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Featured Article

Neuropathologic features of *TOMM40* '523 variant on late-life cognitive decline

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Abstract

Introduction: The study investigated the role of neuropathologies in the relationship between *TOMM40* '523 genotype and late-life cognitive decline.

Methods: Participants were community-dwelling older persons who had annual cognitive assessments and brain autopsies after death. Genotyping used DNA from peripheral blood or postmortem brain tissue. Linear mixed models assessed the extent to which the association of '523 genotype with cognitive decline is attributable to neuropathologies.

Results: Relative to ε3/3 homozygotes with '523-S/VL or '523-VL/VL genotype, both '523-L carriers and ε3/3 homozygotes with '523-S/S genotype had faster cognitive decline. The association of '523-L with cognitive decline was attenuated and no longer significant after controlling for Alzheimer's and other neuropathologies. By contrast, the association of '523-S/S was unchanged.

Discussion: There are two distinct *TOMM40* '523 signals in relation to late-life cognitive decline. One signal primarily acts through AD and other common neuropathologies, whereas the other operates through a different mechanism.

Keywords: Neuropathologies; *TOMM40* '523; Late-life cognitive decline

1. Introduction

Apolipoprotein E (*APOE*) is the best-known susceptibility gene for late-onset Alzheimer disease (AD) [1]. The region on chromosome 19 that harbors the *APOE* gene includes a large haplotype block that contains several other genes including apolipoprotein C1 (*APOC1*) and translocase of outer mitochondrial membrane 40 (*TOMM40*) [2]. Beside *APOE* ε alleles, multiple genetic variations within the block have also been implicated in AD [3–7]. *TOMM40* '523, a poly-T polymorphism at an intronic region of *TOMM40*, is of particular interest. The variable length of poly-T repeat

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at '523 locus is associated with age at disease onset and cognition [7–9]. Notably, the variant is in linkage disequilibrium (LD) with *APOE* genotype. Among Caucasians, *APOE* ε4 is exclusively linked to the long ('523-L) poly-T repeat whereas ε3 can be linked to either short ('523-S) or very long ('523-VL) poly-T repeat. This LD structure has two implications. The close linkage between ε4 and '523-L raises the question whether *TOMM40* '523-L is merely a proxy of ε4 [10]. By contrast, three major '523 genotypes are present in *APOE* ε3/3 homozygotes, namely '523-S/S, '523-S/VL and '523-VL/VL. An earlier study found that age at AD onset varies across these three genotypes [11], and we recently reported that '523-S/S carriers exhibit faster decline in late-life cognition compared with '523-S/VL or '523-VL/VL carriers [12]. These findings suggest that the '523 effect is not fully attributable to the LD with *APOE* variants. However, the neurobiologic or pathobiologic basis underlying these associations remains unclear. Evidence suggests that the ε4 allele is directly involved in the pathogenesis of AD via regulating β-amyloid accumulation, a key neuropathologic feature of the disease [13–15]. In this study, we aimed to determine whether the '523 effect was related to AD or other neuropathologies among persons with *APOE* ε3/3.

To examine the role of common neuropathologies in the relationship between *TOMM40* '523 genotype and longitudinal cognitive decline, we leveraged cognitive, genetic, and neuropathologic data from a large number of community-based older Caucasian Americans who were followed annually for up to 21 years and had undergone brain autopsy after death. We previously reported that AD pathology in general, and β-amyloid in particular, mediates the effect of *APOE* ε4 with cognitive decline and AD dementia [16,17]. Owing to its strong linkage with *APOE* ε4, we first confirm that the same relationship exists for *TOMM40* '523-L. Then, we test the hypothesis that among persons with *APOE* ε3/3, the '523-S/S is also associated with measures of AD pathology. Failure to find such an association, in contrast to a strong association between the *APOE* ε4–'523-L haplotype and AD pathology, would suggest that the two genes have separate and relatively independent effects on cognition and operate through different pathologic mechanisms.

