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A homopolymer polymorphism in the *TOMM40* gene contributes to cognitive performance in aging

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Abstract

Introduction—A highly polymorphic T-homopolymer was recently discovered to be associated with late onset Alzheimer's disease (LOAD) risk and age of onset.

Objective—To explore the effects of the polymorphic polyT tract (rs10524523, referred as '523') on cognitive performance in cognitively healthy elderly.

Methods—181 participants were recruited from local independent-living retirement communities. Informed consent was obtained and participants completed demographic questionnaires, a conventional paper and pencil neuropsychological battery, and the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB). Saliva samples were collected for determination of the *TOMM40* '523' (S, L, VL) and the *APOE* (ε2, 3, 4) genotypes. From the initial sample of 181 individuals, 127 participants were eligible for the association analysis. Participants were divided into three groups based on '523' genotypes (S/S, S/L-S/VL, and L/L-L/VL-VL/VL). Generalized linear models were used to evaluate the association between the '523' genotypes and neuropsychological test performance. Analyses were adjusted for age,

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CONFLICT OF INTEREST DISCLOSURE

Dr. Roses is the CEO of Cabernet Pharmaceuticals, Inc., a pipeline pharmacogenetics consultation and project management company; and CEO of Zinfandel Pharmaceuticals, Inc., who is in alliance with Takeda Pharmaceuticals to perform a delay of onset trial for dementias of the Alzheimer type. Drs. Saunders and Lutz are Members of the Joint Biomarker Committee for the Zinfandel-Takeda Alliance clinical trial and Dr. Saunders is the spouse of Dr. Roses.

sex, education, depression, and *APOE* $\epsilon 4$ status. A planned sub analysis was undertaken to evaluate the association between '523' genotypes and test performance in a sample restricted to *APOE* $\epsilon 3$ homozygotes.

Results—The S homozygotes performed better, although not significantly, than the S/L-S/VL and the VL/L-L/VL-VL/VL genotype groups on measures associated with memory (CANTAB Paired-Associate Learning, and VRM Free Recall) and executive function (CANTAB measures of Intra-Extradimensional set shifting). Follow-up analysis of *APOE* $\epsilon 3$ homozygotes only, showed that the S/S group performed significantly better than the S/VL group on measures of episodic memory (CANTAB Paired-Associate Learning and VRM Free Recall), attention (CANTAB RVP Latency) and executive function (Digit-Symbol substitution). The S/S group performed marginally better than the VL/VL group on Intra-Extradimensional set shifting. None of the associations remained significant after applying a Bonferroni correction for multiple testing.

Conclusions—Results suggest important *APOE*-independent associations between the *TOMM40* '523' polymorphism and specific cognitive domains of memory and executive control that are preferentially affected in early stage AD.

Keywords

TOMM40, polyT polymorphism; cognition; aging; neuropsychological tests; CANTAB; Alzheimer's disease

1. BACKGROUND

The most common form of Alzheimer's disease (AD) is sporadic, late-onset AD (LOAD, age of onset ≥ 65 years) which accounts for the majority of AD cases. Since the original report, 17 years ago, linking $\epsilon 4$ allele of the Apolipoprotein E gene (*APOE* $\epsilon 4$) with LOAD [1], *APOE* $\epsilon 4$ has been the strongest established genetic risk factor for sporadic LOAD. Roses *et al.* (2009) recently demonstrated that *TOMM40*, which encodes the essential, mitochondrial protein import translocase (Translocase of the Outer Mitochondrial Membrane, 40kD), and is adjacent to and in linkage disequilibrium with *APOE*, contributes significantly to the genetic risk and age of onset for LOAD. Using a deep sequencing and phylogenetic analysis approach, it was discovered that the risk locus is a polymorphic polyT tract (rs10524523) positioned within intron 6 of the *TOMM40* gene, referred to as '523'. The variable '523' polyT lengths are predictive of age of disease onset and risk, and may, in fact, explain much of the effect on age of onset previously attributed to the different *APOE* alleles [2, 3]. Three major *TOMM40* '523' alleles are defined by the length of the T homopolymer: 'Short' (S, T = 20), 'Long' (L, T = 21-29) and 'Very Long' (VL, T = 30). The 'long' (L) and 'Very Long' (VL) alleles are associated with higher LOAD risk and earlier age of disease onset in *APOE* $\epsilon 3/4$ (~7 years earlier onset in VL/L vs. S/L) [2, 3] and *APOE* $\epsilon 3/3$ subjects (~9 years earlier onset in VL/VL vs. S/S) [4].

