

# Cognitive Changes Preceding Clinical Symptom Onset of Mild Cognitive Impairment and Relationship to *ApoE* Genotype

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**Abstract:** Background: This study had two goals (1) to evaluate changes in neuropsychological performance among cognitively normal individuals that might precede the onset of clinical symptoms, and (2) to examine the impact of Apolipoprotein E (*ApoE*) genotype on these changes. Methods: Longitudinal neuropsychological, clinical assessments and consensus diagnoses were completed prospectively in 268 cognitively normal individuals. The mean duration of follow-up was 9.2 years ( $\pm$  3.3). 208 participants remained normal and 60 developed cognitive decline, consistent with a diagnosis of MCI or dementia. Cox regression analyses were completed, for both baseline scores and rate of change in scores, in relation to time to onset of clinical symptoms. Analyses were completed both with and without *ApoE-4* status included. Interactions with *ApoE-4* status were also examined. Results: Lower baseline test scores, as well as greater rate of change in test scores, were associated with time to onset of clinical symptoms ( $p < 0.001$ ). The mean time from baseline to onset of clinical symptoms was 6.15 ( $\pm$  3.4) years. The presence of an *ApoE-4* allele doubled the risk of progression. The rate of change in two of the test scores was significantly different in *ApoE-4* carriers vs. non-carriers. Conclusions: Cognitive performance declines prior to the onset of clinical symptoms that are a harbinger of a diagnosis of MCI. Cognitive changes in normal individuals who will subsequently decline may be observed at least 6.5 years prior to symptom onset. In addition, the risk of decline is doubled among individuals with an *ApoE-4* allele.

**Keywords:** Apolipoprotein E genotype, cognitive decline, episodic memory, longitudinal follow-up, mild cognitive impairment, preclinical Alzheimer's disease.

## 1. INTRODUCTION

There is substantial evidence that a subset of older individuals who are cognitively normal have Alzheimer's disease (AD) pathology in their brains, based on both autopsy findings [1-3] and amyloid imaging studies [4-6]. It has been hypothesized that such individuals are at increased risk for developing cognitive decline over time, and that at some point during this 'preclinical' phase of disease, cognitive changes become evident [7], even though clinical symptoms have not yet been reported by the individual or his/her collateral source. Additionally, it has been proposed that Apolipoprotein E (*ApoE*) genotype may alter the rate of cognitive decline during the preclinical phase of AD (see below).

A small number of studies have followed cognitively normal individuals over time and retrospectively examined their cognitive performance to determine if changes in cognition precede the clinical diagnosis of mild cognitive impairment (MCI). These studies have shown that lower baseline test scores in episodic memory predict progression from normal cognition to diagnosis of MCI or AD [8-10]. Tests assessing psychomotor speed [11] have also been associated with time to progress from normal cognition to mild

impairment. A number of studies have also examined the rate of cognitive decline during the preclinical phase of AD. They have found that 3 to 4 years before a diagnosis of MCI, performance on tests of episodic memory [12-15], visuospatial processing [12, 14], executive function [13, 14], and verbal fluency [12] change at a greater rate in cognitively normal individuals who subsequently progress to MCI or AD dementia compared to those who remain normal.

A number of studies have also sought to determine whether cognitive changes evident during the preclinical phase of AD are influenced by the effects of the *E-4* allele of the *ApoE* gene, the major genetic risk factor for late onset AD (see [16] for a meta-analysis). Cross-sectional studies provide evidence for lower cognitive test scores among *ApoE-4* carriers vs. non-carriers. For example, cross-sectional studies of cognitively normal middle-aged and older adults have reported lower performance among *ApoE-4* carriers compared to non-carriers on tests of episodic memory [17], verbal fluency [18], and attention [19]. However, cross-sectional cognitive differences as a function of *ApoE* genotype are not always evident, particularly in studies that largely focus on middle-aged (45 - 65 years) individuals [20-23].

Rate of change in cognitive test scores in relation to *ApoE* genotype has also been examined. Short-term longitudinal studies suggest that episodic memory declines more rapidly among cognitively normal older individuals who are

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*ApoE-4* carriers vs. non-carriers [20, 24-27], while performance on other cognitive tests shows smaller or no differences in the rate of decline in relation to *ApoE-4* genotype [20, 24-27]. However, the follow-up period in these studies has been relatively short, and clinical diagnostic outcomes were not available.

The current study, due, in part, to its large sample size and unusually long follow-up period, can address several issues that remain unresolved by these studies. First, few studies have examined differences in cognitive performance at both 'baseline' (when subjects were first enrolled) as well as in rate of change over time. Second, the outcome has, in all instances, been the diagnosis of MCI (or its equivalent). Since onset of clinical symptoms typically precedes the diagnosis of MCI, it is unclear if cognitive changes are evident even earlier than what has been previously reported. Third, the impact of *ApoE* genotype on cognitive changes during preclinical AD should be elucidated. There appear to be at least two alternative explanations for prior findings related to *ApoE-4* status: (1) it is possible that the lower cognitive scores among *ApoE-4* carriers reflect the fact that a greater proportion of *ApoE-4* carriers than non-carriers are in the preclinical phase of AD, or (2) cognitive changes during the preclinical phase of AD progress more rapidly in *ApoE-4* carriers than non-carriers. If the former were true, one would expect that the degree of cognitive change preceding the emergence of the clinical symptoms of AD would be similar for *ApoE-4* carriers and non-carriers. Whereas, if the latter were the case, one would expect to see a greater rate of decline on test scores among *ApoE-4* carriers in the preclinical phase of AD vs. non-carriers. To determine which of these two alternatives is correct, it is necessary to examine prospective longitudinal cognitive trajectories in cognitively normal adults who later develop clinical symptoms of AD. This was the goal of the present study.