2. Methods

2.1. Study participants

Participants came from two ongoing longitudinal cohort studies of aging and dementia, the Religious Orders Study (ROS) [18] and the Rush Memory and Aging Project (MAP) [19]. ROS and MAP enroll community-dwelling older persons without known dementia. Participants were followed annually for detailed cognitive and clinical assessments; all agreed to brain donation after death. Both studies were approved by the Institutional Review Board of Rush University Medical Center, and written informed consent and an Anatomical Gift Act were provided by each participant. By early January 2017, 1501 participants of European ancestry had died, of which 1326 had undergone brain autopsy (autopsy rate = 88.3%). The present study focused on individuals who had genotype data and longitudinal cognitive assessments (N = 1114). The mean age at death was 89.4 years (standard deviation [SD] = 6.4), 66.6% were females (N = 742), and the mean education was 16.3 years (SD = 3.6).

2.2. APOE *and* TOMM40 *'523 genotyping*

DNA was extracted from peripheral blood and in some cases from frozen postmortem brain tissue. The genotyping were performed at Polymorphic DNA Technologies (Alameda, CA). The vendor was blinded to all clinical and neuropathologic information. *APOE* genotype was based on two polymorphisms (rs429358 and rs7412) at exon 4 of the *APOE* gene. *TOMM40* '523 refers to rs10524523, a homopolymer length polymorphism (poly-T) at intron 6 of the *TOMM40* gene (chr19:44,899,792-44,899,826, human genome reference assembly GRCh38/hg38). The '523 genotype was determined by the length of poly-T repeat, as previously described [20]. Briefly, a '523 short allele ('523-S) has poly-T repeat length less than 20, a long allele ('523-L) has poly-T repeat length between 20 and 29, and a very long allele ('523-VL) has poly-T repeat length of 30 and above.

2.3. Annual cognitive assessments

Participants underwent uniform annual cognitive assessments for up to 22 years (mean = 8.3, SD = 4.5). Cognitive performance was assessed using a battery of 17 tests [21]. Scores from each test were standardized using the baseline mean and SD of the two cohorts. The resulting z-scores were averaged across the tests to obtain a composite measure of global cognition. The composite measure minimizes the floor and ceiling artifacts that are common for individual tests, and similar approach has been applied in many other studies [22–25].

2.4. Postmortem neuropathologic evaluations

At autopsy, we quantified the burdens of common age-related neuropathologies including AD, macroscopic infarcts, microinfarcts, Lewy bodies, hippocampal sclerosis, TDP-43, cerebral amyloid angiopathy, atherosclerosis, and arteriolosclerosis. β-amyloid and phosphorylated PHFtau tangles, two molecular-specific pathologic hallmarks of AD, were assessed in eight brain regions using immunohistochemistry [26]. Percent area positive for β-amyloid was computed for each region using image analysis and averaged across the regions to obtain a summary measure of β-amyloid load. Density of PHFtau tangles per mm^2 was computed for each region using a stereological mapping station and averaged to obtain a summary

measure of PHFtau tangle density. Chronic macroscopic infarcts were recorded during gross examination and verified histologically [27]. Chronic microinfarcts were identified in a minimum of nine regions using hematoxylin and eosin (H&E) staining [28]. The presence of Lewy bodies in neocortical regions was identified using α-synuclein immunostaining [29]. Hippocampal sclerosis refers to severe neuronal loss and astrogliosis of CA1 and/or subiculum and was determined using H&E staining [30]. TDP-43 pathology was assessed in five regions using monoclonal antibodies to phosphorylated TDP-43 and was rated on a four-level scale including no inclusion, inclusion in amygdala, inclusions in amygdala and limbic, or inclusions in amygdala, limbic, and neocortex [31]. Cerebral amyloid angiopathy was assessed in four neocortical regions using monoclonal antibodies to β-amyloid [32]. Amount of amyloid deposition in the vessel walls was scored for each region, and the average scores across the regions were summarized into a four-level scale representing none, mild, moderate, or severe. Atherosclerosis was assessed in the circle of Willis during gross examination and arteriolosclerosis was assessed in anterior basal ganglia using H&E staining, and both were rated on a four-level scale of none, mild, moderate, or severe [33].