Johnson *et al.* demonstrated the significant contribution of '523' to impaired cognition in a clinically normal, late middle-aged cohort by comparing verbal memory measures [5]. Specifically, individuals homozygotes for the '523' VL allele and homozygotes for the '523' S allele differed significantly on retrieval from the primacy region of a verbal list learning task, an aspect of episodic memory that is also affected in early AD. Collectively, these studies suggest the importance of '523' in early AD pathogenesis.

The significant contribution of '523' to cognitive decline and age of dementia onset poses an interesting question regarding influence of the '523' on cognitive performance in normal elderly. In the current study we aim to examine the effects of the '523' variant on cognitive function in normal aging. Specifically, we sought to test the hypothesis that the length of the

'523' polyT is related to robust cognitive function in old age. The prediction is consistent with that seen in age of LOAD onset, *i.e.* the shorter length of the T homopolymer (S) may be related to successful cognitive aging into late old age, evidenced by better performance outcomes on neurocognitive tests of episodic memory and executive function that are typically affected in AD, in those homozygous for the S allele as compared to those homozygous for the VL allele.

2. METHODS

2.1. Participants

A total of 181 individuals from five local independent-living retirement communities in the Research Triangle Park region of North Carolina agreed to participate. Participants were recruited from January 2009 to July 2010 by means of IRB-approved advertisements displayed on bulletin boards, fliers, and presentations describing the study. Participants were scheduled for test sessions during which the study was explained and informed consent was obtained. Personal details, including age, education, ethnicity, medication usage, medical history, and family history were collected. The information collected in the personal questionnaire was used also to determine eligibility for the association analyses describe in 2.4. Exclusion criteria included: self-report of neurological disease (including dementia, MCI, Parkinson's disease, stroke, or TIA), usage of memory medications (e.g. Aricept) or scoring ≤ 23 on the Montreal Cognitive Assessment (MoCA, see test description on section 2.2.). Neuropsychological tests were administered and saliva samples were collected for genetic analysis. Completion of the entire interview and DNA collection typically took 2 to 3 hours. Procedures were administered by a trained psychometrist in a private designated room at each retirement facility. The project was approved by the Duke Institutional Review Board and followed appropriate ethical protocols.

2.2. Neuropsychological Testing

The neuropsychological battery used in this study was designed to measure selective domains of cognition commonly affected by normal aging of the central nervous system (processing speed, attentional processing, inhibitory control) and AD (episodic memory, particularly verbal learning and memory, spatial learning and memory, complex executive functions, and verbal retrieval/fluency). The complete test battery included selected subtests of the computerized Cambridge Neuropsychological Test Automated Battery (CANTAB: Cambridge Cognition, UK), conventional paper and pencil neuropsychological tests typically used in the screening and evaluation of memory complaints, and the Beck Depression Inventory (BDI-II)[6]. The computer battery comprised the following tests: paired associates learning (PAL), spatial working memory (SWM), verbal recognition memory - immediate and delayed (VRM), intra-extradimensional set shifting (IED), rapid visual processing (RVP), pattern recognition memory (PRM), spatial span (SSP) and spatial recognition memory (SRM). The test scores were automatically assigned and stored in the CANTAB computers for later extraction. Total scores for each index were included in the analyses.

The conventional neuropsychological battery, presented in paper and pencil format, included: a) a general cognitive screening examination, the Montreal Cognitive Assessment (MoCA)[7]; b) measures of narrative verbal learning and delayed recall (Green's story recall - immediate and delayed[8]); c) simple visual attention (Trail Making Test Part A; Digit-Symbol Substitution and Symbol Search subtests from the Wechsler Adult Intelligence Scale-III (WAIS-III) [9]); d) visuospatial working memory (Trail Making Test Part B[10, 11]); e) auditory attention (Digit span-forward, a subtest from the WAIS-III[9]); f) auditory working memory (Digit Span backward from the WAIS-III [9]); g) word retrieval including

both lexical fluency (Controlled Oral Word Association test (COWA) [12]) and semantic fluency (animal fluency); and h) tests of inhibitory control (Stroop color-word interference[13]). The tests were double scored prior to data entry by trained psychometrists to ensure scoring accuracy.

