**Title**: Cognitive Reserve and Midlife Vascular Risk: Cognitive and Clinical Outcomes

**Running Head**: Cognitive reserve and cerebrovascular disease

**Authors:** Anja Soldan[1], Ph.D., Corinne Pettigrew[1], Ph.D., Yuxin Zhu[2], Ph.D., Mei-Cheng Wang[2], Ph.D., Rebecca F. Gottesman[1], M.D., Ph.D., Charles DeCarli[3], M.D., Marilyn Albert[1], Ph.D., and the BIOCARD Research Team

**Affiliations:**

1. Department of Neurology, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

2. Department of Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21287

3. Department of Neurology, University of California, Davis, School of Medicine, Davis, CA 95616

**Corresponding Author:** Anja Soldan, Ph.D., Division of Cognitive Neuroscience, 1620 McElderry Street, Reed Hall 1-West, Baltimore, MD 21205, Phone: 410-502-2188, Fax: 410-502-2189, Email: asoldan1@jhmi.edu

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*Objective:* Examine whether cognitive reserve moderates the association of 1) vascular risk factors and 2) white matter hyperintensity burden with risk of clinical progression and longitudinal cognitive decline.

*Methods:* BIOCARD Study participants were cognitively normal and primarily middle-aged (*M*=57 years) at baseline and have been followed with annual cognitive and clinical assessments (*M*=13 years). Baseline cognitive reserve was indexed with a composite score combining education with reading and vocabulary scores. Baseline vascular risk (N=229) was assessed with a composite risk score reflecting five vascular risk factors. Baseline white matter hyperintensity load (N=271) was measured with FLAIR magnetic resonance imaging. Cox regression models assessed risk of progression from normal cognition to onset of clinical symptoms of Mild Cognitive Impairment. Longitudinal mixed effects models measured the relationship of these variables to cognitive decline, using a global composite score, and executive function and episodic memory sub-scores.

*Results:* Both vascular risk and white matter hyperintensities were associated with cognitive decline, particularly in executive function. Higher vascular risk, but not white matter hyperintensity burden, was associated with an increased risk of progression to Mild Cognitive Impairment. Higher cognitive reserve was associated with a reduced risk of symptom onset and higher levels of baseline cognition but did not modify the associations between the vascular risk score and white matter hyperintensities with clinical progression or cognitive decline.

*Interpretation:* Although cognitive reserve has protective effects on clinical and cognitive outcomes, it doesn't mitigate the negative impact of vascular risk and small vessel cerebrovascular disease on these same outcomes.

## Introduction

Vascular risk factors, particularly during midlife, are associated with steeper late-life cognitive decline and increased risk of dementia.[1, 2] For example, among cognitively normal, middle-aged individuals, the presence of hypertension,[1, 3, 4] obesity,[4, 5] or elevated total cholesterol[1, 6] has been linked to subsequent cognitive impairment and decline. Likewise, among cognitively normal middle-aged and older individuals, white-matter hyperintensities (WMH) on magnetic resonance imaging (MRI) scans are associated with cognitive decline.[7-9] WMH are primarily considered markers of small vessel cerebrovascular disease[10] and have been associated with higher levels of cardiovascular risk.[11-14]

Given the importance of midlife cardiovascular disease (CVD) for late-life cognitive functioning, the current study sought to investigate whether cognitive reserve (CR) moderates the effect of midlife vascular risk factors and WMH burden on long-term clinical and cognitive outcomes. CR is thought to be a property of the brain that ameliorates the effects of brain pathology or damage on cognition.[15, 16] It is most commonly measured using proxy variables, such as literacy or educational and occupational attainment. We recently reported that higher CR, measured using a composite score, is associated with lower WMH burden among middle-aged, cognitively normal individuals[17]; however, CR was not associated with a decreased rate of WMH accumulation over time. It remains unclear to what extent CR reduces the impact of midlife measures of WMH and vascular risk on rate of change in cognition.

A number of cross-sectional studies across the clinical spectrum (i.e., cognitively normal, MCI, and dementia) suggest that higher levels of CR may reduce the impact of WMH load[18-22] or vascular risk factors[23-28] on cognitive performance, at least for some cognitive domains. However, few longitudinal studies have examined this issue[29-33] and to our knowledge, none were conducted among individuals who were primarily middle aged and cognitively normal at baseline. Moreover, studies among cognitively normal older individuals (mean baseline age 70-80 years) have been characterized by short follow-up periods (mean 2.5-5 years) and produced

mixed results. One study reported that although a higher level of CR was associated with better cognitive performance, CR did not modify the association between CVD and cognitive trajectories.[31] Another study found that years of education moderated the association between severe WMH and risk of progression to MCI,[29] such that WMH predicted progression only among those with low education (<8 years).

To the extent that midlife vascular risk and WMH could have consequences for long-term cognitive health among individuals with different levels of CR, this may have implications for clinicians and public health efforts focused on reducing or managing CVD. The current study addresses this important question using data from the BIOCARD study. All participants were cognitively normal when first enrolled, primarily middle age (mean 57.9=years) and have been followed for an average of 13 years. The long follow-up period, extensive cognitive testing, and the fact that a substantial number of participants have progressed to MCI or dementia allowed us to examine both the time to clinical symptom onset of MCI, as well as long-term cognitive trajectories. We used global and domain-specific cognitive measures that may be particularly relevant to vascular risk factors and WMH.[21, 22]

## Methods

### Study Design

These data were derived from the BIOCARD study, an ongoing longitudinal study that was initiated in 1995 at the National Institutes of Health (NIH) to identify predictors of progression from normal cognition to mild symptoms of Alzheimer's disease (AD). About 75% of participants had a first-degree relative with AD-dementia. The study was stopped in 2005 for administrative reasons and re-initiated at Johns Hopkins University (JHU) in 2009. While at the NIH, the study included comprehensive neuropsychological testing annually, and the collection of magnetic resonance imaging (MRI) scans, cerebrospinal fluid (CSF) samples, and blood specimens approximately every 2 years. Since the study has been at JHU, the annual evaluations have included clinical and cognitive assessments, and the collection of blood. In

2015, the biennial collection of CSF and MRI scans was re-established, and amyloid imaging was initiated (see Figure 1 for a study timeline). Supplementary Materials 1 provides additional details regarding the study design and participant recruitment. This study was approved by the JHU Institutional Review Board.

As described previously,[34] 349 participants were initially enrolled after providing written informed consent. Participants were excluded at baseline if they were judged to be cognitively impaired, or if they had significant medical problems, including severe CVD, epilepsy, or substance abuse. The current analyses involving WMH included 271 participants (of the 317 with baseline MRI scans); analyses involving vascular risk scores included 229 participants (see Supplementary Materials 2 for reasons for exclusion of participants).

