total effect of e4 carrierson cognition was mediated by white matter lesion volume and cerebral microbleeds. This proportion increased to 25% when total brain parenchymal volume was also considered with these two markers. In analyses separating e4 homozygotes from e4 heterozygotes, the effect on global cognition of specifically e4 homozygosity appeared to be partially mediated by cerebral microbleeds, and particularly lobar microbleeds. There was no evidence of mediation of the e4 effect by cortical or subcortical infarcts.

Interpretation: A small but measurable portion of the e4 effect on cognition is mediated by white matter lesion volume and cerebral microbleeds, consistent with the e4 effect being partially mediated by cerebral amyloid angiopathy.
Introduction

The most well-established genetic risk factor for cognitive decline and dementia due to Alzheimer’s disease (AD) is the e4 allele of the apolipoprotein gene (*APOE*) (1, 2). The major mechanism underlying the e4 effect is thought to be its effect on amyloid-beta accumulation (*Aβ*) in the brain (3). The apoE protein plays an isoform-dependent role in *Aβ* accumulation, such that brain *Aβ* levels are greatest among e4 carriers (4, 5), seemingly because of slower *Aβ* clearance (6). *Aβ* is the major constituent of the hallmark neuritic plaques seen in AD and its accumulation in the brain is considered to trigger a cascade of events that eventually bring about the neuronal degeneration and cognitive impairment characteristic of AD dementia (7).

Many individuals with AD pathologies also have co-existing vascular pathologies (8-11). Autopsy studies have found microinfarcts, macroinfarcts (9, 12) and cerebral amyloid angiopathy (CAA) (10, 13) to be highly prevalent in patients meeting AD neuropathological criteria. In a recent analysis of a large database of autopsy-confirmed neurodegenerative disease cases, there was an increased concurrence of vascular pathology and cerebrovascular disease (CVD) in cases of neuropathologically determined AD compared to other neurodegenerative disorders (10). Similarly, antemortem imaging has documented the frequent presence of MRI markers of cerebral small vessel disease such as microbleeds (14) and white matter lesions (15) among patients with AD dementia. Persons with multiple pathologies have greater odds of dementia incidence and experience accelerated cognitive decline (16-19). The overlap between AD and CVD pathologies is further suggested from epidemiologic research highlighting several common vascular risk factors for the two disorders (20).

A variety of evidence suggests that e4 allele carriership may also affect CVD. ApoE isoforms differentially regulate lipid metabolism in the periphery, with e4 carriers having higher cholesterol and modestly increased risk of coronary heart disease (21, 22). The e4 allele has also been linked with elevated risk of ischemic stroke (23) and greater frequency of cerebral infarction in some but not all studies (24, 25). It has also been proposed that that apoe4 expression may lead to neuronal injury by preferentially activating proinflammatory processes that promote blood brain barrier breakdown (26, 27). The e4 allele is also a strong risk factor for CAA (28, 29), and is also correlated with greater occurrence of white matter lesions and lobar microbleeds, two neuroimaging markers of CAA (24). While *Aβ* is a key feature of the senile plaques of AD, advanced cerebrovascular *Aβ* deposition is seen in CAA. Neuropathological studies suggest that the two types of *Aβ* deposits can occur either relatively independently of one another, or can overlap. Evidence of CAA appears to be present in nearly all brains with AD (30), although
only in approximately 25% of these cases is advanced CAA present (31). Given that these CVD pathologies also strongly predict dementia (32) and poorer cognitive performance among non-demented individuals (13, 33, 34), it is plausible that part of the e4 effect on late-life cognition may be mediated by CVD.

Thus far, such mediation of the e4 effect by brain pathologies has only been assessed in a few autopsy studies (35-38). In general, these studies found little evidence of mediation of the e4 effect by the markers of cerebrovascular pathology assessed (37, 38). However, there have thus far been no population-based studies of this question using antemortem MRI, which allows for in vivo characterization of CVD markers in larger, less selected samples of study participants. Consequently, in this study we used mediation analysis to examine the extent to which the effect of e4 carriership on late-life cognition is mediated by MRI markers of CVD in a large, population-based cohort of older adults.
Methods

Study population

The Age, Gene/Environment Susceptibility–Reykjavik Study (AGES-Reykjavik) is an epidemiologic study of genetic, behavioural and environmental risk factors for late-life disease and disability in the vascular, neurocognitive, musculoskeletal, and metabolic systems (39). Participants in AGES-Reykjavik are drawn from the population-based Reykjavik Study, which began in 1967 and followed a random sample of 30,795 Reykjavik residents born between 1907 and 1935 until 1996. Between 2002 and 2006, 5764 surviving members of the Reykjavik Study cohort were enrolled and underwent baseline assessments for AGES-Reykjavik. Participants completed a structured questionnaire, received clinical and neurological examinations, underwent a series of imaging protocols, and provided laboratory samples for DNA and biomarker analysis. Questionnaire and laboratory data obtained earlier in life during Reykjavik Study assessments were also linked to AGES-Reykjavik data. All participants provided written informed consent. The AGES-Reykjavik Study was approved by the Icelandic National Bioethics Committee (VSN: 00-063), the Data Protection Authority and the institutional review board of the National Institute on Aging.

APOE genotype

APOE genotyping was conducted using microplate array diagonal gel electrophoresis. Participants' APOE genotype was analyzed in two ways: first, as a dichotomous exposure denoting e4 risk allele carriership (\(e4\)carriers [\(e4/e2, e4/e3, e4/e4\) genotypes] versus \(e4\) non-carriers [\(e3/e3, e3/e2, e2/e2\]), and, second, as a three-level exposure based on number of e4 risk alleles carried (\(e4\) homozygotes [\(e4/e4\]), \(e4\) heterozygotes [\(e4/e3, e4/e2\) or \(e4\) non-carriers [\(e3/e3, e3/e2, e2/e2\]).

Magnetic resonance imaging acquisition

MRI sequences and parameters: MR images were acquired on a single, research-dedicated 1.5 T Signa Twinspeed EXCITE system (General Electric Medical Systems, Waukesha, WI). The structural imaging protocol consisted of (1) a T1-weighted, three-dimensional spoiled gradient-echo sequence [time to echo (TE) = 8 milliseconds (ms); time repetition (TR = 21 ms; flip angle (FA) = 30°; field of view (FOV) = 240 mm; matrix = 256 x 256, slice thickness = 1.5 mm], (2) a T2*-weighted gradient-echo type echoplanar sequence [TE = 50 ms; TR = 3050 ms; FA = 90°; FOV = 220 mm; matrix = 256 x 256, slice thickness = 3 mm], (3) a proton density/T2-weighted
fast spin echo sequence [TE1 = 22 ms; TE2 = 90 ms; TR = 3220 ms; echo train length = 8; FA = 90°; FOV = 220 mm; matrix = 256 x 256, slice thickness = 3 mm], and (4) a fluid-attenuated inversion recovery (FLAIR) sequence [TE = 100 ms, TR = 8000 ms, inversion time = 2000 ms, FA = 90°; FOV = 220 mm; matrix = 256 x 256, slice thickness = 3 mm]. All images were acquired to give full brain coverage, and slices were angled parallel to the anterior commissure-posterior commissure line to give reproducible image views in the oblique-axial plane.

*Volume measures:* Volumes of gray matter, white matter, white matter lesions, and cerebral spinal fluid were generated separately using the multispectral MR images and an automatic image analysis pipeline based on the Montreal Neurological Institute pipeline that was optimized for use in the AGES-Reykjavik study. Technical details of aspects of image processing, classification and validation of tissue volumes have been reported elsewhere (40).

**MRI markers**

*Total brain parenchymal volume:* Total brain parenchymal (TBP) volume was computed as the sum of gray matter, white matter, and white matter lesion (see below) volumes obtained from the imaging processing pipeline. In the analysis, TBP volume was represented as a percentage of total intracranial volume, which was calculated as the sum of the TBP volume and cerebral spinal fluid volume.

*White matter lesion volume:* White matter lesion volume was obtained from the imaging processing pipeline, and, like TBP volume, was represented as percentage of total intracranial volume. Because it was highly skewed, it was log-transformed in the analysis.

*Cerebral microbleeds:* Cerebral microbleeds (CMBs) were defined as a focal hypointense lesions visible on the T2*-weighted GRE images and identified by a neuroradiologist who recorded the presence, number, and slice number of microbleeds. Trained raters then viewed the identified images and recorded the anatomical location of the microbleeds. Lobar microbleeds were those located in the frontal, parietal, occipital or temporal lobes, while all other microbleeds were classified as deep.

In our analysis, we categorized CMBs (and subtypes of CMBs) as present or absent, as most individuals did not have microbleeds.

*Infarcts:* Infarcts were defined as defects of the brain parenchyma with signal intensity isointense to that of cerebral spinal fluid on FLAIR, proton density/T2-weighted sequences, and surrounded by an area of hyperintensity on FLAIR images. Cortical infarcts were defined as defects located in the cortical ribbon and subcortical infarcts.
were defined as defects that did not extend to the cortex. Cortical and subcortical infarcts had to have a diameter of at least 4 mm. In our analyses, we categorized cortical and subcortical infarcts as present or absent.

**Cognitive function**

Cognitive functioning obtained at approximately the same time as MRIs were used for this cross-sectional analysis. Participants received a standard neuropsychological test battery that targeted memory, executive function, and processing speed. Composite scores on these three domains were constructed for each participant by combining their scores on individual tests. Similar methods have been used to construct cognitive domain scores in other cohorts (34, 36). The composite memory score was derived from scores on the California Verbal Learning Test (immediate and delayed recall); the composite executive function score was calculated from the Stroop Test (part 3), Digits Backward, and the CANTAB spatial working memory test; and the composite processing speed score was obtained from scores on the Stroop Test (parts 1 and 2), Digit Symbol Substitution Test and Figure Comparison Test. Z-scores were obtained for each individual test \([z\text{-score for test} = (\text{participant’s test score} – \text{mean test score})/\text{standard deviation of test score}]\), and the average of z-scores across tests relevant for a particular domain was taken as the composite score for that domain. An overall, global cognition composite score was calculated from the average of these three domain-specific scores and used as the primary outcome in these analyses.

**Mediation analysis methodology**

*Total, direct and indirect effects.* Mediation occurs when a change in an exposure causes a change in an intermediate variable (the mediator), which in turn causes a change in an outcome (41). Mediation can be assessed statistically by decomposing the effect of an exposure on an outcome (called a *total effect*) into two non-overlapping components: the *indirect effect* and the *direct effect*. In this context, the total effect of e4 exposure is the difference in global cognition z-score between an e4 exposure level and an e4 reference level. The indirect effect is the estimated effect through the measured MRI markers, and the direct effect is the effect operating through pathways not involving the measured MRI markers. An indirect effect of zero would indicate that there is no mediation of the exposure effect; a direct effect of zero would indicate that the exposure effect is entirely mediated. The *proportion mediated (PM)*, an index of the degree to which the exposure effect is mediated, is obtained by dividing the indirect effect by the total effect.
**Counterfactual framework.** In this study, we used an approach to mediation analysis based on the counterfactual framework. In this framework, the average total, direct and indirect effects described above are mathematically defined in terms of counterfactual quantities (42, 43). These effects are identifiable from observed study data under the unmeasured confounding assumptions detailed below and can be estimated using regression models (41, 44, 45). The counterfactual approach offers the additional benefit that valid direct and indirect effect estimates can be obtained even when exposure-mediator interaction is present (i.e., the effect of the exposure on the outcome differs based on the level of the mediator).

**Mediation assumptions.** For direct and indirect effect estimates to be valid, conditional on the observed covariates, there must be no unmeasured confounding of the effects of (i) the e4 allele on cognition, (ii) e4 allele on MRI markers, and (iii) MRI markers on cognition. Additionally, (iv) there should be no confounders of the effect of MRI markers on cognition that are themselves affected by the e4 allele. We addressed (i) and (ii) by conducting our study in the ethnically homogenous AGES-Reykjavik cohort, which protects against confounding due to population stratification. We addressed (iii), by fitting models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of physical activity, body mass index, systolic blood pressure, and total cholesterol (main model). In secondary models, we also additionally adjusted for general health status, depressive symptoms and atrial fibrillation.

