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Family history and *TOMM40* '523 interactive associations with memory in middle-aged and Alzheimer's disease cohorts

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Abstract

Introduction: Family history (FH) of Alzheimer's disease (AD) affects mitochondrial function and may modulate effects of translocase of the outer mitochondrial membrane 40 kDa (*TOMM40*) rs10524523 ('523) poly-T length on memory decline.

Methods: For 912 nonapolipoprotein ε4 middle-aged adults and 365 aged adults across the AD spectrum, linear mixed models gauged FH and *TOMM40* '523 interactions on memory and global cognition between baseline and up to 10 years later. A cerebrospinal fluid mitochondrial function biomarker was also assessed.

Results: For FH negative participants, gene-dose preservation of memory and global cognition was seen for "very long" versus "short" carriers. For FH positive, an opposite gene-dose decline was seen for very long versus short carriers. Maternal FH was a stronger predictor in aged, but not middle-aged, participants. Similar gene-dose effects were seen for the mitochondrial biomarker aspartate aminotransferase.

Discussion: These results may clarify conflicting findings on *TOMM40* poly-T length and AD-related decline.

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Keywords: Alzheimer's disease; Mitochondrial function; Memory; Global function; TOMM40; Aspartate aminotransferase

1. Introduction

The apolipoprotein E (*APOE*) gene is the most replicated genetic risk factor for late-onset Alzheimer's disease (LOAD) [1]. Subjects without the ε4 allele (non-*APOE* ε4s) are considered at neutral risk but show substantial variability for developing Alzheimer's disease (AD), where there is tremendous interest in finding genetic risk factors for non-*APOE* ε4s that strongly predict memory, global decline, and cognitive impairment risk. As mitochondrial dysfunction is associated with LOAD [2], several groups have investigated gene loci that impact mitochondrial bioenergetics, such as translocase of the outer mitochondrial membrane 40 kDa (*TOMM40*).

TOMM40 shares a strong linkage disequilibrium with *APOE* on chromosome 19 [3]. *TOMM40* constitutes the central channel of the outer mitochondrial membrane, through which mitochondria-specific ribosomal preproteins enter for post-translational modification in the inner matrix [4]. Genome-wide association studies have implicated several *TOMM40* single-nucleotide polymorphisms (SNPs) in increased LOAD risk, cognitive decline, and brain atrophy, particularly SNP rs2075650 that has consistently shown such associations [4–7]. In addition, a *TOMM40* variable length deoxythymidine-homopolymer (poly-T) at rs10524523 ('523) within intron 6 has sometimes been shown to associate with LOAD risk. Among non-*APOE* ε4s, the phase-in haplotypes are *APOE* ε3-linked to either a *TOMM40* '523 "short" (S) or "very long" (VL) poly-T length [8]. Some studies find that subjects with the VL allele have worse memory and executive function scores in middle-aged [6] and aged [9] cohorts. To date, several studies have also found that *TOMM40* VL in non-*APOE* ε4s or *APOE* ε3/ε4s predicts memory deficits or earlier age of AD onset [6,8–12]. However, other reports with large samples have found no effect or a benefit of *TOMM40* VL on age of onset and memory decline [13–15].

It may be important to account for other factors that can modulate mitochondrial function, such as AD family history (FH). AD FH is typically defined as maternal or paternal LOAD onset between ages 65 and 80 [16]. Both FH and *TOMM40* influence mitochondrial bioenergetic processes needed for adenosine triphosphate (ATP) synthesis. For example, maternal FH (FHM) is related to reduced cytochrome oxidase activity [17], leading to electron transport chain dysfunction, lower ATP production, and neuronal damage. Like *TOMM40*, FH can increase AD risk independent of *APOE* [18]. To link FH and *TOMM40* with AD, the mitochondrial cascade hypothesis posits that multiple factors determine baseline and age-related decline in mitochondrial function [19]. As neurons in hippocampus and other medial temporal lobe areas have high ATP requirements [16], FH and *TOMM40* might interact to affect mitochondrial dysfunction and contribute to memory and global cognitive decline, as well as cognitive impairment risk.

Our objective was to explore FH and *TOMM40* interactions separately in non-*APOE* ε4 late middle-aged or aged subjects from the Wisconsin Registry for Alzheimer's Prevention (WRAP) study or Alzheimer's Disease Neuroimaging Initiative (ADNI), respectively, to see if FH or FHM modulated *TOMM40* effects in middle-age and along the AD spectrum in old age. The primary outcomes were longitudinal memory decline in both cohorts and global decline in the aged cohort. Previous WRAP and ADNI reports either focused on cross-sectional cognition with fewer subjects [6] or age of onset but not on cognitive or mitochondrial outcomes [13].