**Clinical Assessments and Consensus Diagnostic Procedure**

The annual clinical and cognitive assessments have been described previously (see also Supplementary Materials 1).[34] Consensus diagnoses have been completed annually for each participant. The BIOCARD Clinical Core employed the criteria recommended by the National Institute on Aging and Alzheimer's Association working group for the diagnosis of MCI[35] and dementia due to AD.[36] Briefly, for each case a syndromic diagnosis is first established using (1) clinical data pertaining to the individual's medical, neurological, and psychiatric status; (2) reports of changes in cognition by the individual and by collateral sources (based on the Clinical Dementia Rating scale (CDR)[37]); and (3) evidence of cognitive decline based on longitudinal neuropsychological test performance and comparison to published norms. Participants with contrasting information from the CDR interview and the cognitive test scores received a diagnosis of Impaired Not MCI (i.e., when there was evidence of decline in cognitive testing, but the subject or collateral source had no concerns about cognitive changes in daily life, or vice versa). Second, for subjects judged to be cognitively impaired, the likely etiology of the impairment was determined using all available information, including vascular risks (but not biomarker measures). Multiple etiologies could be endorsed (e.g., AD and vascular disease).

Information from the CDR interview, conducted with both the subject and the collateral source, is used to estimate the age at which the clinical symptoms began, if a subject is judged to be cognitively impaired. The age of onset of symptoms of MCI was the primary clinical outcome variable in this study.

**Cognitive Assessments**

The main cognitive outcome variable in this study was an a-priori derived global cognitive composite score based on four tests previously determined to provide the best combination of cognitive predictors of progression from normal cognition to MCI in the BIOCARD cohort[34], including: Paired Associates immediate recall (Wechsler Memory Scale–Revised; WMS-R), Logical Memory delayed recall (Story A; WMS-R), Boston Naming, and Digit-Symbol Substitution (Wechsler Adult Intelligence Scale–Revised; WAIS-R). To calculate the composite score, these four measures were converted to z-scores (using all available observations) and then averaged, with the requirement that at least 2 of the 4 scores were present at a given time point.

To evaluate domain-specific cognitive performance, two additional scores were calculated, also using z-score averages (based on all observations): (1) a verbal episodic memory composite (using Paired Associates immediate recall and Logical Memory delayed recall); and (2) an executive functioning/speed of processing composite (using Digit-Symbol Substitution and Digit Span backwards (WMS-R)). Cognitive trajectories were calculated from an individual's baseline WMH measure or vascular risk score, through all available follow-up.

**Cognitive Reserve Composite Score**

CR was measured with a composite score that included three commonly used proxy measures reflecting lifetime cognitive experiences: (1) years of education; (2) baseline scores from the National Adult Reading Test[38]; (3) baseline scores on the vocabulary subtest of the WAIS-R[39]. Because the three measures are highly correlated and load onto a single factor in a

factor analysis,[40] they were z-scored (using baseline values only) and then averaged to create the composite score.

**APOE Genotyping and Coding**

APOE genotypes were determined by restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA (Athena Diagnostics, Worcester, MA). APOE4 carrier status was coded dichotomously (i.e., ε4 carriers vs. non-carriers). Analyses including APOE4 excluded APOE ε2/ε4 carriers since these alleles have contrasting effects on dementia risk.[41, 42]

**Summary Vascular Risk Score**

The baseline vascular risk composite score was based on medical records or self-report during a medical history interview. The score was calculated by summing five dichotomous vascular risk factors (each coded as 0 = absent vs. 1 = recent/remote), as previously published[43]: hypertension; hypercholesterolemia; diabetes; current smoking (i.e., within the last 30 days); and obesity (i.e., measured body mass index >30). Because few individuals had 2 (*n*=29) or 3 or more (*n*=7) risk factors, analyses were conducted using both categorical (0, 1, 2, 3, 4) and dichotomous (0 vs. 1+) composite vascular risk scores.

**White Matter Hyperintensity Volumes**

WMH volumes were derived from axial fluid-attenuated inversion recovery (FLAIR) images obtained on a GE 1.5T scanner while the study was at the NIH (TR=9,002, TE=157.5, FOV=256x256, thickness/gap=5.0/0.0 mm, flip angle=90, 28 slices). An automated method, described previously,[44, 45] was used to quantify global WMH volumes (for details, see Supplementary Materials 3). Because the distribution of baseline WMH volumes was skewed, all analyses were run using WMH both as a continuous variable and dichotomized based on quartiles (1=highest quartile; 0=lowest three quartiles).

**Statistical Analyses**

Baseline age was defined as the age at the first FLAIR scan (for WMH) or first vascular risk score, respectively. The mean time from baseline visit to first FLAIR scan was 0.9 years (SD=1.5), and from baseline visit to first vascular risk score was 0.06 years (SD=0.75).

We used Cox regression models to examine the association between baseline CR score and baseline WMH volume or vascular risk score with risk of onset of clinical symptoms of MCI. The models, which were adjusted for left-truncation,[46] compared two groups: (1) participants who were cognitively normal at both baseline and their last visit, and (2) participants who were normal at baseline but were diagnosed with MCI or dementia at last follow-up. For this latter group, the estimated age of clinical symptom onset was used as the outcome variable. The last date of diagnosis was used as the censoring time. Participants with a diagnosis of Impaired Not MCI (N=38 and N=31 for WMH volume and vascular risk analyses, respectively) were included in the cognitively normal group (as they do not meet criteria for MCI). Results were comparable when they were excluded. Hazard ratios (HR) were calculated, which represent the change in relative risk of progression for one-unit change in the predictor. The HRs for all continuous variables can be directly compared because all continuous variables were standardized (i.e., z-scored). Models included the following predictors: baseline age, sex, CR composite score, and baseline WMH volume or vascular risk score, as well as interaction terms (CR x WMH or CR x vascular risk score) for determining if level of CR moderates the relationship between baseline WMH or vascular risk scores and risk of progression.

We used longitudinal linear mixed effects models[47] to examine the association between baseline CR score and WMH volume or vascular risk score on longitudinal cognitive trajectories, using separate models for the three cognitive composite scores. Models were specified with random intercepts and slopes and included linear effects of time (in unit of years). All other continuous variables were standardized before model fitting. Models included terms for baseline age, sex, CR score, WMH volume or vascular risk score, time, and the interaction (cross-product) of each predictor with time. Three-way interactions (CR x WMH or vascular risk score x

time) evaluated whether CR moderates the relationship between baseline WMH or vascular risk scores and cognitive change. If the three-way interaction was not significant, we ran reduced models that excluded this term as well as the corresponding lower order interaction term (i.e., CR x WMH or vascular risk score).

Group differences in baseline characteristics of participants were assessed with two-tailed Wilcoxon rank sum tests for continuous variables or chi-square tests for dichotomous variables. We used a significance level of $p<0.05$, uncorrected for multiple comparisons. All analyses were run in R, version 3.5.0.

## Results

Baseline characteristics for participants included in the WMH and vascular risk analyses are shown in Tables 1 and 2, respectively. Participants who progressed to MCI/dementia tended to be older, have lower baseline cognitive composite scores, lower CR composite scores, and higher baseline WMH volumes and vascular risk scores than those who remained cognitively normal. AD was the most common etiology proposed for MCI/dementia cases (~90%), followed by vascular disease (~50%) (Supplementary Materials 4). See Supplementary Materials 5 for diagnostic frequencies as a function of baseline WMH and vascular risk burden.