**Individual mediator analysis.** We first estimated the degree to which the e4 effect on global cognition was mediated by each MRI marker individually. Under the assumptions mentioned above, a regression-based approach can be used to estimate direct and indirect effects. First, we regressed each MRI marker on e4 status, adjusting for the confounders described in our main model above. Linear regression was used for WML volume and TBP volumes, while logistic regression was used for CMBs, subcortical and cortical infarcts. Both WML volume and TBP volume were expressed as a fraction of intracranial volume to adjust for head size, and WML volume was log-transformed in the analysis. Next, for each MRI marker separately, we performed linear regression of global cognition z-score on e4 status, the MRI marker, a product term denoting the interaction between e4 carri ership and the MRI marker, and the same covariates as above. Direct and indirect effects were estimated by combining the parameter estimates from these models according to the analytic expressions in the literature (44), and bootstrapping with 1000 replications was used to obtain confidence intervals for these estimates.
**Multiple mediator analysis.** In order to estimate the degree to which the effect of e4 carriership on global cognition was mediated by the MRI markers *jointly*, a similar approach was used (46). We considered the markers that showed some evidence of mediation in the individual marker analyses (WML volume, CMBs and TBP volume) together in a multiple mediator model. As a decrease in TBP volume likely reflects brain atrophy arising from both vascular and neurodegenerative processes, we carried out separate analyses examining purely vascular (WML and CMBs), versus vascular and neurodegenerative (WML, CMBs and TBP volume) markers. As in the individual marker analysis, we first separately regressed each MRI marker on e4 status, with adjustment for the same covariates. Next, we fitted a single model, regressing global cognition z-score on e4 status, all MRI markers and all two-way interactions between e4 status and the MRI markers, with adjustment for the same covariates. As above, direct and indirect effect estimates were estimated according to analytic expressions in the literature (47) and bootstrapping was used to obtain confidence intervals.

Given the relative rarity of the e4 homozygous genotype, the e4 carrier versus e4 non-carrier analysis largely reflects comparisons of e4 heterozygotes to e4 non-carriers. To examine effects specific to e4 homozygotes we repeated our analyses separating out e4 homozygotes from e4 heterozygotes, so that mediated effects could be estimated for (i) e4 heterozygotes relative to e4 non-carriers (1e4 vs 0e4), (ii) e4 homozygotes relative to e4 non-carriers (2e4 vs 0e4), and (iii) e4 homozygotes relative to e4 heterozygotes (2e4 vs 1e4).

**Sensitivity analysis.** Finally, we used a published bias formula (48) to determine how much the estimates of direct and indirect effects in our primary individual analyses would change under different degrees of confounding by a hypothetical unmeasured binary confounder (Appendix).

**Secondary analyses**

We also performed a number of secondary analyses. As e4 carriership has been more strongly associated with CAA – and therefore lobar rather than deep microbleeds (24) – we also examined results treating lobar microbleeds as the mediator. We repeated all analyses for each of the three individual cognitive domains: memory, executive function and processing speed. We also examined whether the results obtained when adjusting for the covariates in our main model changed when adjusting for smaller (age, sex, education and smoking status only), or larger sets of covariates (main model covariates, plus general health status, depression and atrial fibrillation).
**Covariates**

We adjusted for age (continuous), sex, education (primary, secondary, college, or university), smoking status (never smoker, former smoker, or current smoker), mid-life physical activity (never, rarely, <1 hour/week, 1-3 hours/week, 4-7 hours/week, or > 7 hours/week), diabetes diagnosis (yes, or no); and mid-life measures of body mass index (continuous), systolic blood pressure (continuous), total cholesterol (continuous) in all models. In secondary analyses, we additionally adjusted for general health status (excellent, very good, good, fair, or poor), depressive symptomatology (yes, or no), atrial fibrillation before AGES-Reykjavik enrolment. (yes, or no). Age, sex, education and general health status were obtained from questionnaire responses. BMI was calculated as weight (kg)/squared height (m$^2$). Diabetes was diagnosed either based on self-reported history of diabetes, fasting glucose level $\geq$ 7 mmol/l, or use of diabetes medications. High depressive symptomatology was defined as scoring $\geq$ 6 on the Geriatric Depression Scale. Mid-life measures were obtained from Reykjavik study assessments.

**Analytic samples**

Of the 5764 individuals included in AGES-Reykjavik, 953 individuals did not receive MRI scans for a variety of reasons, including MRI contraindications, refusal, machine maintenance and home visits and a further 85 did not complete the entire MRI examination. After removing scans due to acquisition artifacts, or failures in post-processing detected during quality control procedures, a sample of 4615 individuals remained. Individuals with missing data on e4 genotype or cognitive testing were excluded in these analyses. Our sample sizes varied between 4000 and 4300 individuals depending on the CVD marker and cognitive domain analyzed. In general, compared to those included, the individuals excluded from these analyses were older, had lower levels of education, and were more likely to be diabetic, current smokers and have higher blood pressure. The distribution of e4 status did not differ between the included and excluded.
Results

Table 2.1 summarizes baseline characteristics of the analytic sample used to investigate mediation of the e4 effect on global cognition by WML volume. In this sample of 4040 participants, the mean age was 76.2 (SD = 5.4), 58.0% of participants were female. Of these 4040 participants, 2896 (71.7%) were e4 non-carriers, 1061 (26.3%) were e4 heterozygotes and 83 (2.0%) were e4 homozygotes. The distribution of baseline characteristics in the analyses focusing on mediation of the e4 effect on global cognition by the other MRI markers of interest was very similar to that shown in Table 2.1.

Table 2.1: Baseline characteristics of analytic sample of n=4040 used in mediation analysis of e4 effect on global cognition by WML volume

<table>
<thead>
<tr>
<th></th>
<th>e4 non-carriers (n = 2896)</th>
<th>e4 carriers (n = 1144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>76.3 (5.4)</td>
<td>75.8 (5.3)</td>
</tr>
<tr>
<td>Female</td>
<td>1702 (58.7)</td>
<td>644 (56.3)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>979 (33.8)</td>
<td>368 (32.2)</td>
</tr>
<tr>
<td>Secondary</td>
<td>1375 (47.5)</td>
<td>558 (48.8)</td>
</tr>
<tr>
<td>College</td>
<td>347 (12.0)</td>
<td>144 (12.6)</td>
</tr>
<tr>
<td>University</td>
<td>195 (6.7)</td>
<td>74 (6.5)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoker</td>
<td>1238 (42.8)</td>
<td>499 (43.6)</td>
</tr>
<tr>
<td>Former Smoker</td>
<td>1324 (45.7)</td>
<td>511 (44.7)</td>
</tr>
<tr>
<td>Current Smoker</td>
<td>334 (11.5)</td>
<td>134 (11.7)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>339 (11.7)</td>
<td>107 (9.4)</td>
</tr>
<tr>
<td>Mid-life SBP, mmHg</td>
<td>131.8 (16.7)</td>
<td>131.2 (16.4)</td>
</tr>
<tr>
<td>Mid-life BMI, kg/m²</td>
<td>25.2 (3.5)</td>
<td>24.9 (3.4)</td>
</tr>
<tr>
<td>Mid-life Cholesterol, mmol/L</td>
<td>6.3 (1.1)</td>
<td>6.5 (1.1)</td>
</tr>
<tr>
<td>Mid-life physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>960 (33.2)</td>
<td>387 (33.8)</td>
</tr>
<tr>
<td>Rarely</td>
<td>710 (24.5)</td>
<td>289 (25.3)</td>
</tr>
<tr>
<td>&lt;1 hr/week</td>
<td>195 (6.7)</td>
<td>77 (6.7)</td>
</tr>
<tr>
<td>1-3 hrs/week</td>
<td>463 (16.0)</td>
<td>176 (15.4)</td>
</tr>
<tr>
<td>4-7 hrs/week</td>
<td>286 (9.9)</td>
<td>113 (10.0)</td>
</tr>
<tr>
<td>&gt;7 hrs/week</td>
<td>282 (9.7)</td>
<td>102 (8.9)</td>
</tr>
</tbody>
</table>

Values are Means (SD) or N (%)
SBP: systolic blood pressure, BMI: body mass index

APOE e4 status and MRI markers: The association between e4 status and MRI markers are reported in Table 2.2.

As a percentage of intracranial volume, mean WML volume was slightly higher among e4 carriers (1.40%) than
among e4 non-carriers (1.33%), and mean TBP volume was slightly lower among e4 carriers (72.2%) than among e4 non-carriers (71.9%). CMBs were only slightly more common among e4 carriers (144/1148 = 12.6%) than among e4 non-carriers (326/2887 = 11.3%), but were markedly more common among e4 homozygotes (17/82 = 21.0%) than among e4 heterozygotes (127/1056 = 11.9%) and e4 non-carriers, largely due to lobar microbleeds being more frequent particularly among e4 homozygotes (17.1%) than e4 heterozygotes (8.7%) and non-carriers (8.0%).

Table 2.2: Association between APOE e4 status and MRI Markers

<table>
<thead>
<tr>
<th></th>
<th>0 e4 alleles (n = 2896)</th>
<th>1 e4 allele (n = 1056)</th>
<th>2 e4 alleles (n = 82)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WML volume, %, mean</td>
<td>1.33</td>
<td>1.38</td>
<td>1.49</td>
</tr>
<tr>
<td>TBP volume %, mean</td>
<td>72.2</td>
<td>71.9</td>
<td>72.9</td>
</tr>
<tr>
<td>Cerebral microbleeds, % any</td>
<td>11.2</td>
<td>11.9</td>
<td>21.0</td>
</tr>
<tr>
<td>Both deep and lobar, %</td>
<td>1.5</td>
<td>2.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Deep, %</td>
<td>4.7</td>
<td>5.0</td>
<td>7.3</td>
</tr>
<tr>
<td>Lobar, %</td>
<td>8.0</td>
<td>8.7</td>
<td>17.1</td>
</tr>
<tr>
<td>Deep only, %</td>
<td>3.2</td>
<td>3.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Lobar only, %</td>
<td>6.5</td>
<td>6.7</td>
<td>14.6</td>
</tr>
<tr>
<td>Subcortical infarcts, %</td>
<td>11.3</td>
<td>11.6</td>
<td>8.5</td>
</tr>
<tr>
<td>Cortical infarcts, %</td>
<td>10.7</td>
<td>11.1</td>
<td>10.8</td>
</tr>
</tbody>
</table>

Ns for each of WML volume (N = 4040), TBP volume (N = 4040), cerebral microbleeds (N = 4025), subcortical infarcts (N=4041), cortical infarcts (N = 4041)

**Individual mediator analysis:** We first modelled e4 status as a two-level exposure comparing e4 carriers to e4 non-carriers. Decomposing this total effect of e4 carriership into direct and indirect effects and adjusting for the covariates above, we found that the effect of e4 carriership on cognition was partially mediated by WML volume and TBP volume (Table 2.3). When WML volume was used as the mediator, the estimated direct effect on global cognition was -0.08 (-0.12, -0.04), while the indirect effect through WML volume was -0.008 (-0.013, 0.000), and the proportion mediated was 9%. When TBP volume was used as the mediator, most of the effect of e4 carriership was similarly direct, although the indirect effect of -0.014 (-0.023, -0.003) and proportion mediated of 16% were both slightly larger than for WML volume. The effect of e4 carriership on global cognition did not appear to be mediated by either CMBs [indirect effect = -0.001 (-0.003, 0.002); PM = 1%], subcortical infarcts [indirect effect = -0.002 (-0.006, 0.004); PM = 2%] or cortical infarcts [indirect effect = 0.00 (-0.005, 0.004); PM = 0%].
Table 2.3: Total, direct and indirect effects in individual mediator analyses, comparing e4 carriers to e4 non-carriers, for all cognitive domains