## 2. SUBJECTS AND METHODS

### 2.1. Study Design

This study was designed to recruit and follow a cohort of cognitively normal individuals who were primarily in middle age. By design, approximately three quarters of the participants had a first degree relative with dementia of the Alzheimer type. The overarching goal of the study was to identify variables among cognitively normal individuals that could predict the subsequent development of mild to moderate symptoms of AD. Toward that end, subjects were administered a comprehensive neuropsychological battery annually. Magnetic resonance imaging (MRI) scans, cerebrospinal fluid (CSF), and blood specimens were obtained every two years. *ApoE* genotype was established for each partici-

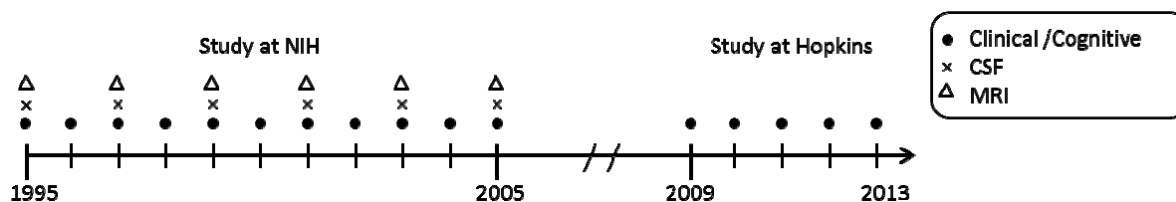
pant after enrollment. The original study was initiated at the NIH in 1995, and was stopped in 2005 for administrative reasons. In 2008, the National Institute on Aging (NIA) and the National Institute of Mental Health (NIMH) decided to re-establish the study. In 2009, a research team at the Johns Hopkins School of Medicine was funded to re-establish the cohort, continue the annual clinical and cognitive assessments, collect blood, and evaluate the previously acquired MRI scans, CSF and blood specimens. All participants who agreed to continued follow-up signed consent forms approved by the Johns Hopkins Institutional Review Board. To our knowledge, this is the only study in participants who were primarily middle aged and cognitively normal at entry, with this set of measures, and with such a long duration of follow-up. The approximate timeline of the study and types of measurements collected each year are shown in (Fig. 1).

### 2.2. Selection of Participants

A total of 354 individuals were initially enrolled in the study. Recruitment was conducted by the staff of the Geriatric Psychiatry Branch (GPB) of the intramural program of the NIMH, beginning in 1995 and ending in 2005. Subjects were recruited via printed advertisements, articles in local or national media, informational lectures, or word-of-mouth.

At enrollment, subjects in the study were admitted to the Clinical Center at the National Institutes of Health (NIH) for 3 days, after providing written informed consent. They received a detailed physical, neurological and psychiatric examination, an electrocardiogram, and standard laboratory studies (e.g., complete blood count, vitamin B12, thyroid function, etc). Mood was assessed with the Hamilton Depression Scale, the Beck Depression Inventory and the Spielberger Anxiety Scale. During the 3-day visit, a neuropsychological battery was administered, a magnetic resonance imaging (MRI) scan was obtained, blood was collected for genetic analysis, and a lumbar puncture was performed.

The GPB staff at the NIH reviewed the results of the clinical and cognitive assessments and excluded participants who were judged to be cognitively impaired, as determined by the cognitive testing or by evidence of clinical symptoms based on reports by collateral sources. Subjects were also excluded who had a history of significant medical problems such as severe cardiovascular disease (e.g., atrial fibrillation), chronic psychiatric disorders (e.g., schizophrenia, alcohol or drug abuse), chronic neurologic disorders (e.g., epilepsy, multiple sclerosis) or severe cerebrovascular disease (based on the MRI scan). Five subjects did not meet the entry criteria and were excluded at baseline, leaving a total 349 participants, who were followed over time. All but 5 of the participants were native English speakers.



**Fig. (1).** Approximate timeline of BIOCARD study indicating the types of measurements obtained each year when the study was at the NIH and since the study has been at Johns Hopkins.

### 2.3. Neuropsychological Assessment

All of the neuropsychological tests included in the battery administered by the Johns Hopkins research team were also administered when participants were examined at the NIH (with one exception, as described below). The battery covered a broad range of cognitive domains, including: memory, executive function, language, visuospatial ability, attention, speed of processing and psychomotor speed. The test battery was as follows: the Logical Memory (raw score and percent Retention) and Paired Associates Subtests of the Wechsler Memory Scale - Revised (WMS-R) [28], the California Verbal Learning Test (CVLT) [29], the Rey-Osterreith Complex Figure (copy and recall) [30], the Trail Making Test (TMT), Parts A and B [31], the Boston Naming Test (30-item version) (BNT) [32], Letter and Category Fluency [33], the Block Design (BD) subtest of the Wechsler Adult Intelligence Scale - Revised (WAIS-R) [34], Digit Span Forward and Backward from the WMS-R [28], the Digit Symbol Test from the WAIS-R [34], the Mini-Mental State Examination (MMSE) [35] and the Lafayette Grooved Pegboard test [36]. The Grooved Pegboard was the only test included in the battery at Johns Hopkins that had not been given previously. Additional neuropsychological tests were given at the NIH, which were not continued, primarily due to the time limitations of the study visit. These included: the complete Wechsler Adult Intelligence Scale- Revised, the complete Wechsler Memory Scale - Revised, a version of the Buschke Cued Selective Reminding test developed by the GPB staff, the Stroop Test, the Delis-Kaplan Executive Function System, and Clock drawing.