### *Baseline CR, WMH Volumes, and Vascular Risk Scores in Relation to Time to Clinical Symptom Onset*

In the Cox regression models, higher baseline CR was significantly associated with approximately a 50% decrease in the relative risk of progression (Table 3). Additionally, higher baseline vascular risk scores (both categorical and dichotomous) were associated with an increased risk of progression to MCI clinical symptom onset (Figure 2). However, neither continuous nor dichotomous baseline WMH volumes were significant predictors of time to clinical symptom onset. Results were similar when APOE4 status was included as an additional predictor. Importantly, there was no evidence that baseline CR modified the relationship between baseline WMH volumes or vascular risk scores and risk of progression (Table 3).

*Baseline CR, WMH Volumes, and Vascular Risk Scores in Relation to Cognitive Trajectories*

Higher vascular risk scores were associated with greater rates of global cognitive decline (Table 4; Figure 3). In the domain-specific analyses, higher baseline WMH volumes and vascular risk scores were associated with greater decline on the executive function/speed composite score (Table 6) but were unrelated to the episodic memory composite (Table 5). Additionally, higher baseline WMH volumes (continuous) were associated with lower levels of global cognitive performance and greater rates of global cognitive decline (Table 4, for dichotomous WMH values, the association did not reach significance, both p<=0.08). Higher CR scores were consistently associated with higher levels of cognitive performance, but not with change in cognitive performance over time, as reflected in the reduced models for the global (Table 4; Figure 3), episodic memory (Table 5), and executive function/speed (Table 6) composite scores. There was no evidence that CR modified the relationship between baseline WMH or vascular risk scores and cognitive trajectories (all three-way interactions, $p \geq .13$; data not shown).

## Discussion

This study found that independent of relatively low levels of midlife vascular risk factors and WMH burden, higher baseline CR scores were consistently associated with a reduction in the risk of symptom onset of MCI and higher level of cognitive performance (both globally and in the domains of executive functioning and episodic memory) among individuals who were cognitively normal at baseline. CR was not, however, associated with rates of cognitive change, in line with prior work.[16] This suggests that the protective effects of CR are equivalent across the observed range of vascular risk and WMH burden.

This is the first study, to our knowledge, to examine interactions between CR and both WMH and vascular risk in relation to longitudinal clinical or cognitive outcomes among largely middle-aged individuals with normal cognition at baseline. Additionally, only two prior studies, conducted among cognitively normal individuals in their 70s, have examined interactions

between CR and WMH on longitudinal cognitive and clinical trajectories. In line with our results, Vemuri et al.[31] reported no interaction between measures of CR and CVD on longitudinal cognitive decline. In contrast, Mortamais et al.[29] found that high WMH burden was associated with risk of MCI/dementia only among individuals with low education (≤8 years) but not high education (8+ years); notably, the same interaction was not significant when education was dichotomized at 12 years. This may be suggestive of a non-linear relationship between CR and WMH with respect to the risk of progression when CR is very low, although additional studies are needed to evaluate this hypothesis.

This study also showed that relatively low levels of midlife vascular risk and WMH were associated with greater rates of global cognitive decline. In line with prior studies, these declines were stronger for executive function/speed of processing compared to episodic memory.[4, 48-50] However, only vascular risk factors (not WMH) were associated with risk of clinical symptom onset. While some prior studies among individuals with baseline ages in their 70s-80s have reported relationships between WMH and subsequent risk of MCI, findings have been mixed[8, 9, 51, 52], possibly reflecting differences in age, WMH burden, or overall levels of neurodegeneration.[45] We hypothesize that a composite measure of vascular risks may have a stronger relationship with clinical and cognitive outcomes because vascular risks have more widespread effects on the brain, including WMH burden, white matter integrity, brain atrophy, and infarcts, among others.[4, 53, 54] Although we did not examine interactions between AD and CVD, we further hypothesize that CVD may lower the threshold for the impact of AD on cognition, given recent findings from this and other cohorts that AD and CVD appear independently associated with the risk of MCI[55] and cognitive decline[56] (also see[57, 58]).

This study has limitations. BIOCARD participants are primarily white, highly educated, and have a strong family history of AD, limiting the generalizability of these findings. Additionally, participants had relatively low levels of vascular risk and WMH burden at baseline.

Therefore, results may differ in samples with greater variability in levels of vascular risk, cerebrovascular disease, or CR.

Taken together, these findings suggest that midlife levels of both CR and vascular risk and cerebrovascular disease have significant and independent consequences on long-term cognitive health. In light of the finding that higher levels of CR are also associated with lower WMH burden at midlife[17], these findings further suggest that CR may impact cognitive and clinical outcomes in two ways: (1) by directly or indirectly reducing cerebrovascular disease (possibly by altering lifestyle factors that reduce vascular risk), and (2) by increasing baseline cognitive performance and thereby delaying the impact of vascular risk and WMH on clinical symptom onset. These findings also suggest that efforts to reduce and manage factors that affect cerebrovascular disease may benefit individuals with different levels of CR equally and may thereby substantially impact the prevalence of cognitive decline and dementia among older persons.

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## Conflicts of Interest

Dr. Soldan reports no disclosures.

Dr. Pettigrew reports no disclosures.

Dr. Zhu reports no disclosures.

Dr. Wang reports no disclosures.

Dr. Gottesman is an Associate Editor for the journal *Neurology*.

Dr. DeCarli is a consultant to Novartis for a trial on heart failure.

Dr. Albert is an advisor to Eli Lilly.

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## Figure Legends

Figure 1. Timeline showing the BIOCARD study design, including the types of data collected each year.[17] Abbreviations: CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; NIH, National Institutes of Health; PET, positron emission tomography.

Figure 2. Survival plots, based on Cox regression model, showing the proportion of subjects who remained cognitively normal over time as a function of their baseline WMH burden (top panel, low = 25$^{th}$ percentile; high = 75$^{th}$ percentile) or vascular risk score (bottom panel, low = 0, high = 1+) and baseline CR score (low = 25$^{th}$ percentile, high = 75$^{th}$ percentile). WMH were not associated with risk of progression. Both high vascular risk and low CR were independently associated with a lower proportion of participants who remain cognitively normal (i.e., higher risk of progression; see text for details).

Figure 3. Estimates with 95% confidence bands from linear mixed effects models predicting longitudinal global cognitive composite scores over time as a function of baseline CR score (low = 25$^{th}$ percentile; high = 75$^{th}$ percentile) for four groups of participants: low baseline WMH burden (25$^{th}$ percentile, panel A); high baseline WMH burden (75$^{th}$ percentile, panel B); vascular risk score = 0 (panel C); and vascular risk score = 1+ (panel D). Estimates are adjusted for baseline age, sex, and their interactions with time. While higher CR scores were associated with higher level of cognitive performance, CR scores did not modify cognitive performance over time, as indicated by the slopes. Both WMH and vascular risk burden were associated with greater cognitive decline.