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4040</td>
<td>-0.09 (-0.13, -0.05)</td>
<td>-0.08 (-0.12, -0.04)</td>
<td>-0.008 (-0.013, 0.000)</td>
<td>9%</td>
</tr>
<tr>
<td>Memory</td>
<td>4136</td>
<td>-0.11 (-0.16, -0.06)</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.009 (-0.017, -0.001)</td>
<td>9%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4283</td>
<td>-0.06 (-0.11, -0.02)</td>
<td>-0.06 (-0.10, -0.01)</td>
<td>-0.008 (-0.014, -0.001)</td>
<td>13%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4250</td>
<td>-0.09 (-0.14, -0.04)</td>
<td>-0.08 (-0.13, -0.04)</td>
<td>-0.009 (-0.016, -0.001)</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Cerebral microbleeds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4025</td>
<td>-0.09 (-0.12, -0.05)</td>
<td>-0.09 (-0.12, -0.04)</td>
<td>-0.001 (-0.003, 0.002)</td>
<td>1%</td>
</tr>
<tr>
<td>Memory</td>
<td>4121</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.001 (-0.003, 0.003)</td>
<td>1%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4267</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.001 (-0.002, 0.002)</td>
<td>2%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4234</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.001 (-0.004, 0.003)</td>
<td>1%</td>
</tr>
<tr>
<td><strong>Subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4041</td>
<td>-0.09 (-0.12, -0.05)</td>
<td>-0.08 (-0.12, -0.05)</td>
<td>-0.002 (-0.006, 0.004)</td>
<td>2%</td>
</tr>
<tr>
<td>Memory</td>
<td>4137</td>
<td>-0.11 (-0.16, -0.06)</td>
<td>-0.10 (-0.16, -0.05)</td>
<td>-0.001 (-0.008, 0.005)</td>
<td>1%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4284</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.06 (-0.11, -0.02)</td>
<td>-0.001 (-0.004, 0.003)</td>
<td>1%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4251</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.002 (-0.006, 0.004)</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4041</td>
<td>-0.09 (-0.12, -0.05)</td>
<td>-0.08 (-0.12, -0.05)</td>
<td>0.000 (-0.005, 0.004)</td>
<td>0%</td>
</tr>
<tr>
<td>Memory</td>
<td>4137</td>
<td>-0.11 (-0.16, -0.06)</td>
<td>-0.11 (-0.16, -0.06)</td>
<td>0.000 (-0.004, 0.004)</td>
<td>0%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4284</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>0.000 (-0.004, 0.003)</td>
<td>0%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4251</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>0.000 (-0.006, 0.006)</td>
<td>0%</td>
</tr>
<tr>
<td><strong>TBP volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4040</td>
<td>-0.09 (-0.13, -0.05)</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.014 (-0.023, -0.003)</td>
<td>16%</td>
</tr>
<tr>
<td>Memory</td>
<td>4136</td>
<td>-0.11 (-0.16, -0.06)</td>
<td>-0.09 (-0.15, -0.04)</td>
<td>-0.014 (-0.025, -0.001)</td>
<td>13%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4283</td>
<td>-0.06 (-0.11, -0.02)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.010 (-0.017, -0.002)</td>
<td>16%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4250</td>
<td>-0.09 (-0.14, -0.04)</td>
<td>-0.07 (-0.12, -0.03)</td>
<td>-0.016 (-0.026, -0.003)</td>
<td>17%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Next, we repeated our analyses estimating mediated effects for the comparisons of (i) e4 heterozygotes to e4 non-carriers (1e4 vs 0e4), (ii) e4 homozygotes to e4 non-carriers (2e4 vs 0e4), and (iii) e4 homozygotes to e4 heterozygotes (2e4 vs 1e4). When WML volume was used as the mediator, the small amount of mediation estimated in each of these three genotype comparisons was similar to that observed when comparing e4 carriers to e4 non-carriers as a whole. When TBP volume was used as the mediator, there was evidence of mediation when comparing e4 heterozygotes to e4 non-carriers \( \text{indirect effect}_{1e4 \text{ vs } 0e4} = -0.014 \) \((-0.024, -0.002)\), but not when comparing e4 homozygotes to e4 non-carriers \( \text{indirect effect}_{2e4 \text{ vs } 0e4} = -0.001 \) \((-0.046, 0.058)\), or e4 homozygotes to e4 heterozygotes \( \text{indirect effect}_{2e4 \text{ vs } 1e4} = 0.028 \) \((-0.024, 0.082)\). There was no evidence of mediation by subcortical or cortical infarcts across any of the three genotype comparisons. (Supplementary Table 2.1-2.4)

There were more substantial differences across genotype comparisons when CMBs was used as the mediator, with evidence of mediation specifically when comparing e4 homozygotes to e4 non-carriers \( \text{indirect effect}_{2e4 \text{ vs } 0e4} = -0.047 \) \((-0.089, 0.015)\), and e4 homozygotes to e4 heterozygotes \( \text{indirect effect}_{2e4 \text{ vs } 1e4} = -0.047 \) \((-0.092, 0.025)\), but not when comparing e4 heterozygotes to e4 non-carriers \( \text{indirect effect}_{1e4 \text{ vs } 0e4} = 0.000 \) \((-0.002, 0.002)\) (Table 2.4).

**Multiple mediator analysis:** Given the evidence of mediation by WML volume, CMBs and TBP volume in either the two- or three-level individual mediator analyses, we next estimated the degree to which these markers jointly mediated the e4 effect on cognition. As decreased TBP volume likely reflects brain atrophy arising from both vascular and neurodegenerative processes, we fit separate models in order to compare the effects mediated by the purely vascular markers (WML volume and CMBs) to the effects mediated by all three markers considered together (Table 2.5). Treating e4 carriership as a two-level variable, we found that the portion of the effect of e4 carriership on global cognition jointly mediated by all three markers \( \text{indirect effect} = -0.021 \) \((-0.033, -0.008)\), PM = 25% was larger than the portion of the e4 effect jointly mediated by the two purely vascular markers alone \( \text{indirect effect} = -0.008 \) \((-0.014, 0.000)\), PM = 9%.

Repeating this analysis for the three genotype comparisons, the portion of the effect on global cognition jointly mediated by the two purely vascular markers WML volume and CMBs when comparing e4 heterozygotes to e4 non-carriers \( \text{indirect effect}_{1e4 \text{ vs } 0e4} = -0.007 \) \((-0.012, 0.000)\) was smaller than the portion mediated when comparing e4 homozygotes to e4 non-carriers \( \text{indirect effect}_{2e4 \text{ vs } 0e4} = -0.068 \) \((-0.118, 0.010)\), or e4 homozygotes to
e4 heterozygotes [indirect effect $e_4$ vs $e_4$ = -0.058 (-0.108, 0.014)]. When also considering TBP volume as a mediator, the effect mediated by these three markers together was larger than the effect mediated by the two purely vascular markers alone when comparing e4 heterozygotes to e4 non-carriers [indirect effect $e_4$ vs $0e_4$ = -0.021 (-0.032, -0.008)], but of similar magnitude when comparing e4 homozygotes to e4 non-carriers [indirect effect $e_4$ vs $0e_4$ = -0.071 (-0.143, 0.015)] or e4 heterozygotes [indirect effect $e_4$ vs $0e_4$ = -0.037 (-0.116, 0.056)] (Supplementary Tables 2.5 and 2.6).

Sensitivity analysis: As explained in the Appendix, if a hypothetical binary unmeasured confounder associated with poorer cognition were more prevalent among e4 carriers, or a confounder associated with better cognition were more prevalent among e4 non-carriers, the indirect effects estimated here would underestimate the true indirect effect. Under the seemingly less likely scenarios of a confounder associated with poor cognition being more prevalent among e4 non-carriers, or a confounder associated with better cognition being more prevalent among e4 carriers, the indirect effect estimated here would overestimate the true indirect effect (Appendix).

Secondary analyses: When using lobar microbleeds as the mediator, the pattern of results was similar to that observed for CMBs in the individual mediator analyses (Table 2.6). A similar analysis could not be conducted for deep CMBs due to insufficient sample size. The pattern of results observed was similar across cognitive domains, and when adjusting for fewer or more covariates.
Table 2.4: Total, direct and indirect effects when cerebral microbleeds was used as the mediator, comparing across number of e4 alleles

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3943</td>
<td>-0.08 (-0.12, -0.04)</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2969</td>
<td>-0.23 (-0.37, -0.08)</td>
<td>-0.18 (-0.33, -0.04)</td>
<td>-0.047 (-0.089, 0.015)</td>
<td>21%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1138</td>
<td>-0.15 (-0.29, 0.00)</td>
<td>-0.10 (-0.24, -0.04)</td>
<td>-0.047 (-0.092, 0.025)</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4038</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3047</td>
<td>-0.22 (-0.39, -0.05)</td>
<td>-0.15 (-0.31, 0.02)</td>
<td>-0.065 (-0.123, 0.012)</td>
<td>30%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1157</td>
<td>-0.12 (-0.29, 0.06)</td>
<td>-0.05 (-0.23, 0.12)</td>
<td>-0.064 (-0.122, 0.018)</td>
<td>55%</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4181</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3152</td>
<td>-0.19 (-0.35, -0.04)</td>
<td>-0.16 (-0.32, 0.01)</td>
<td>-0.038 (-0.078, 0.028)</td>
<td>19%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1201</td>
<td>-0.14 (-0.30, -0.01)</td>
<td>-0.11 (-0.28, 0.04)</td>
<td>-0.035 (-0.073, 0.022)</td>
<td>24%</td>
</tr>
<tr>
<td><strong>Processing speed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4148</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3127</td>
<td>-0.25 (-0.41, -0.08)</td>
<td>-0.22 (-0.37, -0.06)</td>
<td>-0.032 (-0.092, 0.056)</td>
<td>13%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1193</td>
<td>-0.16 (-0.33, 0.03)</td>
<td>-0.12 (-0.28, 0.03)</td>
<td>-0.034 (-0.086, 0.046)</td>
<td>21%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Table 2.5: Total, direct and indirect effects in multiple mediator analyses when WML volume and CMBs are used as mediators, comparing e4 carriers to e4 non-carriers

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume and CMBs as mediators</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4024</td>
<td>-0.09 (-0.12, -0.05)</td>
<td>-0.08 (-0.12, -0.04)</td>
<td>-0.008 (-0.014, 0.000)</td>
<td>9%</td>
</tr>
<tr>
<td>Memory</td>
<td>4120</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.10 (-0.15, -0.04)</td>
<td>-0.010 (-0.017, 0.000)</td>
<td>9%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4266</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.008 (-0.014, -0.001)</td>
<td>13%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4207</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.08 (-0.12, -0.03)</td>
<td>-0.009 (-0.016, -0.001)</td>
<td>10%</td>
</tr>
<tr>
<td><strong>WML volume, CMBs and TBP volume as mediators</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global cognition</td>
<td>4024</td>
<td>-0.09 (-0.12, -0.05)</td>
<td>-0.07 (-0.10, -0.03)</td>
<td>-0.021 (-0.033, -0.008)</td>
<td>25%</td>
</tr>
<tr>
<td>Memory</td>
<td>4120</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.08 (-0.14, -0.03)</td>
<td>-0.022 (-0.036, -0.009)</td>
<td>21%</td>
</tr>
<tr>
<td>Executive function</td>
<td>4266</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.05 (-0.08, -0.01)</td>
<td>-0.018 (-0.028, -0.007)</td>
<td>28%</td>
</tr>
<tr>
<td>Processing speed</td>
<td>4207</td>
<td>-0.09 (-0.13, -0.04)</td>
<td>-0.06 (-0.11, -0.02)</td>
<td>-0.025 (-0.037, -0.009)</td>
<td>28%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.