To establish that FH and *TOMM40* meaningfully interacted to affect mitochondrial processes, cerebrospinal fluid (CSF) levels of a cytosolic aspartate aminotransferase (AST) peptide were gauged. Cytosol AST is an enzyme that facilitates catabolism of glucogenic amino acids through the malate-aspartate shuttle by a final conversion step of aspartate to oxaloacetate, helping to maximize ATP production through glycolysis [20,21]. AST, also called glutamic oxaloacetate transaminase, differs in CSF levels among AD patients and related dementias [22], and shows higher concentrations in AD frontal and parietal cortices [23]. *APOE* ε4 subjects were not considered for this report, as their in-phase *TOMM40* genotype ("long", or L) is different and could confound effects of S and VL genotypes in *APOE* ε3/ε4 subjects.

2. Methods

2.1. Participants

Late middle-aged subjects (91.3% Caucasian, non-Hispanic) were recruited from the WRAP study [24]. Participants were originally recruited from 40 to 65 years. There were 912 non-*APOE* ε4 (129 *APOE* ε2/ε2 or ε2/ε3; 783 *APOE* ε3/ε3) participants with *TOMM40* '523 and neuropsychological data. Cognitive performance was assessed once every roughly 2 years over four waves, from baseline to approximately 7 to 10 years later for a given participant.

Data on aged subjects (92.7% Caucasian, non-Hispanic) was obtained from the ADNI database (adni.loni.usc.edu). The primary goal of ADNI was to test whether serial magnetic resonance imaging, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. Among 365 non-*APOE* ε4s (60 *APOE* ε2/ε2 or ε2/ε3; 305 *APOE* ε3/ε3), *TOMM40* '523 data were available for 146 cognitively normal (CN), 163 MCI, and 56 AD participants. Data were downloaded from baseline and months 6, 12, 24, and 36. All subjects had (1) demographic measures, (2) *TOMM40* '523 poly-T genotype, (3) *APOE* genotype, and (4) neuropsychological performance. We also examined clinical diagnosis at baseline and month 36.

2.2. AD family history

For WRAP, a parental history of AD—defined by clinical diagnosis or autopsy before ages 70 and 75 years for paternal FH and FHM, respectively—was determined as described [25]. For ADNI, the presence of FHM and paternal FH was determined for AD by the participant, informant, or both.

2.3. Protocol approvals, registrations, and patient consents

For WRAP, the University of Wisconsin-Madison Health Sciences Institutional Review Board approved the study, and all participants provided written informed consent. For ADNI, written informed consent was obtained from all ADNI participants at their respective ADNI sites. Site-specific Institutional Review Boards approved the ADNI protocol.

2.4. APOE genotyping

For ADNI, the Biomarker Core at the University of Pennsylvania conducted APOE genotyping [26] as described in white papers (http://adni.loni.usc.edu/). For WRAP, extraction and isoform classification of APOE alleles have been described previously [6]. Participants who were non-APOE ε4 were analyzed. Ancillary analyses were also conducted on APOE ε3/ε3 carriers only or APOE ε2 carriers (Supplementary Text 1), where the pattern of results was similar for APOE ε3/ε3 subjects.

2.5. TOMM40 genotyping

For both WRAP [6] and ADNI [8,11], genotyping of TOMM40 '523 poly-T was conducted as described (Polymorphic DNA Technologies, Inc, Alameda, CA) [6]. Briefly, the '523 poly-T polymorphism was determined using polymerase chain reaction amplification of each region's variant site, followed by corresponding A-peaks to the N values (poly-T length) of each component, relative to the C-peak reference base upstream of the poly-T intron site. Manual electrogram review was also conducted to avoid errors. This process obviated the concern of polymerase chain reaction slippage.

2.6. Cognition, clinical, and mitochondrial biomarker measures

For WRAP, participants underwent a full neuropsychological battery. Although factor analysis derived six cognitive factors [27,28], the focus of this report is on memory—to maintain overlap with ADNI data and reduce type 1 error. Latent memory factors were derived as described in ref [27], which encapsulated immediate and delayed memory. Composite scores based on the latent factor structure were largely derived from Z-scores of longitudinal measures of the immediate memory and delayed recall portions of the Rey Auditory Verbal Learning Test (RAVLT) [29]. RAVLT subcomponents were not considered because they have been investigated elsewhere [6].