### 2.4. Clinical Assessment

Each of the participants also had an annual clinical examination at the NIH. Since the study has been conducted at Johns Hopkins, the annual examination has included the following: a physical and neurological examination, record of medication use, behavioral and mood assessments [37, 38], family history of dementia, history of symptom onset, and a Clinical Dementia Rating (CDR), based on a semi-structured interview [39, 40]. All measures are administered and coded by the evaluating clinician. The clinical assessments given at the NIH covered similar domains (as noted above).

### 2.5. Consensus Diagnoses

Each subject included in these analyses received a consensus diagnosis by the staff of the BIOCARD Clinical Core at Johns Hopkins. This research team included: neurologists, neuropsychologists, research nurses and research assistants. For the participants with evidence of clinical or cognitive dysfunction (i.e., individuals with a CDR score > 0 and/or evidence of decline on cognitive testing), a clinical summary was prepared that included information about demographics, family history of dementia, work history and past history of medical, psychiatric and neurologic disease, record of medication use and results from the neurologic and psychiatric evaluation. The reports of clinical symptoms from the subject and collateral sources were summarized, based on a version of the CDR that incorporates questions targeting the types of problems encountered by very mildly impaired individuals [41]. The results of the neuropsychological testing

were also reviewed. The diagnostic process for each participant was handled in a comparable manner: (1) clinical data were examined pertaining to the medical, neurologic and psychiatric status of the subject, (2) reports of changes in cognition by the subject and by collateral sources were examined, and (3) decline in cognitive performance was established. All cognitive test scores were available during the consensus diagnosis. These data were used to: (1) determine whether the subject had become cognitively impaired, and (2) determine the likely etiology of such impairment. The age at which the clinical symptoms began was based primarily on the reports of clinical symptoms from the subject and from collateral sources reported during the semi-structured CDR interview. The version of the CDR used in the present study was specifically adapted to include questions about the types of problems that very mildly impaired patients experience (as noted above). To the extent possible, the clinical decision about symptom onset did not use the cognitive test scores. As such, our main outcome variable, the estimated age of onset of clinical symptoms, was largely independent of the cognitive test scores (i.e., our predictor variables). These diagnostic procedures are comparable to those implemented in the Alzheimer's Disease Centers program, supported by the NIA. It is acknowledged that, as this process is dependent on the clinical and cognitive data available at any one point in time, some subjects who are diagnosed as having MCI may subsequently be diagnosed as normal or as 'Impaired not MCI'. This change in diagnosis occurred in 4 of the subjects whose data are presented here.

Diagnoses, cognitive testing, and clinical examinations were performed on an annual basis (see Fig. 1) both at the NIH and since the study has been at Johns Hopkins. The age of symptom onset was established for the first visit at which the subject was deemed to be impaired and was reconfirmed on subsequent visits; thus there is a single age of symptom onset for each subject with a diagnosis of MCI or dementia.

### 2.6. APOE Genotyping and Coding

*APOE* genotype was established in all but one of the study participants ( $n = 348$ ). Genotypes were determined by restriction endonuclease digestion of polymerase chain reaction amplified genomic DNA (performed by Athena Diagnostics, Worcester, MA). *ApoE-4* carrier status was coded by creating an indicator variable, with *ApoE-4* carriers coded as 1, if they had at least one  $\epsilon 4$  allele, and non-carriers coded as 0.

### 2.7. Statistical Methods

The primary statistical analyses were designed to determine if performance on any of the cognitive tests administered when the subjects were first enrolled (i.e., at 'baseline') were related to time to onset of clinical symptoms. Exploratory analyses were first conducted based on data for two groups: (1) subjects who were cognitively normal at their last visit ( $n=208$ ), and (2) subjects who received a diagnosis of MCI [42], or dementia of the Alzheimer type [43] ( $n=60$ ) at their last visit (including subjects who are now deceased). A set of Cox regression analyses was then performed, using time to onset of clinical symptoms as the outcome variable. These analyses used baseline test scores and time-dependent-

rate-of-change in scores as covariates; the censoring time was defined as the last date of diagnosis. These models tested whether each of the baseline neuropsychological test scores (adjusted by baseline age and education) were related to time to onset of cognitive impairment, and whether there was a differential rate of change over time in the test scores prior to the onset of clinical symptoms. A second set of analyses was completed, with *ApoE-4* status included as a covariate. The interaction between *ApoE-4* status and both the baseline test score and the rate of change in the test score was also examined. Note that analyses comparing those with a family history of dementia to those without will require longer follow-up, as only one-quarter of the cohort has no family history.

In Cox regression analysis (in which time-to-event' is the outcome of interest), numerous types of 'failure times' can be selected as the outcome variable. The primary requirement is that the outcome measure must properly characterize the progression of the disease in question [44, 45]. In this study, we decided to use the age of onset of clinical symptoms as the 'failure time' of interest, specifically in those subjects who ultimately received a consensus diagnosis of MCI or AD dementia. The estimated age at which the clinical symptoms began was determined during the consensus review process, as described above. We chose to use the age of onset of symptoms as the 'failure time' instead of the date of diagnosis, specifically because of the 4-year gap in the study, during which the subjects were not examined. Using estimated age of onset of symptoms allowed us to generate a 'failure time' measure that was obtained in an identical manner for each subject, including those whose onset of symptoms occurred during the 4-year gap. This would not have been possible if we had used date of diagnosis, since no subjects could have received a consensus diagnosis during the 4-year gap. Additionally, this approach allowed us to exclude subjects whose symptoms were estimated to have begun at, or before, baseline (n=13).