All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
## Table 2.6: Total, direct and indirect effects when lobar microbleeds was used as the mediator, comparing across number of e4 alleles

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3943</td>
<td>-0.08 (-0.12, -0.04)</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2969</td>
<td>-0.22 (-0.37, -0.08)</td>
<td>-0.19 (-0.34, -0.05)</td>
<td>-0.037 (-0.073, 0.026)</td>
<td>16%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1138</td>
<td>-0.14 (-0.29, 0.00)</td>
<td>-0.11 (-0.25, 0.03)</td>
<td>-0.038 (-0.077, 0.024)</td>
<td>26%</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4038</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>0.000 (-0.002, 0.003)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3047</td>
<td>-0.21 (-0.39, -0.05)</td>
<td>-0.17 (-0.35, 0.00)</td>
<td>-0.041 (-0.080, 0.023)</td>
<td>19%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1157</td>
<td>-0.12 (-0.29, 0.06)</td>
<td>-0.08 (-0.26, 0.10)</td>
<td>-0.043 (-0.086, 0.027)</td>
<td>36%</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
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</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4181</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>0.000 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3152</td>
<td>-0.19 (-0.35, -0.04)</td>
<td>-0.16 (-0.32, 0.00)</td>
<td>-0.029 (-0.065, 0.026)</td>
<td>15%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1201</td>
<td>-0.14 (-0.30, -0.01)</td>
<td>-0.12 (-0.27, 0.03)</td>
<td>-0.029 (-0.066, 0.023)</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Processing speed</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4148</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>0.001 (-0.002, 0.002)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3127</td>
<td>-0.25 (-0.40, -0.08)</td>
<td>-0.21 (-0.36, -0.05)</td>
<td>-0.037 (-0.094, 0.047)</td>
<td>15%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1193</td>
<td>-0.16 (-0.33, 0.02)</td>
<td>-0.12 (-0.28, 0.04)</td>
<td>-0.038 (-0.092, 0.042)</td>
<td>24%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.

All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Discussion

In this population-based cohort study, we found that a small but measurable portion of the negative effect of \textit{APOE} e4 carriership on cognition was mediated by WML volume, CMBs and TBP volume. When we compared e4 carriers to e4 non-carriers, about 9\% of the e4 effect was mediated by WML volume and CMBs, and this portion increased to 25\% when WML volume, CMBs and TBP volume were considered together. In analyses separating e4 homozygotes from e4 heterozygotes, we also found that the effect of e4 homozygosity on cognition appeared to be partially mediated by CMBs; this pattern was unique to CMBs among the MRI markers assessed. We found no evidence of mediation of the e4 effect by cortical or subcortical infarcts.

The contribution of brain pathologies to the effect of e4 carriership on AD dementia or late-life cognition has only been investigated in a few autopsy studies. In the Religious Orders Study, the association between e4 allele and both cognitive function and clinical AD diagnosis shortly before death were markedly attenuated after adjustment for measures of neuritic plaques, diffuse plaques and neurofibrillary tangles at autopsy (36). In a separate investigation of 581 individuals, the same investigators found that the e4 effect on onset and rate of cognitive decline was attenuated by measures of amyloid plaque and tangle pathology, but not by measures of macro and microinfarcts (38). A similar investigation of 267 participants from the Nun Study found that the effect of e4 carriership on and all-cause dementia diagnosis was eliminated after adjustment for neocortical tangle pathology, modestly attenuated with adjustment for neocortical neuritic plaques, but unchanged after adjustment for severity of amyloid angiopathy severity, cortical and subcortical infarcts, microinfarcts and degree of atherosclerosis in the circle of Willis (37). Taken together, these autopsy studies suggest that the e4 allele largely works through markers of AD pathology.

The predominantly direct effect of e4 carriership observed here supports these observations. However, unlike these studies, we found some evidence of mediation of the e4 effect by markers of CVD. This is likely partially explained by differences between our study and the autopsy cohorts in the markers of CVD used. While autopsy studies have largely assessed mediation by infarct measures, we additionally were able to use WML volume and CMBs, which have been more consistently associated with e4 carriership than infarct measures. As the magnitude of the e4 effect that is mediated by a particular CVD marker partially depends on the strength of the e4-
marker association, analyses investigating markers that are more strongly associated with e4 carriership will estimate larger mediated effects. A recent systematic review of neuroimaging studies found no association between e4 carriership and (mostly lacunar) brain infarcts [pooled OR = 1.03, 95% CI = (0.90, 1.18)] (24). The findings of no mediation of the e4 effect by infarct measures in both our study and the autopsy studies are consistent with this pattern of associations. The review also noted that e4 carriership was associated with higher WML burden [pooled standardized mean difference (SMD) = 0.047 (0.0006, 0.094)], and greater occurrence of CMBs [pooled OR = 1.24 (1.07, 1.43)], and especially lobar microbleeds (pooled OR = 1.34 (1.09, 1.64), with larger associations among e4 homozygotes (WML pooled SMD = 0.41 (0.17, 0.65); CMBs pooled OR = 1.87 (1.26, 2.78)). Our finding of partial mediation of the e4 effect by WML volume and CMBs, and larger mediated effects when separating out e4 homozygotes, are therefore consistent with these associations.

CMBs and WML volume are two key markers of cerebral small vessel disease (CSVD) (49), which is thought to result primarily from two underlying pathologies: arteriosclerosis and cerebral amyloid angiopathy (CAA) (50). While WMLs are a sign of generalized vascular damage and can occur as a consequence of either of these pathologies, epidemiologic and imaging evidence suggests that microbleeds in lobar areas are strongly suggestive of CAA (51, 52). The e4 allele is a well-established risk factor for lobar microbleeds and CAA in both cognitively normal and AD populations (24, 46, 53, 54). Our finding that the effect on cognition of e4 homozygosity was partially mediated by CMBs, and particularly lobar microbleeds, is therefore consistent with a potential CAA-mediated effect. While it is somewhat surprising that this effect was noticeable only in comparisons separating out e4 homozygotes, other studies have also reported lobar microbleeds or severe CAA to be increased most markedly among e4 homozygotes (34, 54-56). Nonetheless, such a mediating role of CAA is consistent with the known effects of e4 on amyloid deposition in cerebral vasculature, and provides support for the contention that cerebral CMBs and CAA may be a link between the neurodegenerative and vascular pathologies observed in Alzheimer’s disease (51). At a more speculative level, it raises the possibility that CAA pathology might account for some of the substantial difference in impact on AD risk and onset age between one and two copies of the e4 allele.

The remainder of the mediated effect was attributable to lower TBP volume, an indicator of brain atrophy. Brain atrophy on neuroimaging may be a consequence of either vascular or neurodegenerative pathologies. In one autopsy study, the effect of microinfarcts on cognition before death was found to be mediated by total brain weight,
particularly among non-demented individuals (57). Although the relationship between e4 status and microinfarcts is unclear, it may be that some of the TBP volume-mediated e4 effect we observed reflects the downstream effect of cerebrovascular disease (58). On the other hand, brain atrophy also correlates well with degree of neurofibrillary tangle pathology (59), and the e4 effect mediated by TBP volume likely reflects to a substantial extent the effect of AD pathology on cognition. Consequently, the effect jointly mediated by WML volume and CMBs together likely more closely reflects the CVD-mediated effect of e4 carriership, while the effect jointly mediated by these two markers and TBP volume together may provide an upper bound for this CVD-mediated effect.

Population averages of cognitive scores among older individuals reflect longstanding individual differences in cognitive performance and the more recent effects of brain pathologies in a subset of individuals. In a sample such as this one in which most individuals are cognitively normal or only mildly impaired, the estimated total effect of even a strong risk factor such as e4 carriership on cognition will be small. In all mediation analyses, valid estimation of direct and indirect effects requires that strong unmeasured confounding assumptions be met. As AGES-Reykjavik is conducted in an ethnically homogenous population, it is unlikely that ethnicity-related genes will result in major confounding of the e4-MRI marker or e4-cognition relationships. To minimize confounding of the effect of MRI markers on cognition, we compared our results under adjustment for a range of demographic and clinical variables, and found little change in our results. While small effect sizes are typically less robust to confounding, our sensitivity analysis suggests that given the expected direction of such confounding, the indirect effects reported here may underestimate the true mediated effect.

Our study has a number of important strengths. There have thus far been no population-based studies of this research question using antemortem MRI, which allows for in vivo characterization of markers of CVD pathology in a much larger sample than has been available in previous autopsy studies. The availability of neuroimaging also allowed us to capture WML volume and CMBs, aspects of CVD pathology that are not easily measurable in autopsy studies. The large sample size available additionally allowed for e4 homozygotes to be separated from e4 heterozygotes, and for modelling multiple mediators jointly, which would have been impossible in smaller studies (47).

In conclusion, our results show that a portion of the e4 effect on cognition is mediated by MRI markers of CVD pathology. While our finding that the e4 effect largely operates through non-vascular pathways aligns with
previous research and present understanding of the action of apoE in AD and CVD pathogenesis, it is the first to show an effect specifically via markers of CVD pathology. Also of interest in our findings is that the effect of e4 homozygosity on cognition was more substantially mediated by CMBs, particularly lobar microbleeds, perhaps suggesting a greater vascular contribution for these individuals. Similar analyses in other population-based studies will be needed to confirm these findings.

Research on AD biomarkers has inspired an influential theoretical model of AD disease pathogenesis, which, at present, largely focuses on pure AD (60, 61). However, it is becoming clear that AD and CVD share vascular risk factors, and that concurrent CVD pathologies contribute to cognitive impairment in AD patients (10). As CVD pathologies influencing cognition may develop at differing rates on different time scales, their associations with AD dementia risk factors and cognition -- and quantities dependent on these associations such as the mediated effects estimated here - may similarly vary depending on the distribution of disease stages of individuals in a particular study population. Consequently, studies capturing individuals across the clinical spectrum are needed to help us better understand the relationships between established risk factors, CVD pathologies and cognitive impairment, and how these change over the course of the disease. Such research will also inform broader pathogenic models that incorporate both CVD and AD markers and more completely characterize the AD disease process.
Appendix: Sensitivity analysis

Sensitivity analysis approach

The counterfactual approach to mediation analysis emphasizes the importance of adjustment for confounders of the relationship between the mediator and outcome when estimating the direct and indirect effects of an exposure on an outcome. In our analysis, we adjusted for several potential confounders of the effect of MRI markers on cognition, without any substantive change in our estimates of direct and indirect effects of e4 carriersonship on cognition. Nonetheless, these effect estimates will still be biased if there are unmeasured confounders of the relationship between MRI markers and cognition.

One way to assess robustness of these findings to unmeasured confounding is through formal sensitivity analyses that calculate how much estimated direct and indirect effects would be expected to change under different degrees of mediator-outcome confounding. Specifically, given a hypothetical unmeasured confounder of the mediator-outcome relationship, U, with particular associations with the outcome and the exposure, what would the direct and indirect effect estimates be if we were able to also adjust for U? What magnitude of confounding by U would be required for the observed indirect effect to become zero upon adjustment for U?

When U is a binary variable, these calculations can be made using a simple approach described in VanderWeele (62). Using this approach requires specification of two parameters: (i) the magnitude of the association between U and cognition, conditional on APOE e4, the individual MRI marker and the covariates, $b_1$, and (ii) the difference in prevalence of U between e4 carriers and e4 non-carriers, conditional on the MRI marker and other covariates, $b_2$. The bias due to confounding by U is given by the product of these two quantities: $BF = b_1 \times b_2$. The bias-corrected direct effect estimate, $DE_{|U}$ is given by subtracting this bias factor from the unadjusted for U estimate of the direct effect, $DE$, so that $DE_{|U} = DE - BF$, while the bias-corrected indirect effect, $IE_{|U}$ is given by adding the bias factor to the unadjusted for U estimate of the indirect effect, $IE$, so that: $IE_{|U} = IE + BF$. These simple expressions for the bias-corrected estimates require that the U-cognition association represented by $b_1$ is constant across strata of APOE e4 and covariates, conditional on the MRI marker, and that the U-APOE e4 association $b_2$ is constant across strata of the covariates, conditional on the MRI marker.
To capture different degrees of confounding, we specified a range of plausible values for \( b_1 \), and a range of prevalence differences for \( b_2 \). The first parameter, \( b_1 \), represents the strength of the association between \( U \) and cognition. Here, we propose three values for \( b_1 \), that roughly represent “small” (\( b_1 = \pm 0.05 \)), “moderate” (\( b_1 = \pm 0.10 \)) and “large” effects of \( U \) on cognition (\( b_1 = \pm 0.20 \)). The second parameter, \( b_2 \), indexes different strengths of the association between \( U \) and e4 carrierhip. Here, we allow \( b_2 \) to range between -0.5 (i.e., \( U \) is 50 percentage points less common among e4 carriers) to +0.5 (\( U \) is 50 percentage points more common among e4 carriers). Based on the degree of imbalance of \( U \) between e4 carriers and e4 non-carriers, we consider prevalence difference within this broad range to have “small” (\( b_2 = -0.10 \) to -0.01 or \( b_2 = +0.01 \) to +0.10), “moderate” (\( b_2 = -0.20 \) to -0.11, or \( b_2 = 0.11 \) to 0.20) or “large” (\( b_2 <= -0.20 \), or \( b_2 >= 0.20 \)) potential for confounding.