2.3.Genotyping

Saliva sample collection and DNA extraction were performed using the commercially available Oragene DNA Self-Collection Kit (Oragene) according to the manufacture's protocol. DNA concentration and the quality of purification were determined spectrophotometrically. *APOE* genotypes were determined using TaqMan-based allelic discrimination assay (Applied Biosystems). Briefly, *APOE* ϵ 2/3/4 genotypes were established using two separate SNPs: (1) *rs429358* 334T/C (ABI assay ID: C_3084793_20), and (2) *rs7412* 472T/C (ABI assay ID: C_904973_10). The assay was carried out using the ABI 7900HT and genotype analysis was performed by the SNP auto-caller feature of SDS software[14]. *APOE* genotype assignments were defined as described previously [15]. *TOMM40* '523' polyT genotypes were determined based on length variation. The '523' region of each genomic DNA sample was PCR-amplified using a fluorescently labeled primer. Genotypes were determined on an ABI 3730 DNA Analyzer using GeneMapper, version 4.0 software (Applied Biosystems, Foster City, CA) for allelic size assessment. The '523' allele was assigned according to the length of the PCR product[14].

2.4.Data Analysis

Demographic characteristics were compared across genotype groups; continuous variables were assessed with analysis of variance and categorical variables with chi-square statistics. Generalized linear models (GLM) were used to examine the '523' genotypes as predictors of performance of the various neuropsychological tests. Due to the potential for ceiling effects, neuropsychological test scores were transformed prior to analysis. The S-homozygotes (S/S) genotype group was used as the reference group and comparisons were made to the S-heterozygotes (S/L, S/VL) and non-S (L/L, L/VL, VL/VL) groups. Separate GLM models were constructed for each predictor variable of interest. Models were then adjusted for age and other covariates known to influence cognitive performance including *APOE* status, sex, education, and depression as measured by the BDI. As multiple comparisons were made and the nature of the study was exploratory, we report both corrected and uncorrected *p*-values for each analysis. Bonferroni corrected *p*-values by cognitive domain were calculated by dividing an alpha level of 0.05 by the number of tests for each domain. Where multiple scores are listed for the same test, only one test was counted. Because of the strong association between the *APOE* genotype and cognition in aging, and in order to restrict the analysis to a comparison between S and VL genotypes, we carried out a second stage analysis considering only participants having the *APOE* ϵ 3/3 genotype and '523' genotypes S/S, S/VL and VL/VL. All analyses were performed using analytical software SAS 9.3.

3. RESULTS

Volunteer participants included 181 elderly between the ages of 64-93 years old. Saliva samples were provided by 180 participants for genotype analysis of the *APOE* and *TOMM40* '523' polymorphisms. Allele and genotype frequencies were determined in the Caucasian group only as this was the majority in the sample. Figure 1 describes the allele frequencies and genotype distributions for *APOE* (A and B) and *TOMM40* '523' (C and D) in the Caucasian participants. The two genes, *APOE* and *TOMM40*, physically mapped near each other and are in high linkage disequilibrium (LD). We inferred the *APOE* backbone (ϵ 2, 3, 4) of each of the '523' alleles (S, L, VL) in the Caucasian sample. The inferred L allele

was primarily linked to $\epsilon 4$, while the majority of the VL and S alleles showed linkage to $\epsilon 3$. These results were consistent with the previous reports [2, 3].

We next investigated the association between the neuropsychological battery results and '523' genotypes. 177 of the participants who provided DNA completed successfully the traditional neuropsychological battery; 154 of them also completed the CANTAB. Because educational attainment is known to influence cognitive performance, we set aside scores from individuals ($n=7$) who failed to report years of education. Visual acuity is similarly an important component of performance on many of the tests. Therefore, in instances where individuals reported vision problems, we set scores to missing on those tests that required vision for adequate performance (i.e., all computerized tests, Trail Making A & B, Digit-Symbol Substitution and Symbol Search subtests from the WAIS-III, the MoCA, and Stroop color-word items for those reporting colorblindness). We also excluded participants who reported neurological problems or neurodegenerative disorders including stroke, TIA, Parkinson's disease, diagnoses of MCI or dementia ($n=25$). Finally, we excluded participants who reported taking memory medications (e.g. Aricept) or scored ≥ 23 on the MoCA[16], retaining a total of 127 participants eligible for these analyses. The characteristics of the analytic sample are shown in Table 1. The sample ranged in age from 64 to 93, was mostly female (68.5%), and highly educated (average 16.8 years of education). The majority of the sample was White and had spoken English as their first language.