In the Cox regression models, the rate of change was calculated as the slope of the individual changes in test score over time, which was then converted to a z-score, i.e., a 'normed' slope. The following procedure was used: (1) the cognitive test score at each follow-up time (time t) minus the cognitive test score at baseline was calculated, and divided by the difference in the time between these two measurements [(measurement at follow-up time t) - (measurement at baseline 0)] / t. (2) each of these individual slope values were then centered and standardized across subjects; thus, the rate of change had a mean of zero and a variance of 1. The standardized slope values were then used as time-dependent covariates in the Cox regression models, with each subject contributing a standardized slope value for each follow-up assessment that was available (e.g., 0-1, 0-2, 0-3, 0-4, etc). These measurements were not smoothed, but were instead treated as a 'step function', where the rate of change was not varied between the individual follow-up intervals. The baseline test scores were included as covariates in all rate-of-change Cox models.

A multivariate model was also completed to determine whether a combination of baseline cognitive measures could be identified that significantly predicted time to progression

from normal cognition to onset of clinical symptoms. Since individuals in the analyzed dataset were required to be symptom-free at baseline, we used statistical techniques to adjust for left-truncation in the data [46]. For these analyses, tests that were highly correlated with one another were excluded [e.g., of the three scores from the Logical Memory subtest (Immediate recall, Delayed recall and Retention score) only the Logical Memory Retention score was included in the model]. A least absolute shrinkage and selection operator (LASSO) method was also used to exclude variables, in advance, that did not contribute significantly to the model [47, 48].

Additionally, we calculated hazard ratios for each of the significant variables in the univariate baseline models and in the models examining rate of change over time. These analyses were completed with and without the inclusion of *ApoE-4* status. Prior to these analyses, the test scores were converted to z scores (i.e., scores with a mean of 0 and a standard deviation of 1, averaged over the scores for the normal subjects) so that it would be possible to compare the hazard ratios for each test to one another along the same metric. The hazard ratio indicates the change in relative risk per one unit change in the predictor. For example, if the hazard ratio for the Paired Associates Immediate Recall is 0.53, the hazard of clinical symptom onset is reduced by a factor of 0.53 (i.e., by 47%) for each standard deviation increase in this test score. Likewise, a hazard ratio of 1.98 indicates that the hazard of clinical symptom onset is increased by a factor of 1.98 (i.e., 98%) for each standard deviation increase in the measure. All data analyses presented here used R, version 2.14.1.

### 3. RESULTS

#### 3.1. Subject Characteristics

The cognitive data presented here pertain to 268 of the 349 participants (mean duration of follow-up = 9.2 years, SD= 3.3). Their demographic characteristics are shown in (Table 1), as are the characteristics of the cohort as a whole (n=349). The reasons that some subjects were excluded from the analyses were as follows: (1) The onset of symptoms was estimated to have occurred at or prior to baseline (N=13); (2) No follow-up data were available (N=8); (3) Subjects have withdrawn from the study (N=10) or are still considering whether to re-enroll (N=21); and (4) Subjects received a diagnosis of 'Impaired-not-MCI' (N=29). This diagnostic category includes individuals with no declines in cognitive testing but concerns of cognitive decline by the subject or informant, individuals with no concerns of cognitive decline by the subject or informant but evidence of decline on cognitive testing, and those with declines in cognition thought to be the result of non-AD pathology. Of note, when subjects with a diagnosis of 'Impaired-not-MCI' were included among the normal subjects comparable results were obtained.

Table 2 shows the characteristics at baseline of the subjects who remained normal at their last visit (n=208) vs. those who subsequently received a diagnosis of MCI (n=48) or dementia (n=12) (total n=60) (the primary differences between the two groups at baseline related to age). The percentage of subjects who were *ApoE-4* positive was similar in the two groups (33.6% vs. 36.0%). The findings were also

comparable when the one participant who became impaired, and also had a dominant mutation for AD, was excluded.

**Table 1. Participant characteristics at baseline in relation to cohort as a whole.**

Variable	Cohort as a Whole (N=349)	Subjects in Analyses (N=268)
Age, mean years (SD)	57.3 (10.4)	56.9 (10.3)
Gender, females (%)	57.6%	61.6%
Education, mean years (SD)	17.0 (2.4)	17.1 (2.3)
Ethnicity, Caucasians (%)	97.1%	97.0%
<i>ApoE-4</i> carriers (%)	33.6%	36.0%
MMSE, mean score (SD)	29.5 (0.9)	29.6 (0.8)
NART, mean score (SD)	119.6 (7.9)	120.4 (7.6)

Abbreviations: *ApoE-4*, apolipoprotein E-4; MMSE, Mini-Mental State Exam; NART, National Adult Reading Test

**Table 2. Participant characteristics at baseline.**

Variable	Remained Normal (N=208)	Progressed to MCI or Dementia (N=60)
Age, mean years (SD)	55.4 (9.6)	62.4 (10.9)**
Gender, females (%)	63.0 %	56.7%
Education, mean years (SD)	17.3 (2.3)	16.6 (2.3)
Ethnicity, Caucasians (%)	98.6%	91.7%*
<i>ApoE-4</i> carriers (%)	33.2%	45.8%
MMSE, mean score (SD)	29.6 (0.7)	29.4 (1.0)
NART, mean score (SD)	121.5 (6.5)	116.3 (10.0)*