Below, we use the bias formula with these values of \( b_1 \) and \( b_2 \) to illustrate how the results obtained for the analysis of e4 carrierhip as the exposure, WML volume as the mediator and global cognition as the outcome reported in the main text would change under different degrees of confounding.

**Sensitivity analysis results**

In the analysis with e4 carrierhip as the exposure, WML volume as the mediator and global cognition as the outcome reported in the main text, the unadjusted for \( U \) direct effect estimate, \( DE \), was -0.08 (95% CI: (-0.12, -0.04)), while the unadjusted for \( U \) indirect effect estimate, \( IE \), was -0.008 (-0.01, -0.0005)]. The values that the bias-corrected direct, \( DE_U \), and indirect effect, \( IE_U \), would take under different values of \( b_1 \) and \( b_2 \) are shown in Appendix Table 2.1 and Appendix Table 2.2, respectively.

From Appendix Table 2.1, we can see that, if our hypothetical \( U \) were associated with worse global cognition and more prevalent among e4 carriers (\( b_1 < 0 \) and \( b_2 > 0 \)), or was associated with better global cognition and more prevalent among e4 non-carriers (\( b_1 > 0 \) and \( b_2 < 0 \)), then the unadjusted for \( U \) estimate of the direct effect (\( DE = -0.08 \)) would overestimate the true direct effect (\( DE_U > -0.08 \)), and the unadjusted for \( U \) estimate of the indirect effect (\( IE = -0.008 \)) would underestimate the true indirect effect (\( IE_U < -0.008 \)). In these scenarios, the degree of mediation of the e4 effect by WML volume would be underestimated without adjustment for \( U \).

Contrastingly, if \( U \) was associated with worse global cognition and more prevalent among e4 non-carriers (\( b_1 < 0 \) and \( b_2 < 0 \)), or was associated with better global cognition and more prevalent among e4 carriers (\( b_1 > 0 \) and \( b_2 > 0 \))
>0), then the unadjusted for U estimate of the direct effect (DE = -0.08) would underestimate the true direct effect (i.e., $DE_U < -0.08$), while the unadjusted for U estimate of the indirect effect (IE = -0.008) would overestimate the true indirect effect (i.e., $(IE_U > -0.008$). In these scenarios, the degree of mediation would be overestimated without adjustment for U.

Given how small the indirect effect is (-0.008), it is more plausible that unmeasured confounding by U could be made null by confounding. However, as explained above the confounding would need to be in the direction opposite to what would be expected – i.e., a harmful U would need to be common among e4 non-carriers, or a protective U would need to be more common among e4 carriers. If U was associated with better cognition such that the mean cognition for those with U = 1 was 0.05 units higher than for those with U = 0, the prevalence of U would need to be 15.5% percentage points greater among e4 carriers, for the true indirect effect to become zero.
Appendix Table 2.1: How a direct effect estimate of -0.08 would change under different magnitudes of confounding by a binary unmeasured variable U

<table>
<thead>
<tr>
<th>U associated with worse global cognition (b₁ &lt; 0)</th>
<th>U more prevalent among e4 non-carriers (b₂ &lt; 0)</th>
<th>U more prevalent among e4 carriers (b₂ &gt; 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.20</td>
<td>-0.18, -0.16, -0.14, -0.12, -0.10</td>
<td>-0.06, -0.04, -0.02, 0.00, 0.02</td>
</tr>
<tr>
<td>-0.10</td>
<td>-0.13, -0.12, -0.11, -0.10, -0.09</td>
<td>-0.07, -0.06, -0.05, -0.04, -0.03</td>
</tr>
<tr>
<td>-0.05</td>
<td>-0.10, -0.10, -0.09, -0.09, -0.08</td>
<td>-0.07, -0.07, -0.06, -0.06, -0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>U associated with better global cognition (b₁ &gt; 0)</th>
<th>U more prevalent among e4 non-carriers (b₂ &lt; 0)</th>
<th>U more prevalent among e4 carriers (b₂ &gt; 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>-0.05, -0.06, -0.06, -0.07, -0.07</td>
<td>-0.08, -0.09, -0.09, -0.10, -0.10</td>
</tr>
<tr>
<td>0.10</td>
<td>-0.03, -0.04, -0.05, -0.06, -0.07</td>
<td>-0.09, -0.10, -0.11, -0.12, -0.13</td>
</tr>
<tr>
<td>0.20</td>
<td>0.02, 0.00, -0.02, -0.04, -0.06</td>
<td>-0.10, -0.12, -0.14, -0.16, -0.18</td>
</tr>
</tbody>
</table>

In this table, b₁ and b₂ are indices for the strength of confounding by U: b₁ indexes strength of the association between U and global cognition, while b₂ indexes difference in prevalence of U between e4 carriers and e4 non-carriers. If b₁ or b₂ = 0, there is no confounding by U, and the bias-corrected estimate of the direct effect will also be -0.08.

Appendix Table 2.2: How an indirect effect estimate of -0.008 would change under different magnitudes of confounding by a binary unmeasured variable U.

<table>
<thead>
<tr>
<th>U associated with worse global cognition (b₁ &lt; 0)</th>
<th>U more prevalent among e4 non-carriers (b₂ &lt; 0)</th>
<th>U more prevalent among e4 carriers (b₂ &gt; 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.20</td>
<td>0.092, 0.072, 0.052, 0.032, 0.012</td>
<td>-0.028, -0.048, -0.068, -0.088, -0.108</td>
</tr>
<tr>
<td>-0.10</td>
<td>0.042, 0.032, 0.022, 0.012, 0.002</td>
<td>-0.018, -0.028, -0.038, -0.048, -0.058</td>
</tr>
<tr>
<td>-0.05</td>
<td>0.017, 0.012, 0.007, 0.002, -0.003</td>
<td>-0.013, -0.018, -0.023, -0.028, -0.033</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>U associated with better global cognition (b₁ &gt; 0)</th>
<th>U more prevalent among e4 non-carriers (b₂ &lt; 0)</th>
<th>U more prevalent among e4 carriers (b₂ &gt; 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>-0.033, -0.028, -0.023, -0.018, -0.013</td>
<td>-0.003, 0.002, 0.007, 0.012, 0.017</td>
</tr>
<tr>
<td>0.10</td>
<td>-0.058, -0.048, -0.038, -0.028, -0.018</td>
<td>0.002, 0.012, 0.022, 0.032, 0.042</td>
</tr>
<tr>
<td>0.20</td>
<td>-0.108, -0.088, -0.068, -0.048, -0.028</td>
<td>0.012, 0.032, 0.052, 0.072, 0.092</td>
</tr>
</tbody>
</table>

In this table, b₁ and b₂ are indices for the strength of confounding by U: b₁ indexes strength of the association between U and global cognition, while b₂ indexes difference in prevalence of U between e4 carriers and e4 non-carriers. If b₁ or b₂ = 0, there is no confounding by U, and the bias-corrected estimate of the indirect effect will also be -0.008.
### Supplementary Tables

**Supplementary Table 2.1: Total, direct and indirect effects in individual mediator analyses, comparing across number of e4 alleles for global cognition**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3957</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.006 (-0.012, 0.001)</td>
<td>9%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2979</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>-0.21 (-0.34, -0.07)</td>
<td>-0.034 (-0.068, 0.010)</td>
<td>14%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1144</td>
<td>-0.16 (-0.31, -0.02)</td>
<td>-0.14 (-0.27, 0.01)</td>
<td>-0.021 (-0.051, 0.022)</td>
<td>13%</td>
</tr>
<tr>
<td><strong>Subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3958</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.002 (-0.007, 0.004)</td>
<td>1%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2980</td>
<td>-0.24 (-0.38, -0.11)</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>0.001 (-0.022, 0.026)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1144</td>
<td>-0.15 (-0.30, -0.01)</td>
<td>-0.16 (-0.30, -0.02)</td>
<td>0.008 (-0.020, 0.028)</td>
<td>-4%</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3958</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>0.000 (-0.006, 0.005)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2980</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>-0.23 (-0.37, -0.10)</td>
<td>-0.001 (-0.021, 0.019)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1144</td>
<td>-0.16 (-0.30, 0.00)</td>
<td>-0.15 (-0.30, 0.01)</td>
<td>-0.002 (-0.021, 0.019)</td>
<td>1%</td>
</tr>
<tr>
<td><strong>TBP volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3957</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>-0.06 (-0.10, -0.02)</td>
<td>-0.014 (-0.024, -0.002)</td>
<td>19%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2979</td>
<td>-0.25 (-0.40, -0.11)</td>
<td>-0.26 (-0.41, -0.13)</td>
<td>0.001 (-0.046, 0.058)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1144</td>
<td>-0.17 (-0.31, -0.02)</td>
<td>-0.20 (-0.35, -0.06)</td>
<td>0.028 (-0.024, 0.082)</td>
<td>16%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for global cognition.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Supplementary Table 2.2: Total, direct and indirect effects in individual mediator analyses, comparing across number of e4 alleles for memory

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4052</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.09 (-0.14, -0.04)</td>
<td>-0.08 (-0.14, 0.000)</td>
<td>8%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3057</td>
<td>-0.24 (-0.41, -0.06)</td>
<td>-0.21 (-0.37, -0.04)</td>
<td>-0.031 (-0.064, 0.026)</td>
<td>13%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1163</td>
<td>-0.14 (-0.32, 0.04)</td>
<td>-0.12 (-0.30, 0.05)</td>
<td>-0.018 (-0.048, 0.026)</td>
<td>13%</td>
</tr>
<tr>
<td><strong>Subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4053</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.002 (-0.008, 0.006)</td>
<td>2%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3058</td>
<td>-0.24 (-0.38, -0.11)</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>0.003 (-0.040, 0.038)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1163</td>
<td>-0.15 (-0.30, -0.01)</td>
<td>-0.16 (-0.30, -0.02)</td>
<td>0.013 (-0.027, 0.046)</td>
<td>-9%</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4053</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>0.000 (-0.005, 0.004)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3058</td>
<td>-0.23 (-0.43, -0.05)</td>
<td>-0.23 (-0.43, -0.05)</td>
<td>0.000 (-0.019, 0.017)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1163</td>
<td>-0.14 (-0.32, 0.05)</td>
<td>-0.14 (-0.32, 0.06)</td>
<td>0.000 (-0.022, 0.028)</td>
<td>0%</td>
</tr>
<tr>
<td><strong>TBP volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4052</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.015 (-0.026, -0.003)</td>
<td>15%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3057</td>
<td>-0.26 (-0.43, -0.08)</td>
<td>-0.26 (-0.43, -0.08)</td>
<td>0.002 (-0.044, 0.056)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1163</td>
<td>-0.15 (-0.34, -0.03)</td>
<td>-0.18 (-0.37, -0.06)</td>
<td>0.029 (-0.027, 0.087)</td>
<td>-19%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for memory.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Supplementary Table 2.3: Total, direct and indirect effects in individual mediator analyses, comparing across number of e4 alleles for executive function