For ADNI, as described in ref [30], a Z-score general memory composite was derived from longitudinal immediate and delayed RAVLT scores, AD Assessment Schedule–Cognition [31], Mini–Mental State Examination (MMSE), and the Wechsler Memory Scale–Revised Logical Memory II. Total RAVLT scores for immediate memory and delayed recall were separately assessed to establish which aspect of memory function drove the factor result. Global cognition and function were assessed by MMSE and Clinical Dementia Rating–sum of boxes (CDR-sob). Finally, as described in ref [32], a peptide of CSF cytosol AST (IVASTLSNPEL-FEEWTGNVK) was available in 119 ADNI subjects only at baseline, as acquired by the ADNI Biomarker Core using mass spectrometry [32]. No other direct mitochondrial biomarkers were present in existing assays. Values are in arbitrary log units derived from signal intensity.

2.7. Statistical analysis

SPSS 23 (Chicago, IL, USA) was used for analyses. Data were analyzed using mixed-effects regression models. Model equations and both fixed and random terms are described in Supplementary Text 2. Aikake's Information Criterion was used to determine the optimal covariance matrix. In each cohort separately (WRAP or ADNI), for a given Z-score memory factor, we tested all main effects and interactions for FH, TOMM40, and Time as predictors in a single model. A similar approach was used for testing global cognition outcomes in ADNI participants. This approach assessed if FH modified TOMM40 genotype associations, and if these effects on memory decline were the same over time or dynamically changed based on FH and genotype. To explore which memory components drove significant findings for the single ADNI memory factor, immediate and delayed RAVLT scores were assessed. Exploratory analyses were also conducted with FHM, due to FHM's influence on mitochondrial function [17]. We controlled for sex, age, and education. A similar mixed model approach was used to predict baseline CSF AST in ADNI. Logistic regression was used in ADNI data to identify if FH and TOMM40 predicted a greater likelihood of being MCI or AD at baseline versus the CN reference group. Importantly, we did not investigate if TOMM40 and FH modified clinical diagnosis effects on memory because of small sample sizes among subgroups stratified by TOMM40, FH, and APOE.

To minimize type 1 error, as described elsewhere [25], Holm-Bonferroni correction was used to maintain a family-wise error rate of $P < .05$. Briefly, for WRAP, familywise adjusted P values were .025 and .05 for testing 1 model between two latent memory factors, and similarly .025 and .05 for ADNI MMSE and CDR-sob. If a TOMM40*FH or TOMM40*FH*Time interaction was not significant, we separately examined two additional models in FH− and

Table 1
Demographics and neuropsychological characteristics (N or mean ± standard deviation)

| | WRAP | ADNI | | | | | |
| | | CN | | MCI | | AD | |
	CN	FH−	FH+	FH−	FH+	FH−	FH+
Sample size	912	88	58	111	52	39	17
APOE ε2 (%)	14.14	18.18	20.69	9.01	5.77	7.69	5.88
Age	54.20 ± 6.61	75.86 ± 5.04	75.79 ± 4.88	75.36 ± 8.28	76.71 ± 6.63	77.34 ± 7.36	76.82 ± 9.41
Gender	645 F, 285 M	40 F, 48 M	29 F, 29 M	38 F, 73 M	14 F, 38 M	21 F, 18 M	9 F, 8 M
Years of education	16.08 ± 3.12	15.89 ± 2.97	16.55 ± 2.62	15.45 ± 3.17	16.63 ± 2.77	14.23 ± 3.48	16.35 ± 2.40
MMSE	29.29 ± 0.941	29.05 ± 1.00	29.05 ± 1.03	27.04 ± 1.75	27.27 ± 1.76	23.26 ± 2.29	23.94 ± 1.82
CDR-sob	0 ± 0	0.04 ± 0.14	0.03 ± 0.13	1.53 ± 0.77	1.54 ± 1.03	4.22 ± 1.84	4.21 ± 1.50

Abbreviations: AD, Alzheimer's disease; ADNI, Alzheimer's Disease Neuroimaging Initiative; APOE ε2, apolipoprotein ε2; CDR-sob, Clinical Dementia Rating–sum of boxes; CN, cognitively normal; FH, family history; MCI, mild cognitive impairment; MMSE, Mini–Mental State Examination; WRAP, Wisconsin Registry for Alzheimer's Prevention.

FH+ to tests main effects of *TOMM40* and its interaction with Time. This required familywise adjustment of .017, .025, and .050 to yield significance. This approach is more optimal than conventional Bonferroni correction [33-35]. No statistical violations were found for collinearity, normality, outliers, or model overfitting.