Table 1. Baseline characteristics for participants included in the WMH volume analyses. Values reflect mean (SD) [range] unless otherwise indicated.

| | Participants in WMH Analyses | | |
|---|---|---|---|
| | All participants (N = 271) | Remained cognitively normal (n = 211) | Progressed to MCI or dementia (n = 60) |
| Age | 57.9 (10.5) | 56.0 (9.9) | 64.1 (10.3) * |
| Female sex, N (%) | 161 (59.4%) | 130 (61.6%) | 31 (51.7%) |
| White race/ethnicity, N (%) | 264 (97.4%) | 208 (98.6%) | 56 (93.3%) |
| APOE ε4 carrier, N (%) | 86 (31.7%) | 65 (30.8%) | 21 (35.0%) |
| Years of education | 17.1 (2.3) | 17.2 (2.3) | 16.7 (2.4) |
| Mini-Mental State Examination | 29.7 (0.7) | 29.7 (0.6) | 29.6 (0.9) |
| Baseline cognitive composite score | -0.16 (0.7) | -0.02 (0.6) | -0.67 (0.8) * |
| Most recent cognitive composite score | -0.09 (1.0) | 0.20 (0.7) | -1.27 (1.3) * |
| Years of follow-up | 12.9 (3.5) [0.9-17.5] | 12. 9 (3.6) [0.9-17.5] | 13.0 (3.0) [2.0-17.0] |
| No. of cognitive measures over time | 8.1 (3.0) [1-14] | 8.3 (3.0) [1-14] | 7.2 (3.0) [1-12] * |
| Years from baseline to onset of clinical symptoms of MCI | - | - | 6.4 (3.4) |
| Cognitive reserve composite score | 0.00 (0.7) | 0.17 (0.6) | -0.7 (0.8) * |
| WMH volume, $cm^3$ | 3.2 (5.5) [0.03-52.0] | 2.6 (4.6) [0.03-52.0] | 5.2 (7.6) [0.09-44.9] * |
| WMH volume in upper quartile, N (%) | 65 (24.0%) | 42 (19.9%) | 23 (38.3%) * |
| WMH volume in lower three quartiles, N (%) | 206 (76.0%) | 169 (80.1.%) | 73 (61.7%) * |

* Significant difference between outcome groups, $p < .05$.

Abbreviations: WMH, white matter hyperintensity.

Table 2. Baseline characteristics for participants included in the vascular risk score analyses.

Values reflect mean (SD) [range] unless otherwise indicated.

| | Participants in Vascular Risk Score Analyses | | |
|---|---|---|---|
| | All participants (N = 229) | Remained cognitively normal (n = 184) | Progressed to MCI or dementia (n = 45) |
| Age | 56.8 (10.3) | 54.7 (9.7) | 64.5 (8.0) * |
| Female sex, N (%) | 139 (60.7%) | 118 (64.1%) | 21 (46.7%) * |
| White race/ethnicity, N (%) | 222 (96.9%) | 181 (98.4%) | 41 (91.1%) * |
| APOE ε4 carrier, N (%) | 71 (31.0%) | 56 (30.4%) | 15 (33.3%) |
| Years of education | 17.3 (2.2) | 17.3 (2.2) | 17.3 (2.2) |
| Mini-Mental State Examination | 29.6 (0.7) | 29.6 (0.7) | 29.4 (0.9) |
| Baseline cognitive composite score | -0.07 (0.6) | 0.03 (0.6) | -0.52 (0.6) * |
| Most recent cognitive composite score | -0.05 (1.0) | 0.22 (0.7) | -1.26 (1.2) * |
| Years of follow-up | 14.3 (3.8) [0.0-20.7] | 14.1 (4.0) [0.0-20.7] | 14.9 (3.2) [4.2-20.0] |
| No. of cognitive measures over time | 9.7 (3.6) [1-18] | 9.7 (3.7) [1-18] | 9.4 (3.5) [2-16] |
| Years from baseline to onset of clinical symptoms of MCI | - | - | 8.0 (3.9) |
| Cognitive reserve composite score | 0.13 (0.7) | 0.18 (0.7) | -0.05 (0.8) * |
| Vascular risk score | 0.59 (0.8) [0-4] | 0.52 (0.8) [0-4] | 0.89 (0.7) [0-2] * |
| Vascular risk score = 0, N (%) | 130 (56.8%) | 115 (62.5%) | 15 (33.3%) * |
| Vascular risk score = 1+, N (%) | 99 (43.2%) | 69 (37.5%) | 30 (66.7%) * |

* Significant difference between outcome groups, $p < .05$.

Table 3. Cox regression model results for baseline CR scores, and WMH volumes or vascular risk scores, in relation to onset of clinical symptoms of MCI.

| | Estimate (SE) | HR (95% CI) | *p*-value |
|---|---|---|---|
| *WMH Volume – Continuous* | | | |
| CR composite score | -0.651 (0.136) | 0.52 (0.40, 0.68) | < .001 |
| WMH volume | 0.145 (0.132) | 1.16 (0.89, 1.51) | .27 |
| CR x WMH | -0.072 (0.140) | 0.93 (0.70, 1.23) | .61 |
| *WMH Volume – Dichotomous* | | | |
| CR composite score | -0.653 (0.161) | 0.52 (0.38, 0.70) | < .001 |
| WMH volume | -0.088 (0.325) | 0.92 (0.48, 1.75) | .79 |
| CR x WMH | -0.098 (0.263) | 0.91 (0.54, 1.53) | .71 |
| *Vascular Risk Score – Categorical* | | | |
| CR composite score | -0.687 (0.202) | 0.50 (0.34, 0.75) | .001 |
| Vascular risk score | 0.411 (0.180) | 1.51 (1.05, 2.16) | .02 |
| CR x vascular risk score | 0.205 (0.157) | 1.23 (0.90, 1.68) | .19 |
| *Vascular Risk Score – Dichotomous* | | | |
| CR composite score | -0.695 (0.228) | 0.50 (0.32, 0.79) | .002 |
| Vascular risk score | 0.917 (0.347) | 2.50 (1.25, 5.01) | .008 |
| CR x vascular risk score | 0.309 (0.298) | 1.36 (0.75, 2.47) | .30 |

Note: all models adjusted for baseline age and sex.

Abbreviations: CI, confidence interval. CR, cognitive reserve. HR, hazard ratio. MCI, Mild Cognitive Impairment. SE, standard error. WMH, white matter hyperintensity.

Table 4. Longitudinal mixed effects model results for baseline CR scores, and WMH volumes or vascular risk scores, in relation to the global cognitive composite score.

| | WMH Volume – Continuous | | WMH Volume – Dichotomous | |
|---|---|---|---|---|
| | Estimate (SE) | *p*-value | Estimate (SE) | *p*-value |
| Time | 0.010 (0.006) | .08 | 0.014 (0.007) | .03 |
| CR composite score (level) | 0.332 (0.046) | < .001 | 0.334 (0.048) | < .001 |
| WMH volume (level) | -0.093 (0.043) | .03 | -0.227 (0.123) | .07 |
| CR composite score x time (slope) | 0.001 (0.005) | .76 | 0.001 (0.005) | .80 |
| WMH volume x time (slope) | -0.015 (0.005) | .002 | -0.021 (0.012) | .08 |
| | Vascular Risk Score – Categorical | | Vascular Risk Score – Dichotomous | |
| | Estimate (SE) | *p*-value | Estimate (SE) | *p*-value |
| Time | 0.014 (0.006) | .02 | 0.015 (0.006) | .02 |
| CR composite score (level) | 0.273 (0.049) | < .001 | 0.271 (0.049) | < .001 |
| Vascular risk score (level) | -0.001 (0.061) | .99 | -0.051 (0.100) | .61 |
| CR composite score x time (slope) | 0.004 (0.004) | .31 | 0.003 (0.004) | .46 |
| Vascular risk score x time (slope) | -0.017 (0.005) | .001 | -0.025 (0.008) | .002 |

Note: all models adjusted for baseline age, sex, and their interactions with time. All two- and three-way interactions of CR x WMH volume or vascular risk score and CR x WMH volume or vascular risk score x time were not significant (all $p>0.2$) and therefore excluded from the final models.