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4196</td>
<td>-0.05 (-0.09, -0.01)</td>
<td>-0.05 (-0.09, 0.00)</td>
<td>-0.007 (-0.012, 0.001)</td>
<td>13%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3163</td>
<td>-0.21 (-0.36, -0.05)</td>
<td>-0.17 (-0.33, -0.02)</td>
<td>-0.031 (-0.064, 0.015)</td>
<td>15%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1207</td>
<td>-0.15 (-0.29, 0.00)</td>
<td>-0.13 (-0.28, 0.02)</td>
<td>-0.019 (-0.046, 0.021)</td>
<td>13%</td>
</tr>
<tr>
<td><strong>Subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4197</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.001 (-0.004, 0.003)</td>
<td>2%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3164</td>
<td>-0.24 (-0.38, -0.11)</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>-0.002 (-0.026, 0.032)</td>
<td>1%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1207</td>
<td>-0.15 (-0.30, -0.01)</td>
<td>-0.16 (-0.30, -0.02)</td>
<td>0.004 (-0.025, 0.029)</td>
<td>-3%</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4197</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>0.000 (-0.004, 0.004)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3164</td>
<td>-0.20 (-0.34, -0.04)</td>
<td>-0.19 (-0.34, -0.04)</td>
<td>-0.004 (-0.020, 0.018)</td>
<td>2%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1207</td>
<td>-0.15 (-0.29, 0.01)</td>
<td>-0.14 (-0.29, 0.02)</td>
<td>-0.006 (-0.022, 0.019)</td>
<td>4%</td>
</tr>
<tr>
<td><strong>TBP volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4196</td>
<td>-0.05 (-0.09, -0.01)</td>
<td>-0.04 (-0.08, 0.00)</td>
<td>-0.010 (-0.018, -0.001)</td>
<td>20%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3163</td>
<td>-0.22 (-0.39, -0.05)</td>
<td>-0.22 (-0.38, -0.05)</td>
<td>-0.004 (-0.045, 0.042)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1207</td>
<td>-0.16 (-0.30, 0.00)</td>
<td>-0.18 (-0.33, -0.02)</td>
<td>0.018 (-0.026, 0.061)</td>
<td>-11%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for executive function.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
**Supplementary Table 2.4: Total, direct and indirect effects in individual mediator analyses, comparing across number of e4 alleles for processing speed**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WML volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4163</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.07 (-0.12, -0.02)</td>
<td>-0.007 (-0.013, 0.001)</td>
<td>10%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3138</td>
<td>-0.27 (-0.43, -0.10)</td>
<td>-0.23 (-0.38, -0.07)</td>
<td>-0.043 (-0.088, 0.016)</td>
<td>16%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1199</td>
<td>-0.17 (-0.34, 0.01)</td>
<td>-0.15 (-0.30, 0.04)</td>
<td>-0.028 (-0.063, 0.022)</td>
<td>16%</td>
</tr>
<tr>
<td><strong>Subcortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4164</td>
<td>-0.07 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.002 (-0.007, 0.004)</td>
<td>2%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3139</td>
<td>-0.24 (-0.38, -0.11)</td>
<td>-0.24 (-0.38, -0.10)</td>
<td>-0.001 (-0.015, 0.018)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1199</td>
<td>-0.15 (-0.30, -0.01)</td>
<td>-0.16 (-0.30, -0.02)</td>
<td>0.003 (-0.015, 0.017)</td>
<td>-2%</td>
</tr>
<tr>
<td><strong>Cortical infarcts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4197</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.08 (-0.12, -0.03)</td>
<td>0.000 (-0.007, 0.006)</td>
<td>0%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3164</td>
<td>-0.26 (-0.42, -0.10)</td>
<td>-0.26 (-0.41, -0.09)</td>
<td>-0.004 (-0.023, 0.024)</td>
<td>1%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1207</td>
<td>-0.17 (-0.33, 0.01)</td>
<td>-0.16 (-0.32, 0.02)</td>
<td>-0.005 (-0.024, 0.031)</td>
<td>3%</td>
</tr>
<tr>
<td><strong>TBP volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4163</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.06 (-0.11, -0.02)</td>
<td>-0.016 (-0.027, -0.003)</td>
<td>21%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3138</td>
<td>-0.29 (-0.46, -0.11)</td>
<td>-0.28 (-0.45, -0.13)</td>
<td>-0.005 (-0.060, 0.070)</td>
<td>2%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1199</td>
<td>-0.19 (-0.36, 0.01)</td>
<td>-0.21 (-0.39, -0.03)</td>
<td>0.026 (-0.032, 0.087)</td>
<td>-14%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for processing speed.
All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Supplementary Table 2.5: Total, direct and indirect effects in multiple mediator analyses when WML volume and CMBs are used as mediators, comparing across number of e4 alleles

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3942</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>-0.07 (-0.11, -0.03)</td>
<td>-0.007 (-0.012, 0.000)</td>
<td>9%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2968</td>
<td>-0.23 (-0.38, -0.08)</td>
<td>-0.16 (-0.30, -0.04)</td>
<td>-0.068 (-0.118, 0.010)</td>
<td>29%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1138</td>
<td>-0.15 (-0.29, 0.00)</td>
<td>-0.09 (-0.23, 0.05)</td>
<td>-0.058 (-0.108, 0.014)</td>
<td>39%</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4037</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.09 (-0.15, -0.04)</td>
<td>-0.008 (-0.016, 0.002)</td>
<td>8%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3046</td>
<td>-0.22 (-0.41, -0.04)</td>
<td>-0.14 (-0.30, 0.04)</td>
<td>-0.081 (-0.144, 0.008)</td>
<td>37%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1157</td>
<td>-0.12 (-0.29, 0.06)</td>
<td>-0.05 (-0.22, 0.13)</td>
<td>-0.071 (-0.133, 0.014)</td>
<td>61%</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4180</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.05 (-0.09, 0.00)</td>
<td>-0.017 (-0.026, -0.005)</td>
<td>13%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3151</td>
<td>-0.20 (-0.35, -0.05)</td>
<td>-0.14 (-0.31, 0.03)</td>
<td>-0.063 (-0.138, 0.018)</td>
<td>29%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1201</td>
<td>-0.15 (-0.30, -0.01)</td>
<td>-0.10 (-0.26, 0.06)</td>
<td>-0.031 (-0.103, 0.045)</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Processing speed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4147</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.07 (-0.12, -0.02)</td>
<td>-0.008 (-0.014, 0.001)</td>
<td>10%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3126</td>
<td>-0.26 (-0.43, -0.09)</td>
<td>-0.20 (-0.34, -0.04)</td>
<td>-0.062 (-0.138, 0.030)</td>
<td>24%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1193</td>
<td>-0.16 (-0.34, 0.02)</td>
<td>-0.11 (-0.26, 0.05)</td>
<td>-0.052 (-0.116, 0.035)</td>
<td>32%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.

All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
Supplementary Table 2.6: Total, direct and indirect effects in multiple mediator analyses when WML volume, CMBs and TBP volume are used as mediators, comparing across number of e4 alleles

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Total effect (95% CI)</th>
<th>Direct effect (95% CI)</th>
<th>Indirect effect (95% CI)</th>
<th>Proportion mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global cognition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>3942</td>
<td>-0.08 (-0.11, -0.04)</td>
<td>-0.06 (-0.09, -0.02)</td>
<td>-0.021 (-0.032, -0.008)</td>
<td>27%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>2968</td>
<td>-0.25 (-0.40, -0.11)</td>
<td>-0.18 (-0.33, -0.05)</td>
<td>-0.071 (-0.143, 0.015)</td>
<td>28%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1138</td>
<td>-0.16 (-0.32, -0.02)</td>
<td>-0.13 (-0.27, 0.01)</td>
<td>-0.037 (-0.116, 0.056)</td>
<td>23%</td>
</tr>
<tr>
<td><strong>Memory</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4037</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.023 (-0.037, -0.008)</td>
<td>23%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3046</td>
<td>-0.24 (-0.44, -0.06)</td>
<td>-0.16 (-0.34, 0.01)</td>
<td>-0.084 (-0.177, 0.029)</td>
<td>35%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1157</td>
<td>-0.13 (-0.32, 0.05)</td>
<td>-0.08 (-0.27, 0.09)</td>
<td>-0.049 (-0.142, 0.071)</td>
<td>37%</td>
</tr>
<tr>
<td><strong>Executive function</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4180</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.04 (-0.08, 0.01)</td>
<td>-0.017 (-0.026, -0.005)</td>
<td>31%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3151</td>
<td>-0.22 (-0.39, -0.05)</td>
<td>-0.16 (-0.33, 0.02)</td>
<td>-0.063 (-0.138, 0.018)</td>
<td>29%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1201</td>
<td>-0.16 (-0.33, -0.006)</td>
<td>-0.13 (-0.32, 0.03)</td>
<td>-0.031 (-0.103, 0.045)</td>
<td>19%</td>
</tr>
<tr>
<td><strong>Processing speed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1e4 v. 0e4</td>
<td>4147</td>
<td>-0.08 (-0.13, -0.03)</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>-0.024 (-0.037, -0.009)</td>
<td>30%</td>
</tr>
<tr>
<td>2e4 v. 0e4</td>
<td>3126</td>
<td>-0.28 (-0.46, -0.11)</td>
<td>-0.21 (-0.37, -0.06)</td>
<td>-0.070 (-0.158, 0.036)</td>
<td>25%</td>
</tr>
<tr>
<td>2e4 v. 1e4</td>
<td>1193</td>
<td>-0.18 (-0.37, 0.01)</td>
<td>-0.15 (-0.32, 0.01)</td>
<td>-0.031 (-0.119, 0.069)</td>
<td>17%</td>
</tr>
</tbody>
</table>

Values for total, direct and indirect effects indicate changes in z-score for each respective domain.

All models adjusted for age, sex, education, smoking status, diabetes diagnosis; and mid-life measures of body mass index, systolic blood pressure, total cholesterol and physical activity.
References


Chapter 3

Apolipoprotein e4 genotype, cerebral microbleeds and memory: an illustration of the four-way decomposition of a total effect

Gautam Sajeev, Tyler J. VanderWeele, Anand Viswanathan, Sigurdur Sigurdsson, Gudny Eiriksdottir, Thor Aspelund, Rebecca A. Betensky, Francine Grodstein, Vilmundur Gudnason,
Lenore J. Launer, and Deborah Blacker
Abstract

Objective: The most well-established genetic risk factor for poor late-life cognition and dementia due to Alzheimer’s disease is the e4 allele of the apolipoprotein gene. One mechanism by which the e4 allele may influence cognition is through effects involving markers of cerebrovascular disease, such as cerebral microbleeds (CMBs). We investigated the extent to which the e4 effect on memory is mediated by or involves interaction with CMBs.

Methods: This study included 4121 participants in the population-based Age, Gene/Environment Susceptibility–Reykjavik Study. CMBs were ascertained from T2*-weighted magnetic resonance images by trained raters. Composite z-scores for memory were derived from neuropsychological tests. We used a regression-based approach to decompose the total e4 effect on memory into components due to mediation, interaction, both, or neither.

Results: Compared to e4 non-carriers, APOE e4 homozygotes but not e4 heterozygotes had greater occurrence of CMBs, particularly in lobar areas. When comparing e4 heterozygotes to e4 non-carriers, the e4 effect on memory was entirely independent of CMBs. By contrast, when comparing e4 homozygotes to e4 heterozygotes, the entirety of the e4 effect on memory was attributable to interaction between the effects of e4 alleles and CMBs, with about half of this interactive effect involving CMBs arising independently of e4 alleles, and about half involving CMBs arising as a consequence of e4 alleles.

Interpretation: These results tentatively suggest that different mechanisms may underlie the effect on memory of e4 homozygosity versus e4 heterozygosity. As lobar CMBs are characteristic of cerebral amyloid angiopathy, these findings suggest that CAA may play a role specifically in the effect of e4 homozygosity, while mechanisms unrelated to CMBs may underlie the effect of e4 heterozygosity. Similar analyses in other cohorts should seek to further elucidate the contributions of CMBs to the e4 effect on cognition.
Introduction

The most well-established genetic risk factor for poor late-life cognition and Alzheimer’s disease is the e4 allele of the apolipoprotein E (APOE) gene (1). One pathway by which the e4 allele may influence cognition in late-life is through effects on cerebrovascular disease (CVD). Cerebral microbleeds (CMBs) are a neuroimaging marker of CVD indicating leakage of blood from small vessels in the brain (2). CMBs are frequently observed in cerebral amyloid angiopathy (CAA) (3), and are associated with worse late-life cognitive performance (4). The e4 allele is associated with greater prevalence and incidence of CMBs (5, 6), and this association appears to be stronger for CMBs in lobar areas (7). Thus far, there have been no studies of mediation of the e4 effect by CMBs.