3. Results

3.1. Data summary

Participant data for 912 WRAP and 365 ADNI non-*APOE* ε4 participants are listed in Table 1. For WRAP, the sample size was 333 FH− (S/S = 112; S/VL = 143; and VL/VL = 78) versus 579 FH+ (S/S = 177; S/VL = 285; and VL/VL = 117). For ADNI, the sample size was 238 FH− (S/S = 59; S/VL = 124; and VL/VL = 55) versus 127 FH+ (S/S = 28; S/VL = 71; and VL/VL = 28). The roughly 1:2:1 ratio among genotypes is comparable to what has been reported in predominantly Caucasian cohorts [6,8]. All latent factor analyses without *APOE* ε2s remained significant (Supplementary Text 1).

3.2. WRAP: TOMM40 and FH interaction effects on memory performance over 7 to 10 years

We first examined change in immediate and delayed memory factors in late middle-aged *APOE* ε4− WRAP subjects. For immediate memory, a *TOMM40**FH effect ($F = 4.704$, estimate ± standard error [SE] = -0.279 ± 0.192, $P = .009$) showed that *TOMM40* VL was related to better memory among FH− and worse memory among FH+ subjects, regardless of Time (Supplementary Fig. 1). For example, higher scores were seen in FH− VL/VL versus S/S ($D_{Cohen} = 0.352$) and lower scores for FH+ VL/VL versus S/S ($D_{Cohen} = -0.787$). It should be noted here and throughout the report that FH also modified S/S genotype effects. When exploring FHM, a FHM*TOMM40 interaction ($F = 2.935$, estimate ± SE = -0.320 ± 0.248, $P = .050$) showed similar effects.

For the delayed memory factor, a *TOMM40**FH*Time interaction ($F = 3.695$, estimate ± SE = 0.502 ± 0.189,

$P = .025$) indicated a strong effect of VL for either slowing (FH−) or exacerbating (FH+) memory decline for 7 to 10 years (Fig. 1). FH− VL/VL had *less* memory decline compared with S/S ($D_{Cohen} = 1.126$), whereas FH+ VL/VL had more memory decline versus S/S ($D_{Cohen} = -1.322$). Exploratory analyses with FHM revealed a *TOMM40**FHM*-Time interaction ($F = 2.036$, estimate ± SE = 0.323 ± 0.230; $P = .047$) showing similar, albeit weaker effects.

3.3. ADNI: TOMM40 and FH interaction effects on mitochondrial function

Among the subset of ADNI participants (N = 119) who had baseline CSF AST measurements, we assessed if *TOMM40* and FH interacted to affect CSF AST, a biomarker of mitochondrial function. As indicated in Fig. 2A, a FH*TOMM40 interaction ($F = 3.418$, estimate ± SE = -0.263 ± 0.148, $P = .036$) showed a VL effect for higher (FH−) ($D_{Cohen} = 1.077$) or lower (FH+) ($D_{Cohen} = -0.449$) AST. Small sample size for VL/VL genotype precluded formal analysis among FH− ($n = 20$) and FH+ ($n = 15$) subjects, but trended in the expected direction (FH− VL/VL = 12.71 ± 0.03 log units; FH + VL/VL = 12.92 ± 0.06 log units).

3.4. ADNI: TOMM40 and FH interactions for MCI and AD diagnosis odds ratios

For FH and *TOMM40*, no main effects or interactions for risk of MCI or AD versus CN diagnosis were noted. In exploratory analyses for FHM, a *TOMM40**FHM interaction (Wald = 6.154, $P = .046$) showed that for FHM−, S/S and S/VL compared with VL/VL participants had a 4.67 higher odds ratio of being MCI or AD at baseline, whereas for FHM+ the S/S and S/VL genotypes had a 0.215 lower odds ratio. As there were only 102 non-*APOE* ε4 MCI converters to AD by month 36, there was not sufficient sample size to stratify by FH and *TOMM40* to conduct age of onset or logistic regression analyses for stable versus progressive MCI.
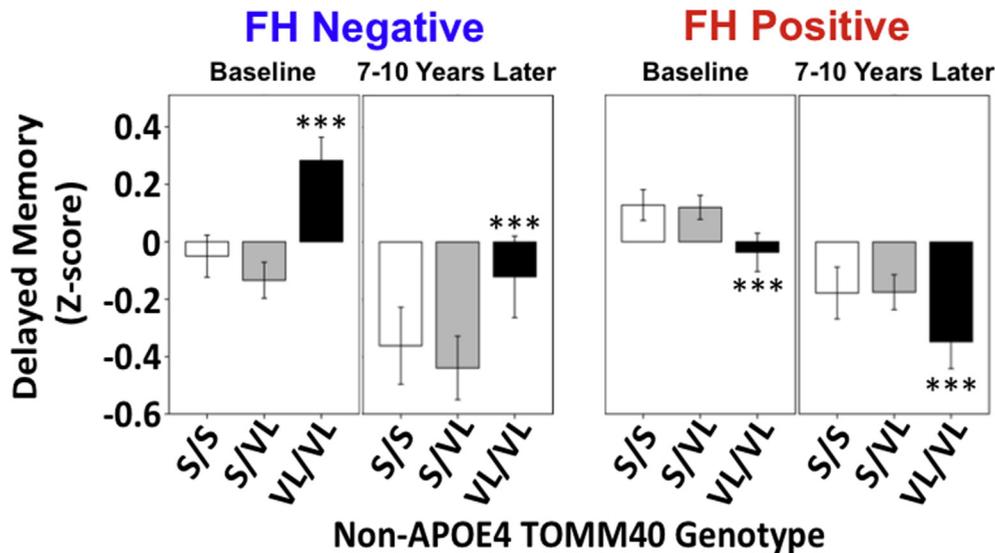
Fig. 1. FH and *TOMM40* '523 effects on delayed memory decline in late middle-age. In the middle-aged WRAP cohort, FH modulates *TOMM40* '523 short (S) versus very long (VL) carriage effects on delayed memory performance. Differences are shown at baseline and the last visit 7 to 10 years later. ***$P < .001$ comparing VL/VL to other genotypes at a given time point. Data are presented as the mean ± standard deviation. Abbreviations: FH, family history; *TOMM40*, translocase of the outer mitochondrial membrane 40 kDa; WRAP, Wisconsin Registry for Alzheimer's Prevention.