2.5. Statistical analysis

Frequency tables and Cohn's κ described the linkage pattern between *APOE* and *TOMM40* '523 genotypes. Analysis of covariance examined the adjusted mean level of continuous pathologic indices by '523 genotype. For each of the binary (or ordinal) pathologic indices, logistic regression tested the '523 association with the odds of having the corresponding pathology (or the odds of having more severe pathology).

To examine the extent to which common neuropathologies contribute to the association between '523 genotype with cognitive decline, we applied linear mixed models with annual global cognition as the longitudinal outcome. The models included a term for time in years before death, which estimates the mean rate of cognitive decline. The predictor of main interest was interaction term between '523 genotype and time, which estimates the genotype association with cognitive decline. We repeated the models three times, (1) without adjustment for neuropathologies; (2) adjustment for AD pathologies; and (3) adjustment for AD and other common neuropathologies. If neuropathologies are involved in the association of '523 genotype with cognitive decline, we expect that the estimate for the interaction term and the corresponding statistical significance would be attenuated after controlling for neuropathologies.

The analyses were performed using SAS/STAT software, version 9.4 for Linux (SAS Institute Inc., Cary, NC, USA). Statistical significance was determined at α level of 0.05, and all the models were controlled for age, sex, and education.

3. Results

3.1. The linkage pattern between APOE and TOMM40 '523

Of the 1114 autopsied individuals included in the study, 60.1% were of *APOE* ε3/3 genotype, 26.6% were ε4 carriers (i.e., ε2/4, ε3/4, or ε4/4) and the rest were ε2 carriers (Table 1). The well-known LD between *APOE* and *TOMM40* '523 was clearly evident (Table 2). Specifically, *APOE* ε4 carriers and *TOMM40* '523-L carriers were highly concordant such that 94.9% of all ε4 carriers had '523-L and 95.3% of all '523-L carriers had ε4 (Cohen's $\kappa = 0.93$, 95% confidence interval [CI] = 0.91–0.96). By contrast, three major '523 genotypes were observed in *APOE* ε3/3 homozygotes, of which '523-S/S accounted for 25.5%, '523-S/VL and '523-VL/VL accounted for 46.7% and 25.7%, respectively. Notably, the '523-L allele was also absent from ε2 carriers in this autopsied sample.

3.2. TOMM40 '523 and cognitive decline

Before examining their association with neuropathology, we first confirm that both '523-L carriers in LD with ε4 and the ε3/3 homozygotes with '523-S/S exhibit faster decline in the current sample, which represents a subset of autopsied individuals used in our prior report [12]. As expected, the results from a linear mixed model (Table 3 Model A) found that compared with ε3/3 homozygotes with '523-S/VL or '523-VL/VL genotype, '523-L carriers declined faster in cognition (estimate = −0.059, standard error [SE] = 0.009, $P < .001$). Furthermore, ε3/3 homozygotes with '523-S/S also had faster decline but with a weaker effect that was approximately 40% that of '523-L (estimate = −0.023, SE = 0.010, $P = .024$).

Table 1
Basic characteristics of the study participants ($N = 1114$) Q10

	Mean (SD) or *N* (%)
Age at death (years)	89.4 (6.4)
Female, *N* (%)	742 (66.6)
Education (years)	16.3 (3.6)
Number of cognitive assessments	8.3 (4.5)
APOE genotype	
ε2/2	6 (0.5)
ε2/3	142 (12.8)
ε2/4	27 (2.4)
ε3/3	670 (60.1)
ε3/4	251 (22.5)
ε4/4	18 (1.6)
TOMM40 '523 genotype	
Short/short (S/S)	199 (17.9)
Short/long (S/L)	141 (12.7)
Short/very long (S/VL)	389 (34.9)
Long/long (L/L)	18 (1.6)
Long/very long (L/VL)	136 (12.2)
Very-long/very long (VL/VL)	231 (20.7)

Abbreviation: SD, standard deviation.