Abbreviations: CI, confidence interval. CR, cognitive reserve. SE, standard error. WMH, white matter hyperintensity.

Table 5. Longitudinal mixed effects model results for baseline CR scores, and WMH volumes or vascular risk scores, in relation to the verbal episodic memory composite score.

| | WMH Volume – Continuous | | WMH Volume – Dichotomous | |
|---|---|---|---|---|
| | Estimate | *p*-value | Estimate | *p*-value |
| Time | 0.024 (0.006) | < .001 | 0.027 (0.007) | < .001 |
| CR composite score (level) | 0.230 (0.053) | < .001 | 0.228 (0.055) | < .001 |
| WMH volume (level) | -0.065 (0.047) | .17 | -0.125 (0.141) | .38 |
| CR composite score x time (slope) | 0.006 (0.005) | .21 | 0.006 (0.005) | .249 |
| WMH volume x time (slope) | -0.006 (0.005) | .22 | -0.016 (0.013) | .21 |
| | Vascular Risk Score – Categorical | | Vascular Risk Score – Dichotomous | |
| | Estimate | *p*-value | Estimate | *p*-value |
| Time | 0.024 (0.006) | < .001 | 0.025 (0.007) | < .001 |
| CR composite score (level) | 0.157 (0.054) | .004 | 0.152 (0.055) | .006 |
| Vascular risk score (level) | -0.007 (0.069) | .92 | -0.092 (0.112) | .41 |
| CR composite score x time (slope) | 0.007 (0.004) | .12 | 0.006 (0.004) | .16 |
| Vascular risk score x time (slope) | -0.010 (0.006) | .06 | -0.015 (0.009) | .09 |

Note: all models adjusted for baseline age, sex, and their interactions with time. All two- and three-way interactions of CR x WMH volume or vascular risk score and CR x WMH volume or vascular risk score x time were not significant (all $p$>=0.13) and therefore excluded from the final models.

Abbreviations: CI, confidence interval. CR, cognitive reserve. SE, standard error. WMH, white matter hyperintensity.

Table 6. Longitudinal mixed effects model results for baseline CR scores, and WMH volumes or vascular risk scores, in relation to the executive function/speed of processing composite score.

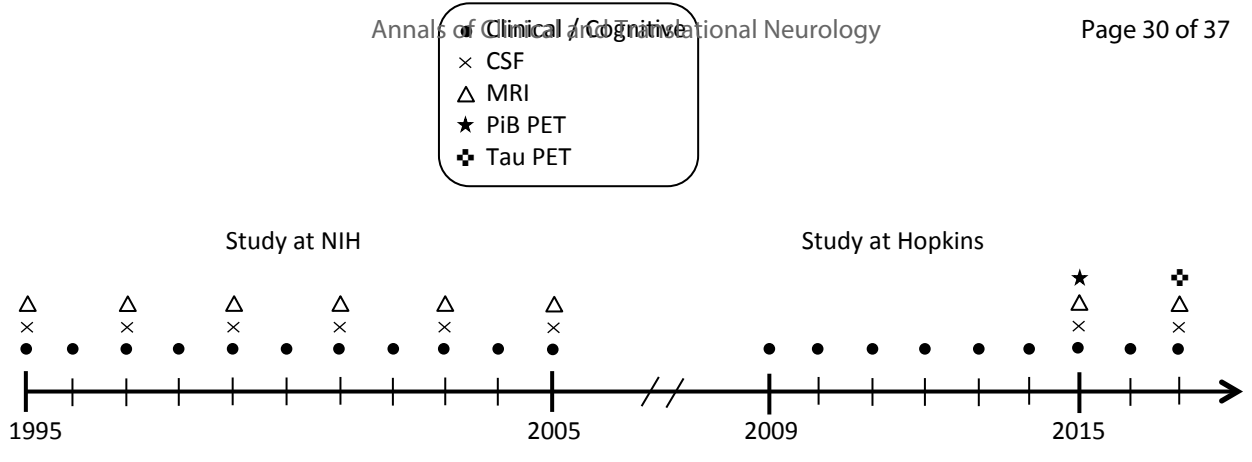
| | WMH Volume – Continuous | | WMH Volume – Dichotomous | |
|---|---|---|---|---|
| | Estimate | *p*-value | Estimate | *p*-value |
| Time | -0.019 (0.004) | < .001 | -0.016 (0.004) | < .001 |
| CR composite score (level) | 0.345 (0.047) | < .001 | 0.342 (0.048) | < .001 |
| WMH volume (level) | -0.056 (0.043) | .20 | -0.145 (0.124) | .24 |
| CR composite score x time (slope) | -0.001 (0.003) | .70 | -0.002 (0.003) | .59 |
| WMH volume x time (slope) | -0.010 (0.004) | .003 | -0.019 (0.008) | .02 |
| | Vascular Risk Score – Categorical | | Vascular Risk Score – Dichotomous | |
| | Estimate | *p*-value | Estimate | *p*-value |
| Time | -0.014 (0.004) | < .001 | -0.013 (0.004) | .001 |
| CR composite score (level) | 0.273 (0.048) | < .001 | 0.263 (0.048) | < .001 |
| Vascular risk score (level) | -0.103 (0.061) | .09 | -0.211 (0.099) | .03 |
| CR composite score x time (slope) | 0.004 (0.003) | .11 | 0.004 (0.003) | .16 |
| Vascular risk score x time (slope) | -0.010 (0.003) | .002 | -0.014 (0.005) | .006 |

Note: all models adjusted for baseline age, sex, and their interactions with time. All two- and three-way interactions of CR x WMH volume or vascular risk score and CR x WMH volume or vascular risk score x time were not significant (all $p>0.1$) and therefore excluded from the final models.

Abbreviations: CI, confidence interval. CR, cognitive reserve. SE, standard error. WMH, white matter hyperintensity.