Abbreviations: *ApoE-4*, apolipoprotein E-4; MMSE, Mini-Mental State Exam; NART, National Adult Reading Test

\*p = .02 \*\*p = .001

### 3.2. Cox Regression Models of Test Scores at Baseline

A set of 17 variables from the cognitive battery were selected for this analysis. Selection of the variables was based on exploratory plots of the change pattern in each of the cognitive measures over time. Table 3 presents the means and standard deviations of these variables at baseline. The mean time from baseline to onset of clinical symptoms was 6.15 (+/- 3.4) years. Univariate Cox regression models were completed for each of the 17 variables at baseline (adjusted for age and education). For 9 of the 17 measures examined, there was a statistically significant association between the baseline measure and age-of-onset of impairment at the p <

.001 level. The hazard ratios for these 9 variables and associated p-values are shown in (Table 4) (Baseline Test Scores). For each of these cognitive variables, after accounting for baseline age and education, each standard deviation increase in the test score was associated with approximately a 40 – 60% reduction in the risk of symptom onset (all *HR* <=0.63).

**Table 3. Mean and standard deviation of cognitive test scores at baseline for subjects who remained Normal vs. Subjects who developed clinical symptoms and were diagnosed with MCI or AD dementia.**

Cognitive Test	Remained Normal	Progressed to MCI or AD Dementia
Logical Memory Immediate	15.28 (2.78)	13.48 (2.99)
Logical Memory Delayed	13.43 (3.26)	11.08 (3.70)
Logical Memory % Retention	87.36 (11.33)	80.63 (20.06)
Paired Associate Immediate	21.03 (2.79)	18.88 (3.12)
Paired Associate Delayed	7.69 (0.63)	7.31 (0.99)
CVLT Total Trials 1-5	53.93 (8.90)	48.79 (10.43)
CVLT Short Delay Free Recall	11.53 (2.92)	10.68 (3.30)
CVLT Long Delay Free Recall	12.19 (2.78)	10.74 (3.30)
CVLT Short Delay % Retention	86.97 (18.18)	85.19 (18.06)
Rey Figure Copy	33.89 (2.22)	32.83 (2.89)
Rey Figure Recall	19.22 (6.10)	14.66 (6.54)
Block Design Subtest	34.48 (8.18)	27.47 (8.72)
Boston Naming % Correct	97.02 (4.51)	93.67 (6.63)
Category Fluency Animals	24.11 (4.86)	21.25 (4.30)
Digit Symbol	55.66 (11.33)	45.78 (9.85)
Trail Making, Part A Time in sec	30.22 (10.74)	46.78 (28.45)
Trail Making, Part B Time in sec	65.41 (19.92)	104.00 (52.23)

Abbreviations: CVLT, California Verbal Learning Test

A second set of Cox regression models were performed, additionally including *ApoE-4* status as a predictor, as well as the interaction between *ApoE-4* status and the baseline cognitive score. The interaction term was not significant for any of these 9 measures (all p > 0.1), but *ApoE-4* status was significant in all models (all *HR* >=1.98, all p < 0.01, see Table 5, Baseline Test Scores). These results suggest that baseline cognitive performance and *ApoE-4* status are independently associated with the time to onset of clinical symptoms, with *ApoE-4* status increasing the risk of progressing from normal cognition to symptom onset by about 100% (i.e., a doubling of risk).

**Table 4. Hazard ratios for univariate analyses of baseline and rate of change in cognitive test scores in relation to onset of clinical symptoms.**

Cognitive Test	Baseline Test Scores		Time-Dependent Rate of Change in Scores	
	Hazard Ratio* (95% CI)	p-value	Hazard Ratio* (95% CI)	p-value
Logical Memory Immediate	0.59 (0.44-0.80)	0.0005	0.58 (0.36-0.93)	0.024
Logical Memory Delayed	0.48 (0.36-0.65)	0.0001	0.54 (0.34-0.86)	0.009
Logical Memory % Retention	0.56 0.44-0.71	0.0001	0.54 (0.38-0.76)	0.001
Paired Associate Immediate	0.53 (0.41-0.70)	0.0001	0.37 (0.24-0.57)	0.001
Paired Associate Delayed	0.63 (0.49-0.82)	0.0004	0.63 (0.43-0.92)	0.016
Key Figure Recall	0.62 (0.46-0.82)	0.0008	0.49 (0.38-0.63)	0.001
Block Design Subtest	0.53 (0.38-0.74)	0.0001	0.40 (0.19-0.83)	0.014
Boston Naming Test	0.57 (0.43-0.74)	0.0001	0.69 (0.55-0.85)	0.001
Digit Symbol Test	0.41 (0.29-0.58)	0.0001	0.47 (0.35-0.64)	0.001

\*The hazard ratios are for standardized scores (adjusted for age at baseline and education), and are presented per one standard deviation increase in each cognitive test.  
Abbreviations: CI, confidence interval

**Table 5. Hazard ratios for univariate analyses of baseline and rate of change in cognitive test scores and ApoE-4 status in relation to onset of clinical symptoms.**