In order to evaluate the association between performance in neuropsychological tests and '523' genotype, we defined three categories of '523' genotypes: S-homozygotes (S/S; $n=25$), S-heterozygotes (S/L, S/VL; $n=63$), and non-S (L/L, L/VL, VL/VL; $n=39$). There were no significant differences in demographic characteristics between the groups. Mean test scores, standard deviations, and results of the GLM models comparing genotype groups are shown in Table 2. Test scores tended to be skewed due to generally uniform good performance on all measures. Prior to analysis, scores were transformed by taking either the log, square root, or Tukey transformation of each score in order to normalize the score distributions. Each transformed score was then evaluated to determine if the skew was significant [17]. Scores with non-significant skew were retained for the analysis. Skewed scores, including the Trail Making Test Part A # of errors and CANTAB subscale scores were excluded. The first column in Table 2 lists each test grouped by cognitive domain. The first set of tests listed under each domain are from the paper/pencil battery; the second set are from the CANTAB. In the next three columns, mean scores and standard deviations for each test are listed by genotype group. The p -values from GLM analyses, adjusted for age, sex, education, BDI, and *APOE* $\epsilon 4$ status (coded as 0 vs. 1 or more $\epsilon 4$ alleles due to the small number of $\epsilon 4$ homozygotes) are listed next for each comparison. The last column lists the Bonferroni corrected p -value threshold for each cognitive domain. The overall p -value for all comparisons was 0.002. Differences were observed between the '523' genotype groups' mean scores, however, none reached statistical significance at the Bonferroni-corrected p -value level or the $p=0.05$ level. Differences on the IED were most noticeable, the S/S group had 4 fewer errors than the S/L-S/VL group on IED and 9 fewer errors on IED compared to the L/L-L/VL-VL/VL group. On the PAL, the S/S group had 3 fewer errors on average than the S/L-S/VL group and two fewer than the L/L-L/VL-VL/VL group. Also, the S/S group performed slightly better than the other groups on the VRM (Table 2).

The effects of '523' might be confounded by *APOE* ϵ status, therefore we re-analyzed the data using a restricted sample of individuals with the *APOE* $\epsilon 3/3$ genotype ($n=82$). In order to keep '523' genotype groups defined clearly by the S and VL alleles, we dropped one participant who had $\epsilon 3/3$ genotype and carried the L allele. We retained 82 $\epsilon 3/3$ individuals with S/S ($n=21$), S/VL ($n=39$), and VL/VL ($n=22$) genotypes at the '523' locus. Table 3 lists the p -values for the GLM models for selected tests comparing the S/S group to the S/VL and

the VL/VL groups. In the restricted analyses, the differences in memory between the S/S and the S/VL groups on the PAL ($p=0.01$ mean trails; $p=0.02$ mean errors) and VRM ($p=0.03$ free recall items correct) tests became significant. The S/S group also performs significantly better on the RVP test (attention; $p=0.04$) and Digit Data (executive function; $p=0.03$). Differences between the S/S group and the VL/VL group are suggested on the Trail Making test part A and IED but not significant. After Bonferroni adjustment none of the associations remained significant (Table 3).

4. DISCUSSION

We tested the association between ‘523’ genotypes and neuropsychological function across a number of broad processing domains in a group of cognitively normal older adults and found significant differences in test performance. In comparisons with all ‘523’ genotypes, and adjusting for *APOE* $\epsilon 4$ status, we attempted to disentangle the effects of *APOE* and *TOMM40*. Findings in these analyses were not particularly strong due to the small sample size. However, a few items of interest suggested possible associations in the initial analysis, *e.g.*, the number of errors on the paired associates learning (PAL) test, the number of items recalled in a test of free recall (VRM), and the ratio of errors to trials in the intra-extra dimensional set shifting task (IED).