**Supplemental Files: Supplementary Materials**

**Contents:**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PiB PET
Tau PET
Study at NIH
Study at Hopkins
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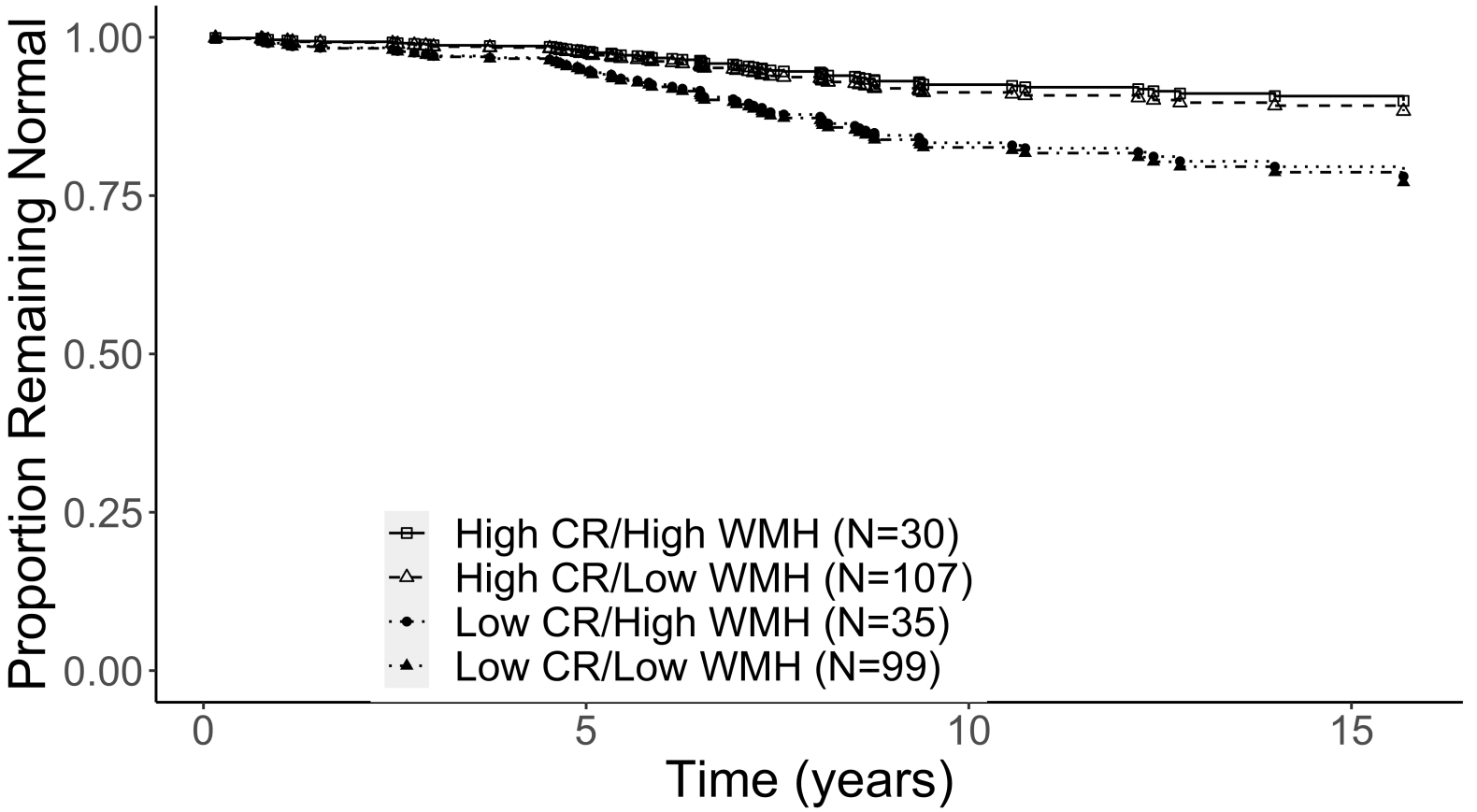
2005
2009
2015

A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0.00
0
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10
15
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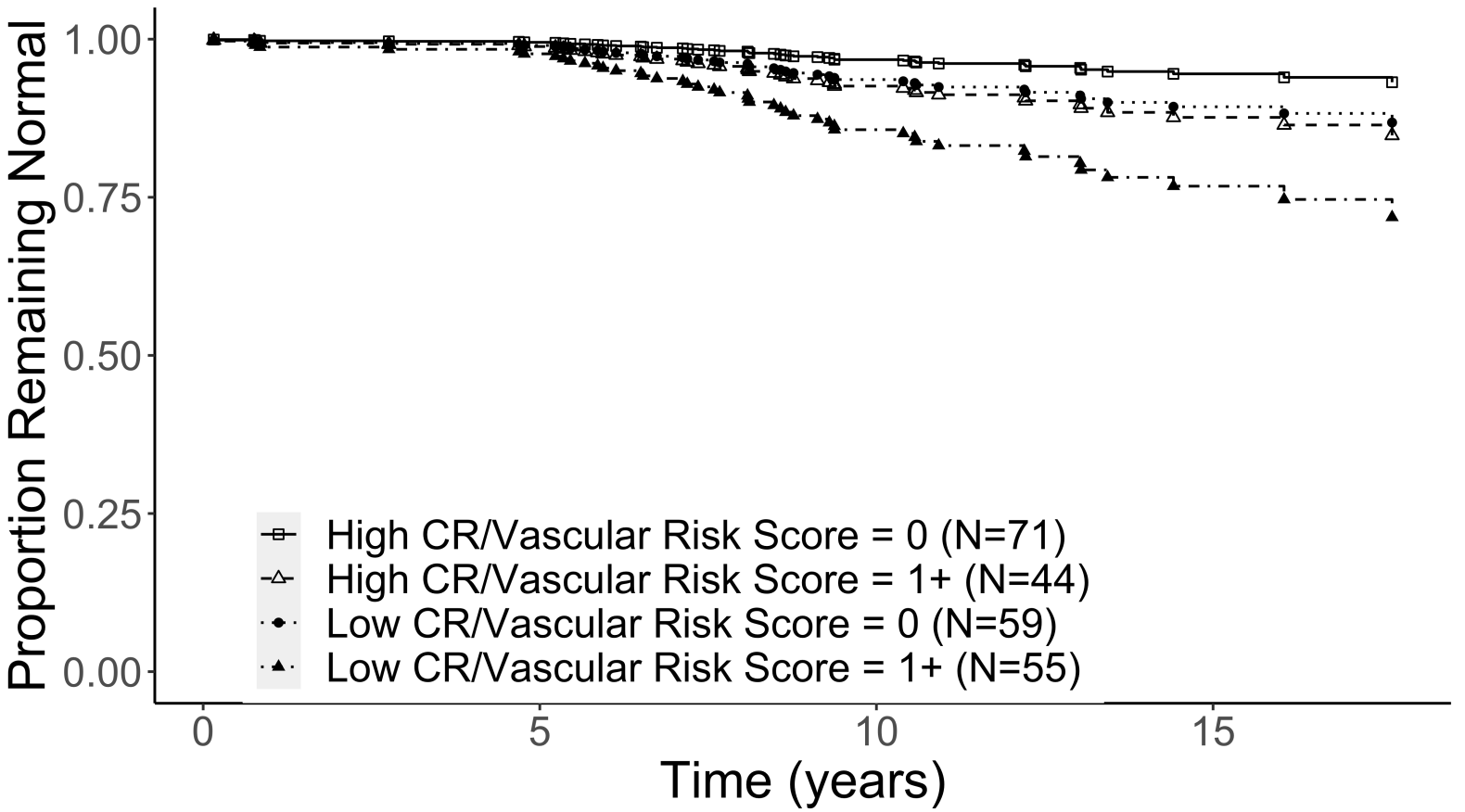
Low CR/Low WMH (N=99)