Cognitive Test	Baseline Test Scores		Time-Dependent Rate of Change in Scores	
	Hazard Ratio* (95% CI)	p-value	Hazard Ratio* (95% CI)	p-value
Logical Memory Immediate <i>ApoE-4</i>	0.61 (0.47-0.81)	0.001	0.56 (0.36-0.89)	0.014
	2.35 (1.32-4.17)	0.004	2.25 (1.31-3.87)	0.003
Logical Memory Delayed <i>ApoE-4</i>	0.51 (0.39-0.68)	0.001	0.53 (0.33-0.83)	0.006
	2.18 (1.24-3.85)	0.007	2.12 (1.23-3.64)	0.007
Logical Memory % Retention <i>ApoE-4</i>	0.59 (0.46-0.75)	0.001	0.55 (0.40-0.76)	0.001
	1.98 (1.14-3.45)	0.015	1.78 (1.04-3.05)	0.036
Paired Associate Immediate <i>ApoE-4</i>	0.55 (0.42-0.70)	0.001	0.38 (0.25-0.60)	0.001
	2.39 (1.35-4.26)	0.003	1.87 (1.05-3.32)	0.033

(Table 5) contd....

Cognitive Test	Baseline Test Scores		Time-Dependent Rate of Change in Scores	
	Hazard Ratio* (95% CI)	p-value	Hazard Ratio* (95% CI)	p-value
Paired Associate Delayed <i>ApoE-4</i>	0.64 (0.52-0.79)	0.001	0.64 (0.45-0.93)	0.020
	2.77 (1.56-4.91)	0.001	2.31 (1.34-3.96)	0.002
Rey Figure Recall <i>ApoE-4</i>	0.57 (0.43-0.75)	0.001	0.46 (0.36-0.61)	0.001
	2.75 (1.56-4.84)	0.001	2.74 (1.65-4.55)	0.001
Block Design Subtest <i>ApoE-4</i>	0.54 (0.40-0.74)	0.001	0.40 (0.19-0.82)	0.013
	2.35 (1.35-4.07)	0.002	2.16 (1.28-3.65)	0.004
Boston Naming Test <i>ApoE-4</i>	0.63 (0.51-0.78)	0.001	0.68 (0.55-0.85)	0.001
	2.00 (1.16-3.43)	0.013	1.80 (1.06-3.06)	0.031
Digit Symbol Test <i>ApoE-4</i>	0.44 (0.31-0.61)	0.001	0.47 (0.34-0.67)	0.001
	2.01 (1.17-3.47)	0.012	1.94 (1.18-3.20)	0.009

\*The first line for each cognitive test is the hazard ratio for the standardized scores of each cognitive test, adjusted by - baseline age, education and *ApoE-4* status (i.e., *ApoE-4* positive or *ApoE-4* negative). The second line for each cognitive test is the hazard ratio, comparing those who were *ApoE-4* positive to those who were *ApoE-4* negative, adjusted by - the standardized scores of the cognitive test, baseline age and education.  
Abbreviations: CI, confidence interval

### 3.3. Cox Regression Models of Rate of Change in Test Scores

For each of these 9 measures, univariate Cox regression models were also completed, with baseline test scores and time-dependent-rate-of-change in scores as covariates (adjusted for baseline age and education). As shown in (Table 4), the time-dependent rate of change in scores was significant in all models (all  $HR \leq 0.069$ , all  $p < 0.025$ ), indicating that there was a differential rate of change over time for participants who remained cognitively normal compared to those who progressed to MCI or AD for all 9 of these test scores, prior to the onset of clinical symptoms. For each one standard deviation increase in the rate of change over time (i.e., decrease in test score), the risk of progressing from normal cognition to symptom onset increased by 30 – 60%. *ApoE-4* status was significant, when included in the models for each of these 9 measures ( $p < 0.03$ ).

When *ApoE-4* status and the interaction between *ApoE-4* status and the rate of change were added to these models, *ApoE-4* status was again significant in all models (all  $HR > 1.78$ , all  $p < 0.04$ ) and the interaction term was significant for 2 of the 9 measures [i.e., the Boston Naming Test ( $p=0.003$ ) and the Logical Memory Retention Score ( $p \leq 0.05$ )]. The hazard ratios for *ApoE-4* and the cognitive variables are shown in (Table 5) (Time-Dependent Rate of

Change in Scores). These findings suggest that with the exception of two cognitive measures, the association between the rate of change in cognitive performance and the time to onset of clinical symptoms is comparable for *ApoE-4* carriers and non-carriers. By comparison, for the Boston Naming Test and the Logical Memory Retention Score, there was evidence that *ApoE-4* carriers declined at a greater rate relative to non-carriers prior to the onset of clinical symptoms.

### 3.4. Cox Regression Multivariate Model

Lastly, we completed a Cox multivariate model with baseline test scores as covariates (adjusted for age and education) in which the 9 variables mentioned above were included. Four of the 9 variables in the multivariate model were statistically significant (as well as age), as was the overall model ( $p < 0.0001$ ). The significant cognitive tests were: the Digit Symbol Test ( $p = 0.0001$ ), Paired Associates immediate recall ( $p = 0.008$ ), Logical Memory Retention ( $p = 0.003$ ), and the Boston Naming Test ( $p = .001$ ). When *ApoE-4* status was added to this model, the same 4 cognitive variables remained significant and *ApoE-4* status was also significant ( $p < 0.005$ ). The hazard ratios and p-values for significant variables in this model are shown in (Table 6).