Restricting the sample to individuals with *APOE* $\epsilon 3/3$ only and comparing S and VL homozygotes and heterozygotes, our results became more evident. Differences were found in memory, attention, and executive function. The PAL test on the CANTAB provides measures of episodic memory and learning and is particularly sensitive to early memory changes. We found that on average, the S/S group had 7 fewer errors than the S/VL group and 3 fewer errors than the VL/VL group on PAL. The S/VL group had the fewest items correct on VRM free recall number of items correct, about 2 fewer than the S/S group on average. The Trail Making Test Part A is a gauge of attention level. Although the difference was not significant, S/S group performed 7 seconds faster on average than the VL/VL group. On a second measure of attention, Rapid visual information processing (RVP latency) from the CANTAB, there were significant differences in latency time between the S/S group and the S/VL group. In executive function, the S/S group performed better on a digit-symbol substitution task than the S/VL group. Finally, the IED is a test of rule acquisition and shifting attention and is a measure of executive function. This was marginally significant in the initial analysis and less so in the restricted analysis although the VL/VL group had on average 11 more total errors on IED than S/S.

Collectively we showed that in cognitively normal participants, the S/S homozygotes had better cognitive performance than those with the AD-risk VL allele. Moreover, the L and VL ‘523’ genotypes in this group of normal elderly showed mild deficits in cognitive domains that are typically affected in early AD. These findings are in line with a previous study that found significant associations between memory and learning functions deficits that occur in LOAD, and the ‘523’ genotype in normal late middle-aged subjects[5]. Together these studies imply that the *TOMM40* ‘523’ effect on cognition in normal subjects might be attributed to its association with AD risk. Further studies to elucidate the functional contribution of the ‘523’ polymorphism to the etiology of AD will be important.

Although research into the cognitive effects associated with the *TOMM40* ‘523’ alleles is relatively recent, there have been numerous neuropsychological studies demonstrating early cognitive changes associated with later onset of AD. These studies suggest that in addition to the expected deficits in memory, the pre-clinical phase of cognitive decline preceding the emergence of AD involves other cognitive impairments[18] including attention and executive function[19-21] [22, 23]. Similarly, a recent study examined neuropsychological

functioning using the CANTAB in adults with mild cognitive impairment (MCI) and reported deficits in working memory attention and executive measures [24]. Interestingly, our study found that these particular cognitive domains are associated with the AD-risk '523' genotypes.

Our study's strength lies in the extensive neuropsychological battery that was administered which included conventional and computerized measures allowing for greater sensitivity in detecting subtle cognitive variations. The battery comprehensively covered cognitive domains of memory, attention, language, and executive function, all commonly affected early in AD. To date there are few studies that explore the effects of the '523' genotype on cognitive function. The current study is one of the first to focus on the S allele, suggesting it offers some protection, akin to early studies of the *APOE* ϵ 2 allele. Our results demonstrate subtle but detectable differences between groups of individuals categorized by their '523' genotypes exclusive of *APOE* genotype that warrant follow up investigation in a larger group.

There are few limitations to the study that should be noted. First and foremost is the small sample size which limited our power to detect associations. Multiple comparisons were made in this study. Although we downplayed corrections for these comparisons due to the exploratory nature of the work, we reported corrected *p*-values and by that standard, none of our results were significant. The study sample is relatively homogenous (elderly, white, highly educated, and mostly female), thus the findings cannot be generalized to the broader population. One benefit of a homogenous sample, however, is the potential for controlling bias due to unmeasured variables. Another limitation is the lack of clinical assessments to determine the cognitive status of study participants. Nonetheless, the presence of symptomatic AD or other dementias is not likely as all individuals were living independently in the community at the time of the interview. In addition, individuals with self-reported clinical diagnosis of neurological diseases, history of stroke or TIA, dementia diagnoses, or the use of memory medications (e.g. Aricept) were excluded from the study. Finally, because of the age range of the participants (64-93) we were unable to investigate the early deleterious effects of the VL allele, thought to occur prior to age 65.

In the current study we showed that the '523' genotypes may affect cognitive performance in elderly. The study is cross sectional, showing a snapshot of cognitive performance in relation to '523' genotypes. It will be of great interest to evaluate the effect of the '523' polymorphism on rate of cognitive decline in aging. Therefore, a follow up longitudinal study to assess the '523' influence on cognitive changes and decline in our study's participants is underway. Interestingly, a recent genome wide association study identified a significant association between the *TOMM40* gene and survival into old age [25]. It will be important to assess the association of the '523' polymorphism in that cohort as well as in other large nonagenarian cohorts in order to get a better understanding of the genetic factors that contribute to longevity and successful cognitive aging.

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Figure 1.