Methodologies for mediation and interaction analysis in epidemiology have expanded substantially over the last few years (8, 9). Recently, VanderWeele showed that when considering a single exposure and a mediator with which the exposure may also interact, the total effect of an exposure on an outcome can be decomposed into four distinct components: the effect due to neither mediation nor interaction, the effect due to mediation alone, the effect due to interaction alone, and the effect due to both mediation and interaction (10).

In this brief report, we illustrate the use of this four-way decomposition by quantifying how much of the effect of the e4 allele on memory is attributable to mediation and interaction involving CMBs. This analysis, with a specific focus on the four-way decomposition (one of the first ever applications of this technique), arose out of a broader study of mediation of the e4 effect by several CVD markers, which is reported in more detail elsewhere (Chapter 2).
Methods

Study population and analytic sample

The Age, Gene/Environment, Susceptibility-Reykjavik Study (AGES-Reykjavik) is a cohort study focusing on vascular, neurocognitive, musculoskeletal and metabolic disorders (11). Between 2002 and 2006, AGES-Reykjavik enrolled 5764 survivors of the population-based Reykjavik Study, an investigation of cardiovascular disease that began in 1967 and followed a random sample of 30,795 residents born between 1907 and 1935. AGES-Reykjavik participants completed a structured questionnaire, clinical examinations, imaging protocols, and provided laboratory samples for DNA and biomarker analysis in this study. Data obtained during Reykjavik Study assessments were used as mid-life measures. This analysis focuses on the baseline assessment of the AGES-Reykjavik study. The analytic sample was restricted to 4121 individuals with complete data on APOE genotype, CMBs, memory and covariates as described below.

Assessment of APOE, cerebral microbleeds and memory

APOE genotyping was done via microplate array diagonal gel electrophoresis. CMBs were defined as focal hypointense lesions and identified as present on T2*-weighted gradient-echo type sequence MR images by a neuroradiologist, and their number and location were characterized by trained raters (12). In our analyses, we categorized CMBs as present or absent, because most participants did not have microbleeds. All participants received a standard neuropsychological test battery. Z-scores were obtained for each individual test and averaged across different combinations of tests in order to get composite domain-specific scores for memory, executive function and processing speed. The composite memory score was based on the California Verbal Learning Test (immediate and delayed recall) and was used as the outcome measure here.

Components of the four-way decomposition

The total effect of e4 exposure is the difference in memory z-score between an e4 exposure level and an e4 reference level. This total effect can be decomposed into four non-overlapping components: the controlled direct effect, the pure indirect effect, the reference interaction, and the mediated interaction (10).
The controlled direct effect (CDE) represents the effect of e4 exposure on cognition that is independent of CMBs (i.e. the effect that would remain if there were no CMBs). The remaining three components represent portions of the e4 effect that involve CMBs, either as a variable that mediates the e4 effect, as a variable with which e4 interacts, or as a variable that both interacts with e4 and mediates the e4 effect on cognition. The pure indirect effect (PIE) represents the portion of the e4 effect on memory that arises due to the effect on memory of e4-induced CMBs.

There will be interaction on the additive scale between the effects of e4 and CMBs on cognition when the effect on cognition of e4 and CMBs together is different than the sum of the effect of e4 alone and the effect of CMBs alone ($INT_{add} \neq 0$). The two interaction components are functions of this additive interaction measure. The reference interaction ($INT_{ref}$) represents the portion of the e4 effect on cognition that requires the joint presence of CMBs, with CMBs arising independently of e4 exposure. The mediated interaction ($INT_{med}$) represents the portion of the e4 effect on cognition that requires the joint presence of CMBs, with CMBs arising as a consequence of e4 exposure.

These four components are related to the standard two-way decomposition used in mediation analyses in the following way: The sum of the mediated interaction and pure indirect effect give the overall mediated effect (also called the natural indirect effect, total indirect effect or just the indirect effect), while the sum of the controlled direct effect and the reference interaction gives the overall direct effect (also called the natural direct effect, pure direct effect or just the direct effect) (13, 14).

Mediation assumptions

For the empirical estimates of the four components obtained in our analysis to have the causal interpretations described above, several assumptions are required. We require that conditional on covariates there be no unmeasured confounding of the effects of (i) e4 on memory, (ii) e4 on CMBs, and (iii) CMBs on memory, and that (iv) none of the CMB-memory confounders themselves be affected by the e4 alleles. We addressed assumptions (i) and (ii) by conducting our study in the ethnically homogenous AGES-Reykjavik cohort, which protects against confounding due to population stratification. For (iii), we adjusted for potential confounders of the relationship between CMBs and cognition using available data on demographic variables and risk factors for CVD. In the
analyses, we adjusted for age, sex, education, diabetes, smoking status, and midlife measures of physical activity, body mass index, systolic blood pressure, and total cholesterol.

**Statistical analysis**

We used a regression-based approach to obtain estimates of the four components. First, we regressed CMBs on e4 status and the covariates using logistic regression. We then regressed the memory z-score on e4 carriership, CMBs and a product term denoting the interaction between e4 status and CMBs using linear regression. In all models, we adjusted for the covariates listed above. We obtained estimates of the four components by combining parameters from these two models according to the analytic expressions provided by VanderWeele, and obtained confidence intervals for the effect estimates by repeating the process for 1000 bootstrap samples. In the main text, we present results comparing (i) e4 heterozygotes to e4 non-carriers (i.e. 1 vs. 0 e4 allele), and (ii) e4 homozygotes to e4 heterozygotes (i.e. 2 vs. 1 e4 allele). Results for e4 carriers versus e4 non-carriers, and e4 homozygotes versus e4 non-carriers are shown in Supplementary Table 3.1.

**Results**

The characteristics of the analytic sample (n = 4121) are described in detail elsewhere (Chapter 2). Briefly, 57.8% of participants were female, and their mean age was 76.3 (SD = 5.4). CMBs were approximately twice as prevalent among e4 homozygotes (18/83 = 21.7%) than among e4 heterozygotes (128/1074 = 11.9%) and e4 non-carriers (333/2964 = 11.2%).

The results of the four-way decomposition are shown in Table 3.1. When comparing e4 heterozygotes to e4 non-carriers (i.e. 1 vs. 0 e4 allele), the total effect on memory was entirely attributable to the controlled direct effect component, i.e. the e4 effect is independent of CMBs in that the e4 allele has an effect on memory even without the presence of CMBs, and does not affect memory by changing CMBs. Comparisons of all e4 carriers to e4 non-carriers gave similar results (Supplementary Table 3.1).

Contrastingly, when comparing e4 homozygotes to e4 heterozygotes (i.e. 2 vs. 1 e4 allele), the total effect seemed to about half attributable to the reference interaction ($INT_{ref}=-0.06$ [95% CI: (-0.11, -0.02)] and half
attributable to the mediated interaction \((INT_{med} =-0.06 (-0.12, 0.02))\). Interaction thus contributed to almost all of the effect i.e. the effect for 2 vs. 1 e4 allele only operates when CMBs are present; when CMBs are present and there is an effect then in about half of the instances, the CMBs are brought about by the e4 alleles and in about half of the instances the CMBs are there independently of the e4 alleles.

Table 3.1: Four-way decomposition results, comparing e4 heterozygotes to e4 non-carriers, and e4 homozygotes to e4 heterozygotes

<table>
<thead>
<tr>
<th></th>
<th>e4 heterozygotes vs e4 non-carriers</th>
<th>e4 homozygotes vs e4 heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1 vs. 0 e4 alleles)</td>
<td>(2 vs. 1 e4 alleles)</td>
</tr>
<tr>
<td></td>
<td>(n = 4038)</td>
<td>(n = 1157)</td>
</tr>
<tr>
<td>Total Effect</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.12 (-0.29, 0.06)</td>
</tr>
<tr>
<td>4-way decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlled Direct Effect ((CDE))</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>0.01 (-0.18, 0.20)</td>
</tr>
<tr>
<td>Pure Indirect Effect ((PIE))</td>
<td>0.00 (-0.002, 0.002)</td>
<td>0.00 (-0.02, 0.02)</td>
</tr>
<tr>
<td>Reference Interaction ((INT_{ref}))</td>
<td>0.01 (-0.01, 0.02)</td>
<td>-0.06 (-0.11, -0.02)</td>
</tr>
<tr>
<td>Mediated Interaction ((INT_{med}))</td>
<td>0.00 (-0.003, 0.003)</td>
<td>-0.06 (-0.12, 0.02)</td>
</tr>
<tr>
<td>Proportion of effect due to each component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDE (neither mediation nor interaction)</td>
<td>106%</td>
<td>-7%</td>
</tr>
<tr>
<td>PIE (mediation alone)</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>INT_{ref} (interaction alone)</td>
<td>-6%</td>
<td>52%</td>
</tr>
<tr>
<td>INT_{med} (mediation and interaction)</td>
<td>0%</td>
<td>54%</td>
</tr>
<tr>
<td>2-way decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct Effect ((PDE))</td>
<td>-0.10 (-0.15, -0.05)</td>
<td>-0.05 (-0.23, 0.12)</td>
</tr>
<tr>
<td>Indirect Effect ((TIE))</td>
<td>0.00 (-0.002, 0.002)</td>
<td>-0.06 (-0.12, 0.02)</td>
</tr>
</tbody>
</table>

All models adjusted for age, sex, education, diabetes, smoking status, and midlife measures of physical activity, body mass index, systolic blood pressure, and total cholesterol.

In a standard analysis of interaction (Table 3.2), the interaction is substantial for the heterozygous comparison \((INT_{add2v1} = -0.57 (-1.01, -0.15))\), but small for the homozygous comparison \((INT_{add1v0} = 0.06 (-0.12, 0.23))\), again reinforcing the role of interaction only for the homozygous comparison.
Table 3.2: Interaction results, comparing e4 heterozygotes to e4 non-carriers, and e4 homozygotes to e4 heterozygotes

<table>
<thead>
<tr>
<th>Interaction results</th>
<th>e4 heterozygotes vs e4 non-carriers</th>
<th>e4 homozygotes vs e4 heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additive interaction measure ((INT_{add}))</td>
<td>0.06 (-0.12, 0.23)</td>
<td>-0.57 (-1.01, -0.15)</td>
</tr>
</tbody>
</table>

**Stratum-specific effect estimates**

<table>
<thead>
<tr>
<th></th>
<th>e4 heterozygotes vs e4 non-carriers</th>
<th>e4 homozygotes vs e4 heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference e4, no CMBs (ref)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exposed e4, no CMBs</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>0.01 (-0.19, 0.21)</td>
</tr>
<tr>
<td>Reference e4, CMBs</td>
<td>-0.07 (-0.16, 0.02)</td>
<td>-0.01 (-0.15, 0.14)</td>
</tr>
<tr>
<td>Exposed e4, CMBs</td>
<td>-0.12 (-0.26, 0.02)</td>
<td>-0.57 (-0.94, -0.20)</td>
</tr>
</tbody>
</table>

**CMB effect within e4 strata**

<table>
<thead>
<tr>
<th></th>
<th>e4 heterozygotes vs e4 non-carriers</th>
<th>e4 homozygotes vs e4 heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference e4</td>
<td>-0.07 (-0.16, -0.02)</td>
<td>-0.01 (-0.15, 0.14)</td>
</tr>
<tr>
<td>Exposed e4</td>
<td>-0.01 (-0.15, 0.13)</td>
<td>-0.58 (-1.00, -0.17)</td>
</tr>
</tbody>
</table>

**E4 exposure effect within CMB strata**

<table>
<thead>
<tr>
<th></th>
<th>e4 heterozygotes vs e4 non-carriers</th>
<th>e4 homozygotes vs e4 heterozygotes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CMBs</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>0.01 (-0.19, 0.21)</td>
</tr>
<tr>
<td>CMBs</td>
<td>-0.05 (-0.20, 0.11)</td>
<td>-0.57 (-0.96, -0.17)</td>
</tr>
</tbody>
</table>

All models adjusted for age, sex, education, diabetes, smoking status, and midlife measures of physical activity, body mass index, systolic blood pressure, and total cholesterol.