**Table 6. Hazard Ratios for Significant Variables in Multivariate Analysis of Baseline Test Scores in Relation to Onset of Clinical Symptoms.**

Cognitive Test	Baseline Test Scores	
	Hazard Ratio* (95% CI)	p-value
Logical Memory % Retention	0.75 (0.59-0.95)	0.017
Paired Associate Immediate	0.67 (0.51-0.88)	0.005
Boston Naming Test	0.70 (0.54-0.93)	0.011
Digit Symbol Test	0.52 (0.37-0.72)	0.001
<i>ApoE-4</i>	2.43 (1.32-4.49)	0.005

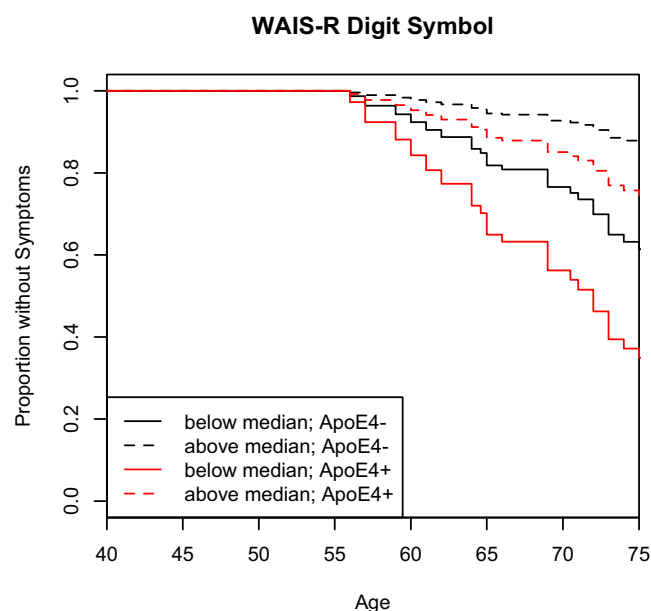
### 3.5. Cox Survival Curves

Survival curves from the Cox regression models were plotted to facilitate visualization of the results. These curves were derived from a Cox regression model for each variable, by categorizing the baseline variable into two groups (normal at follow-up vs. MCI or Dementia at follow-up), using the median score at baseline as a cut-off. As an example, Fig. (2) presents the survival plot for the Digit Symbol test, showing the impact of *ApoE-4* status.

## 4. DISCUSSION

In this study, longitudinal cognitive evaluations were conducted in a large group of normal individuals, some of whom subsequently were diagnosed with MCI or dementia (i.e. 17%). Approximately one-third of the subjects in both groups were carriers of the *ApoE-4* allele, likely due to the over-recruitment of those with a family history of AD. Our data show that lower cognitive test scores at baseline and a greater rate of decline in test scores over time are significantly associated with time to onset of clinical symptoms, which were a harbinger of a diagnosis of MCI. Increased risk of progressing from normal cognition to onset of clinical symptoms was associated with lower baseline scores on several tests of episodic memory (i.e., Paired Associates immediate recall and Logical Memory Retention) as well as multifactorial tests of processing speed, where better memory ability leads to improved performance (e.g., Digit Symbol Test). Lower performance on tests in other cognitive domains was also associated with an increased risk of developing clinical symptoms among cognitively normal individuals, most notably performance on the Boston Naming Test (which was significant in both the univariate and multivariate models). Importantly, when both cognitive performance and *ApoE-4* genotype were taken into account, the relative risk of progression from normal cognition to mild impairment was doubled (i.e., increased by 100%) for individuals with one or more *e4* alleles. The rate of cognitive decline

was higher in *ApoE-4* carriers than in non-carriers for two of the nine tests.



**Fig. (2).** The survival plot for the Digit Symbol Test, based on the Cox regression model. This is one of the measures that was associated with time to onset of clinical symptoms (both with respect to the baseline score and the rate of change over time). The solid line represents scores below the median at baseline, and the dashed line represents scores above the median at baseline. The lines in black represent the scores of those who were *ApoE-4* negative and the lines in red represent the scores of those who were *ApoE-4* positive. The y axis represents the proportion of subjects who remained without symptoms. The plot starts at age 50 and is truncated at 75 years of age, since few participants remained unimpaired after this age, making the estimates unreliable after that age. Note that this survival curve is not age adjusted.

These findings are notable for several reasons. First, given that lower baseline scores (obtained on average 6.5 years prior to symptom onset) were associated with time to symptom onset suggests that about 6.5 years prior to symptom onset, cognitive performance of individuals who progress is already lower than that of individuals who remain normal over the same time period. Since the onset of clinical symptoms typically precedes the diagnosis of MCI by several years, this extends previous findings that used the date of clinical diagnosis of MCI as the outcome. Second, the average age of the cohort was 56.9 years at baseline. Since previous studies have been conducted among individuals who were over the age of 65, this also extends the age at which cognitive changes may be observed among normal individuals who will subsequently develop MCI. Third, these findings suggest that the primary reason that test scores tend to be lower in cognitively normal individuals who are *ApoE-4* carriers is that they are further along the preclinical trajectory of AD than non-carriers. That is, for an individual with a particular cognitive test score (i.e., at baseline or rate of change in score, adjusted by age and education) the risk of onset of symptoms is almost doubled for an individual who is *ApoE-4* positive. Two cognitive tests had a differential rate



of change in *ApoE-4* carriers vs. non-carriers (i.e., the Boston Naming test and Logical Memory Retention), thus there is only modest evidence to support the hypothesis that *ApoE-4* carriers progress at a more rapid rate than non-carriers during the preclinical phase of AD. These results may also be of relevance to clinical drug trials, as they highlight the importance of taking into account participants' *ApoE-4* genotype when evaluating drug efficacy in relation to cognitive change [see 49 for a discussion of this issue].