3.5. ADNI: TOMM40 *and FH interactions on memory performance over 3 years*

Unlike WRAP, the *TOMM40**FH*Time interaction was nonsignificant among ADNI participants, perhaps because
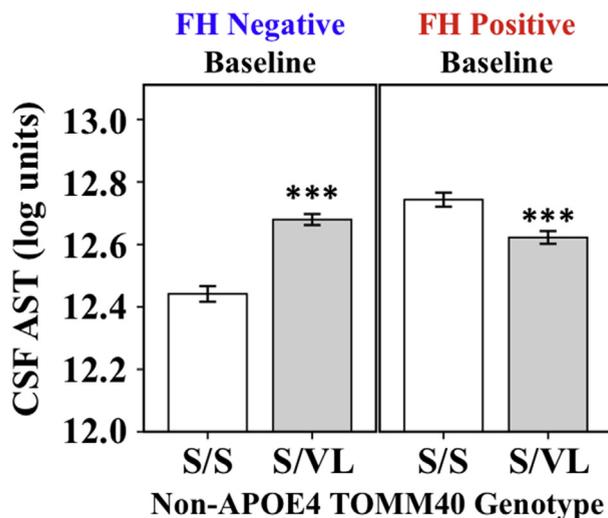
Fig. 2. FH and *TOMM40* '523 effects on a biomarker of mitochondrial function in old age. For the aged ADNI cohort, the effect of FH status and *TOMM40* '523 genotype on CSF aspartate aminotransferase (AST), a putative biomarker of mitochondrial function, was examined. Very long/very long (VL/VL) subjects were formally excluded from FH negative ($n = 20$) and FH positive ($n = 15$) groups because of low sample size. Exploratory analysis showed that FH negative and FH positive VL/VL subjects had significantly higher AST levels than short/short (S/S) subjects. ***$P < .001$ comparing S/VL to S/S. Data are presented as the mean ± standard deviation. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE* ε4, apolipoprotein ε4; CSF, cerebrospinal fluid; FH, family history; *TOMM40*, translocase of the outer mitochondrial membrane 40 kDa.

of lower power. When stratified by FH status, FH− subjects showed a *TOMM40**Time effect ($F = 2.465$, estimate ± SE = $−0.349 ± 0.096$, $P = .012$), whereas FH+ subjects had a *TOMM40* main effect ($F = 3.453$, estimate ± SE = $0.228 ± 0.070$, $P = .008$). FH− *TOMM40* genotypes did not vary at baseline, whereas 3 years later S/S carriers showed a decline of more than 0.3 standard deviations, with no decline evident in S/VL or VL/VL ($D_{Korr} = 0.497$). FH+ *TOMM40* VL/VL carriers, meanwhile, showed a gene-dose decrease in memory performance relative to S/S or S/VL that did not vary over time ($D_{Cohen} = 0.430$).