B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0.25
0.00
0
5
10
15
Time (years)
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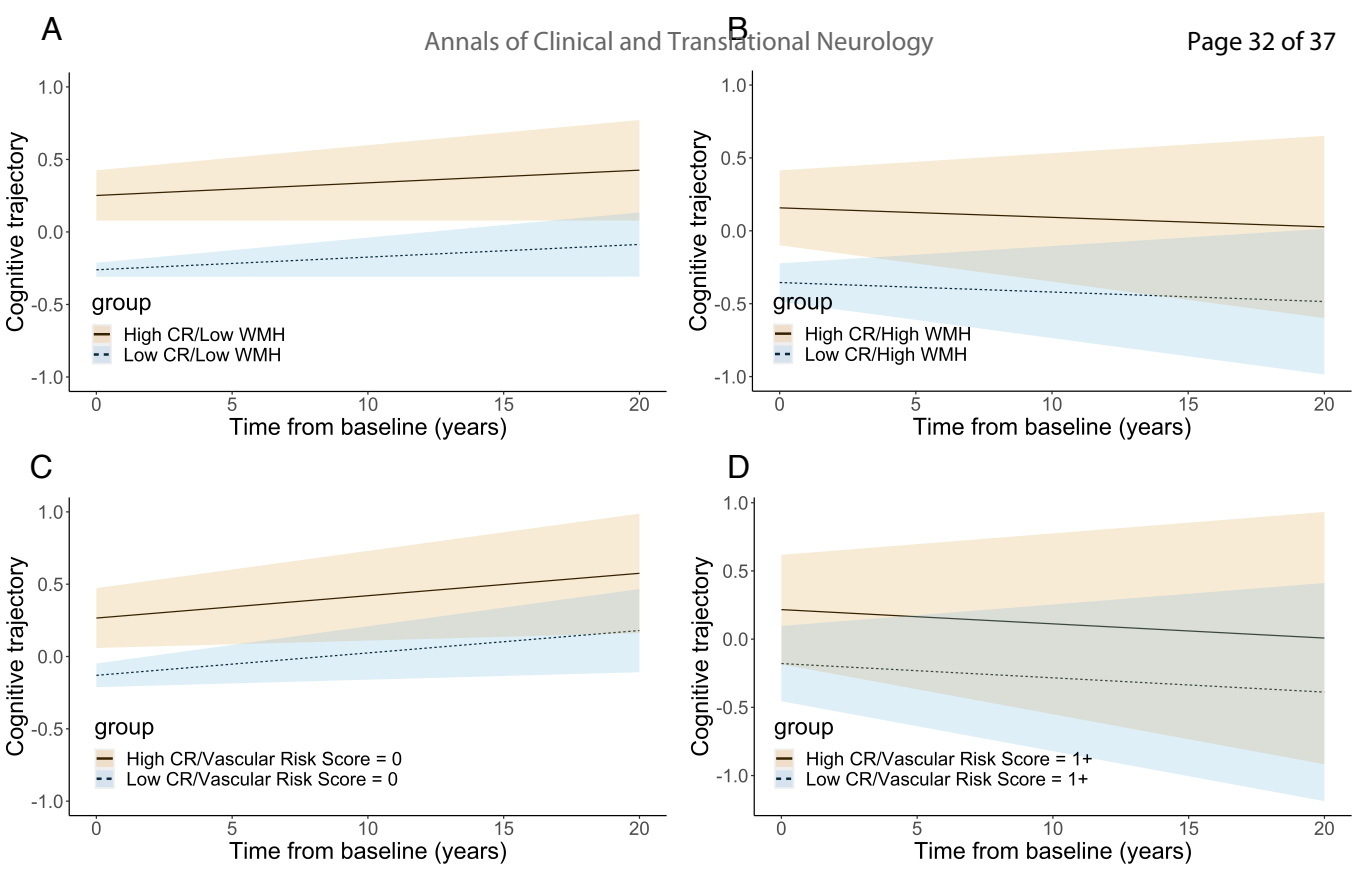
Low CR/Vascular Risk Score = 1+ (N=55)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15
20
Time from baseline (years)
B
Cognitive trajectory
group
High CR/High WMH
Low CR/High WMH
Time from baseline (years)
C
Cognitive trajectory
group
High CR/Vascular Risk Score = 0
Low CR/Vascular Risk Score = 0
Time from baseline (years)
D
Cognitive trajectory
group
High CR/Vascular Risk Score = 1+
Low CR/Vascular Risk Score = 1+
Time from baseline (years)

# Supplementary Materials

## Cognitive Reserve and Midlife Vascular Risk: Cognitive and Clinical Outcomes

Anja Soldan, Ph.D., Corinne Pettigrew, Ph.D., Yuxin Zhu, Ph.D., Mei-Cheng Wang, Ph.D., Rebecca F. Gottesman, M.D., Ph.D., Charles DeCarli, M.D., Marilyn Albert, Ph.D., and the BIOCARD Research Team

**Contents:**

**Supplementary Materials 1:**
**Study Design, Participant Selection, Clinical Assessments, and Consensus Diagnosis Procedure, and Cognitive Assessments**

*Study design and participant selection*

The data presented here were derived from the BIOCARD study, which is an ongoing longitudinal prospective cohort study designed to identify variables among cognitively normal individuals that predict subsequent development of mild to moderate symptoms of AD. As reported previously,[1] the BIOCARD study was initiated in 1995 at the National Institutes of Health (NIH) with study recruitment conducted by the staff of the Geriatric Psychiatry Branch of the intramural program of the National Institute of Mental Health. At their baseline visit, all participants completed a comprehensive evaluation that included a physical and neurological examination, neuropsychological testing, an electrocardiogram, and standard laboratory studies. Participants were excluded if they were judged to be cognitively impaired, as determined by the cognitive testing or by evidence of clinical symptoms based on reports by collateral sources. Participants were also excluded if they had significant medical problems (such as severe cardiovascular disease (e.g., atrial fibrillation), severe cerebrovascular disease (based on MRI), chronic psychiatric disorders (e.g., schizophrenia, alcohol or drug abuse), or chronic neurologic disorders (e.g., epilepsy, multiple sclerosis)). Five individuals did not meet the entry criteria and were excluded at baseline. After providing written informed consent, a total of 349 cognitively normal, primarily middle aged (*M* = 57.3 years, *SD* = 10.4, range = 20.0-85.8) participants were enrolled over time, beginning in 1995 and ending in 2005. By design, approximately 75% of the cohort had a first degree relative with dementia of the Alzheimer type.

While the study was at the NIH, participants were administered a comprehensive neuropsychological battery and clinical assessment annually, which included a physical and neurological examination, record of medication use, and behavioral and mood assessments. Blood, CSF, and MRI scans were obtained approximately every two years. In 2005, the study was stopped for administrative reasons. The study was reinitiated in 2009 when a research team from the Johns Hopkins University (JHU) School of Medicine was funded to re-establish the cohort and continue annual cognitive and clinical assessments, collect blood, and evaluate the previously acquired cognitive and biomarker data. In 2015, biennial collection of CSF and MRI scans was re-established, and the acquisition of positron emission tomography (PET) scans using Pittsburgh Compound B (PiB) was begun. Tau PET imaging was initiated in 2017. See Figure 1 in the manuscript for a study timeline. This study was approved by the JHU Institutional Review Board, and data collection is ongoing.