These findings are consistent with recent estimates of the onset of cognitive decline prior to diagnosis among individuals with a dominant AD mutation [50]. The strength of the findings is emphasized by the fact that increased risk of progression from normal cognition to onset of clinical symptoms were observed both with regard to baseline cognitive performance, and also in relation to rate of change over time in cognitive test scores. We cannot rule out the possibility that the lower baseline performance in our participants reflects lifelong lower cognitive abilities, as has been suggested by others [51]. However, the fact that the rate of change was also predictive of time to develop clinical symptoms suggests that the baseline scores likely reflect an actual decline.

Our findings complement and extend those of prior studies. A number of prior studies have demonstrated differences in rate of change in cognitive test scores 4-6 years prior to diagnosis of MCI [12-15]. Likewise, baseline test scores obtained in normal individuals up to 6 years prior to a diagnosis of MCI have been significantly associated with outcome [8-11].

A number of cross-sectional and short-term longitudinal studies have reported lower cognitive performance [17-19] and a greater rate of cognitive decline in cognitively normal *ApoE-4* carriers than non-carriers [20-24], particularly for tests of episodic memory.

It is also noteworthy that the relationships observed in this study are consistent with those that have examined cognitive change among individuals in the symptomatic phase of AD. For example, comparable findings have been reported in a number of longitudinal studies of non-demented individuals that have included both cognitively normal participants and subjects with MCI. These studies have found both lower baseline test scores [52-55] and greater rate of decline in test scores [56] among individuals who subsequently developed dementia, compared to those who remained non-demented. Such cognitive differences were evident 5 to 15 years prior to the diagnosis of dementia and encompassed several cognitive domains, including episodic memory.

Likewise, cross-sectional studies and short-term longitudinal studies of non-demented individuals (i.e., including both cognitively normal individuals and those with MCI) reported lower episodic memory scores and greater declines in episodic memory over time for *ApoE-4* carriers vs. non-carriers [57-63]. The results of studies comparing performance on tests other than episodic memory in non-demented *ApoE-4* carriers vs. non-carriers are more variable. Some have reported differences between the groups [22, 57, 61, 64] while others have not [58-60].

The multivariate analysis showed that in addition to two tests of episodic memory, the Boston Naming Test, which

assesses semantic memory, and the multifactorial Digit Symbol test, represented the best combination of predictors of progression to cognitive impairment in this study. These findings are consistent with reports of differential brain atrophy in medial temporal lobe brain regions during the preclinical phase AD, particularly in the entorhinal cortex and hippocampus, which are involved in episodic memory performance [e.g., 65]. These findings further suggest that AD pathology may extend into other brain regions during this phase of the disease, such as the anterior and middle temporal cortex (which has been associated with Boston Naming performance [66, 67]. Performance on the Digit Symbol test benefits from episodic memory as well as a number of other cognitive skills, so it is unclear whether the association between performance on this test and risk of progression reflects pathology in the medial temporal lobe or in other regions.

The study results must be interpreted in the context of its limitations. First, the subjects are well educated, primarily Caucasian, with the majority having a family history of dementia, so the results may not generalize to the U.S. population at large. Second, the results may depend on the specific neuropsychological tests used. While many of the individual tests were significant in the univariate models, only a small set remained significant in the multivariate model, suggesting that a different set of initial tests might alter the findings in the multivariate model. Additionally, it remains unclear if other aspects of cognition not covered by our cognitive battery are also associated with the progression to clinical symptom onset and show differential rates of decline for *ApoE-4* carriers and non-carriers. Third, the present work is limited to *ApoE* genotype and it remains unclear if the effects of *ApoE* single nucleotide polymorphisms (SNPs) influence cognitive change during preclinical AD [70]. Lastly, it is important to acknowledge the challenges of estimating age of symptom onset. While we used a semi-standardized instrument administered to both the subject and an informant, with specific targeted questions to assess onset of symptoms, the determination was ultimately based on the judgment of skilled clinicians. Additionally, although the test scores were not used to establish age of symptom onset, they were available to the clinician, thus making it unclear whether they influenced the decision making process in some way. It should be noted, however, that we have recently published data on CSF and MRI changes in relation to symptom onset in this cohort [65, 68], suggesting that this measure is biologically meaningful and related to variables above and beyond cognitive test scores.

Taken together, these findings are consistent with the hypothesis outlined in the working group report on 'preclinical AD' suggesting that decline in cognitive performance is detectable during the phase of AD when pathology is developing, but clinical symptoms have not yet been reported [7], and that genetic factors might influence the rate of decline. Interestingly, the working group report also hypothesized that more difficult or targeted cognitive tests might be more useful for demonstrating cognitive decline during the 'pre-clinical' phase of AD than the standard cognitive measures, particularly for the purposes of identification of individuals at risk. While our analyses do not include such comparisons, they nonetheless do demonstrate that standard cognitive tests

are quite sensitive to declines in cognition during pre-clinical AD, and suggest that these tests might be useful indicators of which cognitively normal individuals will go on to subsequently develop clinical symptoms over a period of years.

Additionally, the hypotheses summarized in the working group report proposed that cognitive decline would follow alterations in both amyloid and tau levels in CSF, as well as volumetric changes on MRI scans. With additional follow-up of the participants it should soon be possible to address the question of the sequence in which changes in these biomarkers occur, and how our findings might compare with proposed hypothetical models [69].

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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