Analyses with FHM had similar, stronger effects. FHM− showed a *TOMM40**Time effect ($F = 2.352$, estimate±SE = $−0.318 ± 0.094$, $P = .017$), where, for example, S/S but not VL/VL carriers evinced decline ($D_{Korr} = 0.378$) (Fig. 3, left panels). Among FHM+, a main effect of *TOMM40* ($F = 4.284$, estimate ± SE = $0.350 ± 0.139$, $P = .014$) illustrated a clear gene-dose effect on overall memory performance that did not change for more than 3 years (Fig. 3, right panel). For example, VL/VL versus S/S carriers had lower memory scores ($D_{Cohen} = 0.607$).

3.6. ADNI: TOMM40 *and FH interaction effects on RAVLT over 3 years*

As ADNI has one memory factor including immediate and delayed components, we did follow-up analyses to see if the RAVLT encoding or retention portions explained results for the factor. For RAVLT immediate memory (i.e., trials 1–5 total), a *TOMM40**FH*Time ($F = 2.203$, estimate ± SE = $9.124 ± 2.942$, $P = .027$) pattern similar to the general memory factor emerged. VLs of both FH
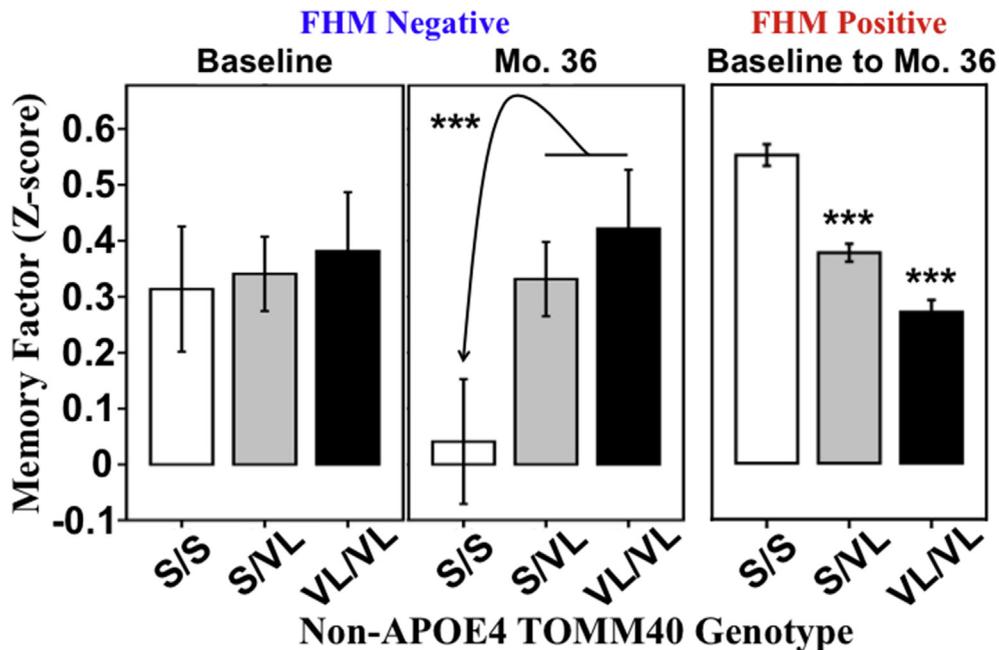
Fig. 3. FH and *TOMM40* '523 effects on memory decline in old age. For the aged ADNI cohort, maternal FH (FHM) status modulates the effect of TOMM40 '523 very long (VL) carriage on general memory performance. TOMM40 '523 genotypes showed relative changes in memory decline for FHM negative participants over time, whereas FHM positive participants showed gene-dose differences that remained constant from baseline to month 36. ***$P < .001$ for genotype differences relative to other genotypes at a given time point. Data are presented as the mean ± standard deviation. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE* ε4, apolipoprotein ε4; FH, family history; Mo., month; S, short; *TOMM40*, translocase of the outer mitochondrial membrane 40 kDa.

groups did not differ at baseline. However, over 3 years, FH− S/S showed progressive decline, whereas VL/VL showed little to no decline ($D_{Korr} = 0.378$). The opposite pattern was observed for FH+ VL/VL versus S/S, where VL/VL showed greater decline over time ($D_{Korr} = -0.248$).

For FHM, a similar *TOMM40**FHM*Time interaction ($F = 2.008$, estimate ± SE = 8.412 ± 3.558, $P = .042$) showed no FHM differences at baseline, but VL gene-dose preservation (FH−) or decline (FH+) over 3 years (Supplementary Fig. 2). For example, FH− VL/VL versus S/S had progressively better scores ($D_{Korr} = 0.601$) but FH+ VL/VL versus S/S had progressively worse scores ($D_{Korr} = 0.271$).

RAVLT delayed memory was not affected by FH or FHM, *TOMM40*, or their interaction with or without Time. These results suggest that immediate verbal memory encoding rather than delayed retention may underlie the statistical effect on the ADNI memory factor.