Genotypes of *TOMM40* '523' and *APOE* were determined using DNA extracted from Saliva samples. *APOE* genotypes-ε2, 3, 4-were assigned as described previously [15], and *TOMM40* '523' genotypes-S, L, VL-were defined based on fragment length polymorphism. Allele and genotypes frequencies were determined for the Caucasian sample (98% of total participants): **(A)** *APOE* allele frequencies; **(B)** *APOE* genotype frequencies; **(C)** *TOMM40* '523' allele frequencies; **(D)** *TOMM40* '523' genotype frequencies.

Table 1
Demographic Characteristics n=127

Characteristic	Range	n (%)
Age, mean(SD)	64-93	80.6 (6.0)
Sex, female		87 (68.5)
Education, mean(SD)	12-20	16.8 (2.3)
English as 1 st language		120 (94.5)
Caucasian		126 (99.2)
<i>APOE</i>		
0 ε4 alleles		101 (79.5)
1 ε4 allele		25 (19.7)
2 ε4 alleles		1 (0.8)
<i>TOMM40</i>		
SS		25 (19.7)
S/L, S/VL		63 (49.6)
L/L, L/VL, VL/VL		39 (30.7)
MoCA		27.4 (2.4)
BDI-II		4.9 (4.1)

Abbreviations: SD=Standard deviation; MoCA=Montreal Cognitive Assessment; BDI-II=Beck Depression Inventory, 2nd Edition.

Values are number (%) unless indicated as mean (SD).

Table 2
Mean scores by group and p-values for Generalized Linear Models of '523' genotypes for n=127 participants' neuropsychological test scores

	‘523’ Genotype Group, Mean (SD)				p-value	
Cognitive Domain Test	S/S n=25	S/VL, S/L n=63	VL/VL, L/L, L/VL n=39	S/S vs. S/VL, S/L	S/S vs. VL/VL, L/L, L/VL	Corrected p-value threshold by domain
Global Cognition						
MoCA	27.0 (2.3)	27.4 (2.4)	27.7 (2.4)	0.5589	0.2916	0.05
Memory						
Story Recall – Immediate	46.2 (9.5)	47.4 (8.9)	46.2 (8.0)	0.9896	0.5934	0.01
Story Recall – Delayed	33.1 (10.8)	33.4 (11.6)	33.0 (9.6)	0.7606	0.6510	
PAL Mean Errors to Success	6.8 (6.0)	10.1 (10.7)	9.1 (7.9)	0.0907	0.2707	
PAL Mean Trials to Success	3.3 (1.5)	4.3 (2.9)	4.1 (2.4)	0.0736	0.1653	
PAL Total Trials	17.3 (4.0)	19.7 (6.3)	19.0 (4.4)	0.0689	0.1486	
PRM Percent Correct	78.1 (10.1)	79.2 (8.7)	79.7 (8.4)	0.9163	0.8022	
PRM Mean Correct Latency	2462.8 (452.6)	2559.5 (613.6)	2786.9 (598.7)	0.9615	0.2145	
SRM Percent Correct	71.0 (13.5)	72.8 (10.2)	68.8 (10.7)	0.4611	0.6686	
SRM Mean Correct Latency	2974.5 (717.2)	3018.0 (1184.8)	3224.9 (931.5)	0.7331	0.8179	
VRM Free Recall Total Correct	7.7 (3.1)	5.7 (3.8)	6.8 (3.4)	0.0663	0.5785	
VRM Recognition Total Correct Less False Positives	33.5 (2.4)	31.8 (5.8)	33.3 (3.9)	0.4915	0.5270	
VRM Free Recall – Immediate Total Correct	8.5 (2.8)	7.6 (2.7)	8.5 (2.7)	0.2910	0.9250	
Attention						
# Colors read	60.7 (10.1)	63.0 (11.4)	61.1 (9.0)	0.2449	0.6723	0.008
# Words read	90.7 (13.4)	92.9 (13.3)	91.8 (10.4)	0.2401	0.4740	
Forward -# times Correct	9.9 (1.8)	9.8 (2.3)	9.9 (1.8)	0.8044	0.6440	
Trails A seconds	34.4 (9.6)	36.7 (11.5)	37.4 (10.9)	0.4152	0.3382	
RTI 5-choice movement time	651.1 (149.4)	626.5 (181.7)	636.8 (142.9)	0.5986	0.9679	
RTI 5-choice reaction time	466.7 (73.8)	493.2 (122.1)	482.1 (78.5)	0.4546	0.6754	
RVP Probability of a hit	0.82 (0.1)	0.77 (0.2)	0.75 (0.17)	0.7321	0.3849	