*Clinical assessments and consensus diagnosis procedure*

The clinical and cognitive assessments in the BIOCARD study have been completed annually, first at the NIH and subsequently at JHU.[1] Clinical assessments include a physical and neurological examination, record of medication use, behavioral and mood assessments, family history of dementia, history of symptom onset, and a Clinical Dementia Rating[2] (CDR®) based on a semi-structured interview.

All consensus diagnoses used in study analyses are based on procedures implemented by the staff of the JHU BIOCARD Clinical Core, with diagnoses completed prospectively for all JHU visits and retrospectively for all NIH visits. All cases are handled in a manner comparable with those employed in the National Institute on Aging Alzheimer's Disease Centers program.

First, a syndromic diagnosis is established (e.g., normal, mild cognitive impairment, dementia), using three sources of information: (1) clinical data pertaining to the medical, neurological, and psychiatric status of the individual; (2) reports of changes in cognition by the individual and by collateral sources based on a semi-structured interview (the CDR); and (3) decline in cognitive performance, based on review of longitudinal testing from multiple domains (and comparison to published norms).

Second, if a subject is deemed to be impaired, a decision is made about the likely etiology of the syndrome, based on the medical, neurologic, and psychiatric information collected at each visit, as well as medical records obtained from the subject, where necessary. More than one etiology can be endorsed for each subject (e.g., AD and vascular disease, PD and depression).

This consensus diagnosis procedure follows the diagnostic recommendations incorporated in the NIA/AA working group reports for the diagnosis of MCI[3] and dementia due to AD.[4] A diagnosis of 'Impaired Not MCI' is also utilized, reflecting this incorporation in the AD Centers Program diagnostic procedures. Within the context of this study, this diagnosis typically reflects contrasting information from the CDR interview and the cognitive test scores (i.e., the subject or collateral source expressed concerns about cognitive changes in daily life but the cognitive testing did not show changes, or visa versa). Diagnoses (and determination of likely etiology) are made without knowledge of biomarker measures.

The estimated age of onset of clinical symptoms is established separately, based primarily on the semi-structured interview (the CDR) with the subject and the collateral source. The age of symptom onset is established for the first visit at which the subject is deemed to be impaired and is reconfirmed on subsequent visits; thus, there is a single age of symptom onset for each subject with a diagnosis of MCI or dementia.

*Cognitive Assessments*

The annual cognitive battery administered at the NIH and at JHU covers all major cognitive domains, including memory, executive function, language, visuospatial ability, attention, speed of processing and psychomotor speed. The complete battery has been described previously[1].

*References*

1. Albert M, Soldan A, Gottesman R, et al. Cognitive changes preceding clinical symptom onset of mild cognitive impairment and relationship to ApoE genotype. Curr Alzheimer Res. 2014;11(8):773-84.
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**Supplementary Materials 2:**
**Reasons participants were excluded from the analyses**

Participants were successively excluded from the WMH volume analyses for the following reasons: (1) baseline FLAIR scans for deriving WMH volumes were missing (n = 35); (2) the estimated age of onset of clinical symptoms of MCI was determined to be at or prior to their baseline WMH volume measure (n = 14); (3) participants have not yet re-enrolled, or have withdrawn from the study (n = 29).

Participants were successively excluded from the vascular risk score analyses for the following reasons: (1) missing baseline vascular risk scores (n = 94); (2) the estimated age of onset of clinical symptoms of MCI was determined to be at or prior to their baseline vascular risk score (n = 24); (3) participants have not yet re-enrolled, or have withdrawn from the study (n = 1); (4) missing cognitive reserve composite score (n = 1).

**Supplementary Materials 3:**
**White matter hyperintensity volume calculation**

An automated method was used to quantify a measure of global WMH volume from each scan (for additional details, see https://www.alz.washington.edu/WEB/adni_proto.pdf). Briefly, following skull removal, images were nonlinearly registered to a minimal deformation template adapted for age range of 60 years and above. Following field inhomogeneity bias correction, an Expectation-Maximization algorithm was used to segment gray, white, and CSF tissues. WMH measures were calculated based on a combination of FLAIR and 3D T1 images using a modified Bayesian probability structure based on histogram fitting. Likelihood estimates of the native image were calculated through histogram segmentation and thresholding. All segmentation was initially performed in standard space. The resulting probability likelihood values of WMH at each voxel in the white matter were then thresholded at 3.5 SD above the mean to create a binary WMH mask, which was then back-transformed to native space for tissue volume calculation.

**Supplementary Materials 4:**
**Distribution of etiologies proposed to underly the clinical diagnoses of MCI or dementia**

As shown in the table below, the majority of participants who progressed to MCI or dementia had probable or possible AD as a primary or contributing etiology (i.e., N=54 and N=38 in the WMH and vascular risk analyses, respectively, which corresponds to 90% and 84% of progressors in the analysis). Among those with probable or possible AD, approximately half were judged to also have vascular disease as primary or contributing (i.e., N=25/54 or 46% and N=18/38 = 53% in the WMH and vascular risk score analyses, respectively).

| | Participants who progress to MCI or dementia in WMH Analyses (N=60) | Participants who progress to MCI or dementia in Vascular Risk Score Analyses (N=45) |
|---|---|---|
| N with probable or possible AD | 54 | 38 |
| N with vascular disease | 29 | 25 |
| N with depression | 12 | 12 |
| N with medication usage | 4 | 2 |
| N with substance use/abuse | 1 | 0 |
| N with other etiologies | 11 | 8 |

*Note: For both columns, the N sums more than the total N with MCI or dementia because more than one etiology can be endorsed for each participant.*

**Supplementary Materials 5:**
**Distribution of etiologies proposed to underly the clinical diagnoses of MCI or dementia as a function of baseline WMH and vascular risk burden**

Among progressors with probable or possible AD as a primary or contributing etiology, there was a higher tendency for those with high baseline WMH volumes and high vascular risk scores to be diagnosed as also having vascular disease contributing compared to participants with low baseline WMH or vascular risk scores. For example, among progressors in the WMH analysis, 13/25 or 52% of those with high WMH at baseline were subsequently diagnosed as having AD and vascular contributions compared to 8/29 or 27% of those with low baseline WMH. This suggests that participants with greater WMH or vascular risk burden at baseline are more likely to eventually progress to MCI/dementia with vascular disease contributing. However, the differences in the eventual diagnoses as a function of baseline WMH and vascular risk scores did not reach statistical significance due to the small sample size.

| | Participants who progress to MCI or dementia in WMH Analyses (N=60) | Participants who progress to MCI or dementia in Vascular Risk Score Analyses (N=45) |
|---|---|---|
| N with probable or possible AD but NOT vascular disease | 29 | 18 |
| N with probable or possible AD AND vascular disease | 25 | 20 |
| (N with probable or possible AD but NOT vascular disease) and high baseline WMH volume | 8 | 4 |
| (N with probable or possible AD AND vascular disease) and high baseline WMH volume | 13 | 10 |
| (N with probable or possible AD but NOT vascular disease) and vascular risk score >=1 | 11 | 12 |
| (N with probable or possible AD AND vascular disease) and vascular risk score >=1 | 11 | 12 |