3.7. ADNI: TOMM40 *and FH effects on global decline over 3 years*

For CDR-sob, no effects were significant when considering FH. For FHM, a *TOMM40**FHM*Time interaction ($F = 2.187$, estimate ± SE = 2.854 ± 0.961, $P = .025$) showed that global decline varied among *TOMM40* groups based on FHM status. For FHM−, a marginally lower CDR-sob at baseline was seen between VL/VL versus S/S, where by 3 years later S/VL and VL/VL genotypes showed

less decline compared with S/S ($D_{Korr} = 0.286$) (Fig. 4, left panel). In contrast, FHM+ VL/VL carriers did not differ at baseline from S/S, but showed more progressive decline after 3 years ($D_{Korr} = -0.337$) (Fig. 4, right panel).

For MMSE, a *TOMM40**FH*Time effect ($F = 1.960$, estimate ± SE = 1.179 ± 0.662, $P = .048$) revealed a weak pattern similar to CDR-sob. For instance, FH− VL/VL versus S/S subjects showed less progressive decline ($D_{Korr} = 0.1$). However, FH + VL/VL did not differ from S/S at baseline or over the course of 3 years. For FHM, a similar *TOMM40**FHM*Time interaction ($F = 2.452$, estimate ± SE = 1.091 ± 0.660, $P = .012$) showed that VL/VL versus S/S carriers had marginally higher MMSE scores at baseline, where 3 years later the S/S genotype showed greater decline compared with S/VL or VL/VL ($D_{Korr} = 0.125$) (Supplementary Fig. 3, left panel). FHM+ participants did not differ at baseline or up to 3 years later (Supplementary Fig. 3, right panel).

4. Discussion

In this report, we found in middle-aged and aged cohorts that FH altered *TOMM40* '523 poly-T genotype associations on memory, and additionally in aged participants for both global decline and cognitive impairment risk. Specifically, VL genotype was related to ameliorative (FH−) or deleterious (FH+) effects on memory factors, RAVLT immediate memory, CDR-sob, and MMSE scores. FHM had stronger effects relative to FH in the old age cohort.
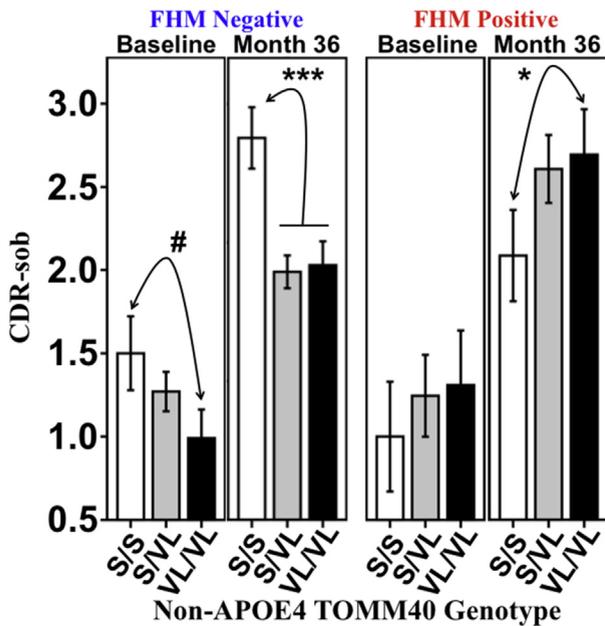
Fig. 4. FH and TOMM40 '523 effects on CDR-sob in old age. For the aged ADNI cohort, maternal FH (FHM) status modulates the effect of TOMM40 VL carriage on global function using CDR-sob. [#], [*], [***] = $P < .076$, .05, or .001 when comparing CDR-sob scores between genotypes at a given time point. Data are presented as the mean ± standard deviation. Abbreviations: ADNI, Alzheimer's Disease Neuroimaging Initiative; *APOE* ε4, apolipoprotein ε4; CDR-sob, Clinical Dementia Rating–sum of boxes; FH, family history; S, short. TOMM40, translocase of the outer mitochondrial membrane 40 kDa; VL, very long.