Cognitive Domain Test	'523' Genotype Group, Mean (SD)				p-value	
	S/S n=25	S/VL, S/L n=63	VL/VL, L/L, L/VL n=39	S/S vs. VL/VL, L/L, L/VL	S/S vs. VL/VL, L/L, L/VL	Corrected p-value threshold by domain
RVP Latency	446.5 (74.3)	439.87 (77.3)	440.3 (79.7)	0.2765	0.1754	
Language						
Semantic Memory - #Animals in 60sec	17.6 (5.7)	17.9 (4.2)	18.9 (5.4)	0.7740	0.0792	0.025
COWA Total – sum of C, L, F	44.3 (14.6)	41.5 (11.4)	40.4 (12.2)	0.5982	0.3777	
Executive						
Backward –# times Correct	7.1 (2.0)	7.0 (1.8)	6.8 (2.1)	0.8270	0.6018	0.007
Colorword # colors as words read	33.0 (8.5)	31.2 (9.5)	30.4 (8.7)	0.5835	0.4137	
Digit Data	55.2 (9.9)	58.8 (13.1)	56.1 (12.5)	0.1391	0.4548	
Symbol Data	24.8 (6.4)	26.3 (6.7)	24.7 (6.2)	0.2776	0.7083	
Trails B Errors	0.51 (0.72)	0.78 (1.2)	0.62 (1.15)	0.5837	0.7423	
Trails Making Test Part B seconds	76.8 (18.9)	82.6 (29.3)	93.9 (39.4)	0.8543	0.2544	
IED Ratio – Errors/Trials	0.25 (0.10)	0.26 (0.08)	0.31 (0.10)	0.4577	0.0520	
IED Total Errors	22.8 (12.3)	26.7 (13.3)	32.1 (16.0)	0.3242	0.0597	
SSP Span	5.2 (0.6)	5.2 (0.85)	5.1 (0.83)	0.8538	0.7612	
SSP Total Errors	14.5 (6.0)	12.8 (4.5)	13.8 (6.8)	0.3034	0.6236	
SWM Strategy	27.5 (3.9)	27.3 (4.0)	26.6 (4.8)	0.9189	0.4208	
SWM Total Errors	37.0 (20.3)	38.1 (16.9)	35.8 (16.5)	0.7377	0.6151	

Models are adjusted for age, sex, years of education, Beck Depression Inventory-II, and APOE status (0 e4 alleles vs. 1 or more).

Abbreviations: COWA=Controlled Oral Word Association; IED= Intra-Extra Dimensional set shift; MoCA=Montreal Cognitive Assessment; PAL=Paired Associates Learning; PRM=Pattern Recognition Memory; RTI=Reaction Time; RVP=Rapid Visual Information Processing; SD=Standard Deviation; SSP=Spatial Span; SRM= Spatial Recognition Memory; SWM=Spatial Working Memory; VRM= Verbal Recognition Memory

Table 3

Adjusted from *APOE* 3/3 subsample n=82

Cognitive Domain Test	'523' Genotype Group			p-value		Corrected p-value threshold
	S/S n=21	S/VL n=39	VL/VL n=22	S/S vs. S/VL	S/S vs. VL/VL	
Memory						
PAL Mean Errors to Success	5.7 (4.3)	12.6 (13.1)	8.7 (6.2)	0.0204	0.2357	0.01
PAL Mean Trials to Success	3.1 (1.3)	5.0 (3.5)	4.1 (2.0)	0.0163	0.1928	
VRM Free Recall Items Correct	8.3 (2.5)	5.9 (3.4)	6.5 (3.5)	0.0325	0.2504	
Attention						
RVP Latency	460.2 (69.8)	420.3 (71.0)	439.1 (80.7)	0.0475	0.1460	0.008
Executive						
Digit Data	53.9 (10.1)	61.8 (13.8)	53.7 (10.9)	0.0349	0.6961	0.007
IED Ratio Errors/Trials	0.25 (0.1)	0.26 (0.08)	0.32 (0.09)	0.8674	0.0771	
IED Total Errors	22.6 (11.5)	12.6 (13.1)	34.5 (16.8)	0.9168	0.0905	

Models are adjusted for age, sex, years of education, and Beck Depression Inventory-II

Abbreviations: IED= Intra-Extra Dimensional set shift; PAL=Paired Associates Learning; RVP= Rapid Visual Information Processing; VRM=Verbal Recognition Memory