**Discussion**

In these data, the negative effect of the e4 allele on memory was entirely independent of CMBs for e4 heterozygosity (1 vs. 0 e4 allele), but with homozygosity (2 vs. 1 e4 allele), the effect was largely attributable to the joint presence of CMBs, arising either as a consequence of e4 homozygosity, or independently of the e4 alleles. The difference between the homozygous and heterozygous comparisons arises from (i) the greater prevalence of CMBs exclusively among e4 homozygotes and (ii) from differences in their respective additive interaction measures.

No other studies have specifically examined interaction of the effects of \(APOE\) e4 and CMBs on cognition. However, with respect to mediation, there is, in fact, evidence suggestive of CMB frequency being increased specifically, or at least more substantially, among e4 homozygotes. In the Rotterdam Scan Study, compared to e3 homozygotes, e4 homozygotes had a higher incidence of new CMBs [OR = 4.43 (1.44, 13.6)], while e4 carriers as a
whole did not [OR = 1.19 (0.69, 2.05)] (5). A recent review found CMBs to be more strongly associated with e4 homozygosity [summary OR = 1.87 (1.26, 2.78], than with e4 carriership [summary OR = 1.24 (1.07, 1.43)] (7). CMBs, particularly those in lobar areas, are indicative of advanced CAA, and the greater frequency of CMBs among e4 homozygotes in this study is largely due to greater frequency of lobar CMBs (Chapter 2). These results suggest that CAA-related mechanisms may play a role specifically in the effect of e4 homozygosity while other mechanisms may underlie the effect of e4 heterozygosity.

These conclusions are reliant on the strong assumptions required for mediation analyses. We make these assumptions more likely to be met by setting this study in the ethnically homogenous AGES-Reykjavik cohort, and adjusting for multiple demographic and clinical risk factors. However, given the small effect sizes, it is possible that moderate amounts of residual confounding could explain the indirect effects observed. While the results are largely driven by the small fraction of e4 homozygotes in the sample, the pattern was observed only for CMBs, and not for other CVD markers (Chapter 2). Replication of these interesting findings in other studies is needed.

AGES-Reykjavik offers the benefits of in vivo characterization of CMBs, and a population-based cohort that is substantially larger than the autopsy samples available previously. This allowed for investigation of effects specific to e4 homozygotes, an important strength given the relative rarity of this genotype.

The study also has interesting methodological implications. That mediated and interactive effects involving CMBs were observed in the pairwise genotype-specific comparisons, but not under a dominant model of inheritance for APOE e4 suggests that future studies might similarly benefit from examining genotype-specific comparisons when sample size permits. This may be especially important in in studies of mediation and interaction. Finally, these results also highlight the key advantage of the four-way decomposition: the ability to separate the two interaction components (interaction when the exposure does, versus does not, also change the mediator), which are otherwise subsumed under the direct and indirect effects.(10) The approach taken here may therefore be useful in a variety of settings in which environmental factors are suspected to mediate and/or modify genetic effects.
Appendix: The four-way decomposition

In order to define the components of the four-way decomposition, we utilize the counterfactual framework. Let $A$ be a binary variable denoting the exposure, $Y$ be a continuous variable denoting the outcome, and $C$ be a set of baseline covariates. Let $Y_a$ denote the counterfactual value of the outcome that would be observed had exposure $A$ been set to $a$. If $A = 1$ denotes exposure and $A = 0$ denotes lack of exposure, the average total effect of the exposure $A$ on the outcome $Y$, conditional on covariates $C$, is defined by $E[Y_1 | C] - E[Y_0 | C]$. This total effect is identifiable from observational data under the assumption of no unmeasured exposure-outcome confounding within levels of the covariates $C$.

A third variable, $M$ may be a potential mediator of interest. Let $M$ be a binary variable, with $M = 1$ denoting presence of the mediator and $M = 0$ denoting absence of the mediator. We can similarly define $M_a$ be the counterfactual value of the mediator that would have been observed had exposure $A$ been set to $a$. We also define $Y_{am}$ to refer to the counterfactual value of the outcome that would have been observed had exposure $A$ been set to $a$, and mediator $M$ been set to $m$.

Using this notation, it can be shown that the total exposure effect can be decomposed into four non-overlapping components. For a binary exposure $A$ and binary mediator $M$, this four-way decomposition is given by:

$$Y_1 - Y_0 = (Y_{10} - Y_{00}) + (Y_{0M1} - Y_{0M0}) + (Y_{11} - Y_{10} - Y_{01} + Y_{00})(M_0) + (Y_{11} - Y_{10} - Y_{01} + Y_{00})(M_1 - M_0)$$

The four components, in the order in which they appear in the decomposition above, are the controlled direct effect ($Y_{10} - Y_{00}$), the pure indirect effect ($Y_{0M1} - Y_{0M0}$), the reference interaction ($(Y_{11} - Y_{10} - Y_{01} + Y_{00})(M_0)$), and the mediated interaction ($(Y_{11} - Y_{10} - Y_{01} + Y_{00})(M_1 - M_0)$). These components are identifiable from observed data under the no unmeasured confounding assumptions detailed in the main text, and their average values can be estimated using regression models as described in VanderWeele (10).

The controlled direct effect (CDE) is defined as the effect of the exposure on the outcome when the mediator, $M$ is set at a particular value, $m$; when $M$ is set to 0, it represents the effect of the exposure in the absence of the mediator. For a binary exposure $A$, the average CDE when $M$ is set to 0 is given by $E[Y_{10} - Y_{00} | C]$. 

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The pure indirect effect (PIE) is defined as the difference between the outcome were the exposure absent and the mediator what it would have been in the presence of exposure and the outcome were the exposure absent and the mediator what it would have been in the absence of exposure. For a binary exposure A, the average PIE is given by \( E[Y_{0M1} - Y_{0M0} | C] \). When it is identified, it can also be written as \( E[Y_{01} - Y_{00} | C] E[M_1 - M_0 | C] \).

The effect of the exposure A may also depend on the level of this potential mediator, M. There will be interaction on the additive scale if the joint effect of the exposure and the mediator on the outcome is different than the sum of the effect of the exposure alone and the effect of the mediator alone. For binary exposure and a binary mediator, there is said to be additive interaction if the counterfactual contrast given by \( E[Y_{11} - Y_{00} | C] - \{E[Y_{10} - Y_{00} | C] + E[Y_{01} - Y_{00} | C]\} \) is non-zero. This additive interaction contrast can be more simply written as by \( E[Y_{11} - Y_{10} - Y_{01} + Y_{00} | C] \).

The reference interaction (\( INT_{ref} \)) and mediated interaction (\( INT_{med} \)) components are both functions of this additive interaction contrast. When it is identified, the average reference interaction is equal to \( E[Y_{11} - Y_{10} - Y_{01} + Y_{00} | C] E[M_0 | C] \), and captures the portion of the exposure effect that requires the joint presence of the mediator, with the mediator arising independently of the exposure. When it is identified, the average mediated interaction is equal to \( E[Y_{11} - Y_{10} - Y_{01} + Y_{00} | C] E[M_1 - M_0 | C] \), and captures the portion of the exposure effect that requires the joint presence of the mediator, but with the mediator itself arising as a consequence of the exposure.

These components correspond to the effect due to neither interaction nor mediation (\( CDE \)), the effect due to mediation alone (\( PIE \)), the effect due to interaction alone (\( INT_{ref} \)), and the effect due to both mediation and interaction (\( INT_{med} \)). The relation of this four-way decomposition to other mediation and interaction decompositions in the literature is discussed in detail in VanderWeele (10).
Supplementary Tables

Supplementary Table 3.1: Four-way decomposition and mediation and interaction results, comparing e4 carriers to e4 non-carriers, and e4 homozygotes to e4 non-carriers

<table>
<thead>
<tr>
<th></th>
<th>e4 carriers vs e4 non-carriers (1 or 2 vs. 0 e4 alleles) (n = 4121)</th>
<th>e4 homozygotes vs e4 non-carriers (2 vs. 0 e4 alleles) (n = 3047)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Effect</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.22 (-0.39, -0.05)</td>
</tr>
<tr>
<td>4-way decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controlled Direct Effect (CDE)</td>
<td>-0.10 (-0.16, -0.05)</td>
<td>-0.10 (-0.28, 0.08)</td>
</tr>
<tr>
<td>Pure Indirect Effect (PIE)</td>
<td>0.00 (-0.003, 0.002)</td>
<td>-0.01 (-0.02, 0.01)</td>
</tr>
<tr>
<td>Reference Interaction (INT&lt;sub&gt;ref&lt;/sub&gt;)</td>
<td>0.00 (-0.02, 0.02)</td>
<td>-0.05 (-0.09, -0.01)</td>
</tr>
<tr>
<td>Mediated Interaction (INT&lt;sub&gt;med&lt;/sub&gt;)</td>
<td>0.00 (-0.003, 0.003)</td>
<td>-0.06 (-0.11, 0.02)</td>
</tr>
<tr>
<td>Proportion of effect due to each component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDE (neither mediation nor interaction)</td>
<td>98%</td>
<td>46%</td>
</tr>
<tr>
<td>PIE (mediation alone)</td>
<td>1%</td>
<td>4%</td>
</tr>
<tr>
<td>INT&lt;sub&gt;ref&lt;/sub&gt; (interaction alone)</td>
<td>1%</td>
<td>24%</td>
</tr>
<tr>
<td>INT&lt;sub&gt;med&lt;/sub&gt; (mediation and interaction)</td>
<td>0%</td>
<td>26%</td>
</tr>
<tr>
<td>2-way decomposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure Direct Effect (PDE)</td>
<td>-0.11 (-0.16, -0.05)</td>
<td>-0.15 (-0.31, 0.02)</td>
</tr>
<tr>
<td>Total Indirect Effect (TIE)</td>
<td>0.00 (-0.003, 0.003)</td>
<td>-0.07 (-0.12, 0.01)</td>
</tr>
<tr>
<td>Interaction results</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive interaction measure (INT&lt;sub&gt;add&lt;/sub&gt;)</td>
<td>-0.01 (-0.18, 0.17)</td>
<td>-0.50 (-0.89, -0.08)</td>
</tr>
<tr>
<td>Stratum-specific effect estimates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference e4, no CMBs (ref)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Exposed e4, no CMBs</td>
<td>-0.10 (-0.16, -0.05)</td>
<td>-0.10 (-0.29, 0.09)</td>
</tr>
<tr>
<td>Reference e4, CMBs</td>
<td>-0.07 (-0.16, 0.02)</td>
<td>-0.07 (-0.16, 0.02)</td>
</tr>
<tr>
<td>Exposed e4, CMBs</td>
<td>-0.18 (-0.32, -0.05)</td>
<td>-0.67 (-1.03, -0.31)</td>
</tr>
<tr>
<td>CMB effect within e4 strata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference e4</td>
<td>-0.07 (-0.16, 0.02)</td>
<td>-0.07 (-0.16, 0.02)</td>
</tr>
<tr>
<td>Exposed e4</td>
<td>-0.08 (-0.22, 0.06)</td>
<td>-0.57 (-0.97, -0.16)</td>
</tr>
<tr>
<td>E4 exposure effect within CMB strata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No CMBs</td>
<td>-0.10 (-0.16, -0.05)</td>
<td>-0.10 (-0.29, 0.09)</td>
</tr>
<tr>
<td>CMBs</td>
<td>-0.11 (-0.27, 0.04)</td>
<td>-0.60 (-0.97, -0.23)</td>
</tr>
</tbody>
</table>

All models adjusted for age, sex, education, diabetes, smoking status, and midlife measures of physical activity, body mass index, systolic blood pressure, and total cholesterol
References