4.1. FH and TOMM40 *interact to affect memory decline in late middle-age*

Within WRAP, Johnson et al. [6] originally found gene-dose associations (S/S, S/VL, VL/VL) with worse RAVLT total recall and retrieval from primacy, where S/S versus VL/VL subjects showed differences of over half a standard deviation (Cohen's $D = 0.599$ to 0.718). Comparatively, by 7 to 10 years later, we found for the delayed memory factor that FH− S/S and VL/VL differed by a Cohen's $D = 1.126$, where for FH+ that difference was Cohen's $D = -1.322$. These findings were stronger than that for the immediate memory factor, perhaps because delayed memory is a more sensitive cognitive feature for detecting decline related to AD [36]. Caselli et al. [10] noted that non-*APOE* ε4 S/S versus VL/VL subjects showed less RAVLT decline in middle-age, but a flat slope after age 60 years, indicating that initial differences in cognitive performance abated over time. Yet, in this report, VL/VL homozygotes differed from other genotypes at baseline (mean age = 54.20) and showed less (FH−) or more (FH+) decline by the last available study visit (mean age = 63.21).

4.2. FH and TOMM40 *interact to affect memory decline in old age*

FH and *TOMM40* poly-T genotype showed similar effects in ADNI. Effects were stronger in FHM than FH, which

may be because of age-related mitochondrial dysfunction [37]. Unlike WRAP, *TOMM40* VL effects between FH− and FH+ groups were similar at baseline, but over time showed progressive gene-dose differences in memory and global decline. These results may explain the null ADNI findings of Jun et al. [14], and in particular Cruchaga et al. [13] noting later age of onset among *APOE* ε3 homozygotes with VL carriage. Indeed, given the opposing effects we see for FH− and FH+ in ADNI, *TOMM40* VL effects would largely cancel out when collapsed across FH status. Hayden et al. [9] also found a mild but significant gene-dose response (S/S, S/VL, VL/VL) for improved global function. Comparably, we found that FH− VL carriers showed less decline in CDR-sob and MMSE scores.

4.3. FH and TOMM40 *affect mitochondrial function in old age*

Importantly, among a subset of ADNI subjects, FH modified VL carriage associations of CSF AST, a biomarker of mitochondrial function [20,21] that is higher in AD CSF [22] and cortical tissue [23]. As glucose metabolism decreases in AD, alternative sources of energy are used to sustain energy-intensive synaptic processes. Cytosolic AST may through the malate-aspartate cycle maximize ATP production [21], as well as maintain ATP and antioxidative capacity in neural cells [38].

4.4. Limitations and future work

There are serious limitations that should be addressed. First, the sample size for some subgroups in ADNI was small, particularly for FH+ and FHM+ subjects, which may explain nonsignificant trends related to decline. We intend to re-examine these effects in additional AD cohorts. Nonetheless, we consistently found that for FH−, VL carriage was associated with lower AD risk, less mitochondrial dysfunction via the CSF biomarker AST, and less memory and global decline over time, whereas the opposite pattern was seen for FH+. Although FH and *TOMM40* may affect mitochondrial function, it is not clear if FH is modulating *TOMM40* function by epigenetic, environmental, or other factors. It is clear that FHM affects cytochrome oxidase [17] production that impacts AD progression, and that SNPs for several mitochondrial genes contribute to a higher odds ratio for AD [39] in ADNI. Although FH in WRAP and ADNI both modified *TOMM40* poly-T effects, FHM exerted modest effects in middle age but more potent associations in the aged cohort. Mitochondrial dysfunction is thought to increase with age [37], where the influence of FHM on mitochondrial bioenergetics may only be present in older subjects evincing damage because of reactive oxygen species. Despite these limitations, this report may clarify why the *TOMM40* '523 literature has shown beneficial, null, and/or detrimental effects. We propose that the proportion of

participants with FH+ may influence *TOMM40* '523 effects on memory, age of onset, and other outcomes. Our future work will investigate additional mitochondrial biomarkers and examine impact on neural, CSF biomarker, and metabolic outcomes.

Acknowledgments

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2017.03.009.

RESEARCH IN CONTEXT

1. Systematic review: We searched for articles in PubMed using the keywords: translocase of outer mitochondrial membrane 40; Alzheimer's disease; mitochondrial function, aspartate aminotransferase; memory; preclinical AD; poly-T; rs10524523. We focused on human work to examine translocase of the outer mitochondrial membrane 40 kDa (TOMM40) '523 genotype and effects on memory decline and Alzheimer's disease (AD) risk.

2. Interpretation: This is the first human study to demonstrate that AD family history modifies the effect of TOMM40 '523 genotype on memory, global cognition, and a biomarker of mitochondrial function. Findings were similar across two separate cohorts of cognitively normal middle-aged and aged subjects across the AD spectrum. Varying proportions of AD family history in subjects may explain mixed findings in the TOMM40 '523 literature.

3. Future directions: Data from other observational AD cohorts will be collected to confirm results. Higher sample size will allow AD age of onset and mild cognitive impairment conversion risk analyses. Effects on neural, metabolic, and biomarker outcomes will be assessed.

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