Table 2
Distribution of *TOMM40* '523 genotype by *APOE*

Frequency row percent column percent	*TOMM40* '523-S/S	*TOMM40* '523-S/VL	*TOMM40* '523-VL/VL	*TOMM40* '523-S/L	*TOMM40* '523-L/VL	*TOMM40* '523-L/L
APOE ε2*	27	70	51	0	0	0
	18.24	47.30	34.46	0.00	0.00	0.00
	13.57	17.99	22.08	0.00	0.00	0.00
APOE ε3/3	171	313	172	5	9	0
	25.52	46.72	25.67	0.75	1.34	0.00
	85.93	80.46	74.46	3.55	6.62	0.00
APOE ε4†	1	6	8	136	127	18
	0.34	2.03	2.70	45.95	42.91	6.08
	0.50	1.54	3.46	96.45	93.38	100.00

Abbreviations: S/S, short/short; S/VL, short/very long; VL/VL, very-long/very long; S/L, short/long; L/VL, long/very long; L/L, Long/long.
*ε2 consists of ε2/2 and ε2/3.
†ε4 consists of ε2/4, ε3/4, and ε4/4.

3.3. TOMM40 *'523 and neuropathologies*

Next, we examine the genotype associations with β-amyloid load and PHFtau tangle density. The distributions of both indices by '523 genotypes showed that average level of β-amyloid and PHFtau tangle pathology was noticeably elevated among '523-L carriers, similar to *APOE* ε4. Burdens of these pathologies were similar by the '523-S/S status (Fig. 1A and 1B). In analysis of covariance models adjusted for demographics, compared with the reference group (i.e., ε3/3 homozygotes with '523-S/VL or '523-VL/VL genotype), the levels of β-amyloid load and PHFtau tangle density on average were higher in '523-L carriers (both P's $< .001$). By contrast, we did not observe difference in AD pathologies for ε3/3 homozygotes with '523-S/S (Table 4).

Similar results were observed in relation to other common age-related neuropathologies (Table 4). Briefly, '523-L carriers were more likely to have macroscopic infarcts (odds ratio [OR] = 1.45, 95% CI = 1.07–1.98) and hippocampal sclerosis (OR = 1.85, 95% CI = 1.13–3.02). In addition, they had greater odds of having more advanced TDP-43 pathology (OR = 2.03, 95% CI = 1.51–2.73) and amyloid angiopathy (OR = 3.77, 95% CI = 2.84–5.00). Notably, the association with these non-AD pathologies persisted even after the adjustment for β-amyloid load and PHFtau tangle density. By contrast, we did not find significant association of '523-S/S with any of the neuropathologic indices examined (all P's $> .05$).

Table 3
TOMM40 '523 genotypes, neuropathologies, and cognitive decline

	Model A	Model B	Model C
	Estimate (SE), *P*	Estimate (SE), *P*	Estimate (SE), *P*
Age	0.0001 (0.0006), .927	0.0014 (0.0006), .013	0.0027 (0.0006), <.001
Male	0.022 (0.008), .008	0.005 (0.008), .517	0.007 (0.007), .312
Education	0.0026 (0.0011), .018	0.0021 (0.0009), .028	0.0017 (0.0009), .050
Amyloid load	-	−0.006 (0.003), .051	−0.006 (0.003), .074
PHFtau tangle density	-	−0.038 (0.003), <.001	−0.033 (0.003), <.001
Macroscopic infarcts	-	-	−0.024 (0.007), <.001
Microinfarcts	-	-	0.004 (0.007), .560
Lewy bodies	-	-	−0.057 (0.009), <.001
Hippocampal sclerosis	-	-	−0.041 (0.011), <.001
TDP-43	-	-	−0.010 (0.003), <.001
CAA	-	-	−0.008 (0.004), .043
Atherosclerosis	-	-	−0.017 (0.004), <.001
Arteriolosclerosis	-	-	−0.007 (0.004), .048
'523-S/S	−0.023 (0.010), .024	−0.019 (0.009), −.031	−0.022 (0.008), .007
'523-L	−0.059 (0.009), <.001	−0.024 (0.008), .003	−0.011 (0.008), .160

Abbreviations: CAA, cerebral amyloid angiopathy; SE, standard error.
NOTE. Estimates in each column were obtained from separate linear mixed models. The estimates came from the interaction terms with time in years before death, which refer to the associations of corresponding predictors with annual rate of decline. Model A was controlled for demographics only; Model B was controlled for demographics and AD (amyloid and tangle) pathologies; and Model C was controlled for demographics, AD, and other non-AD pathologies.

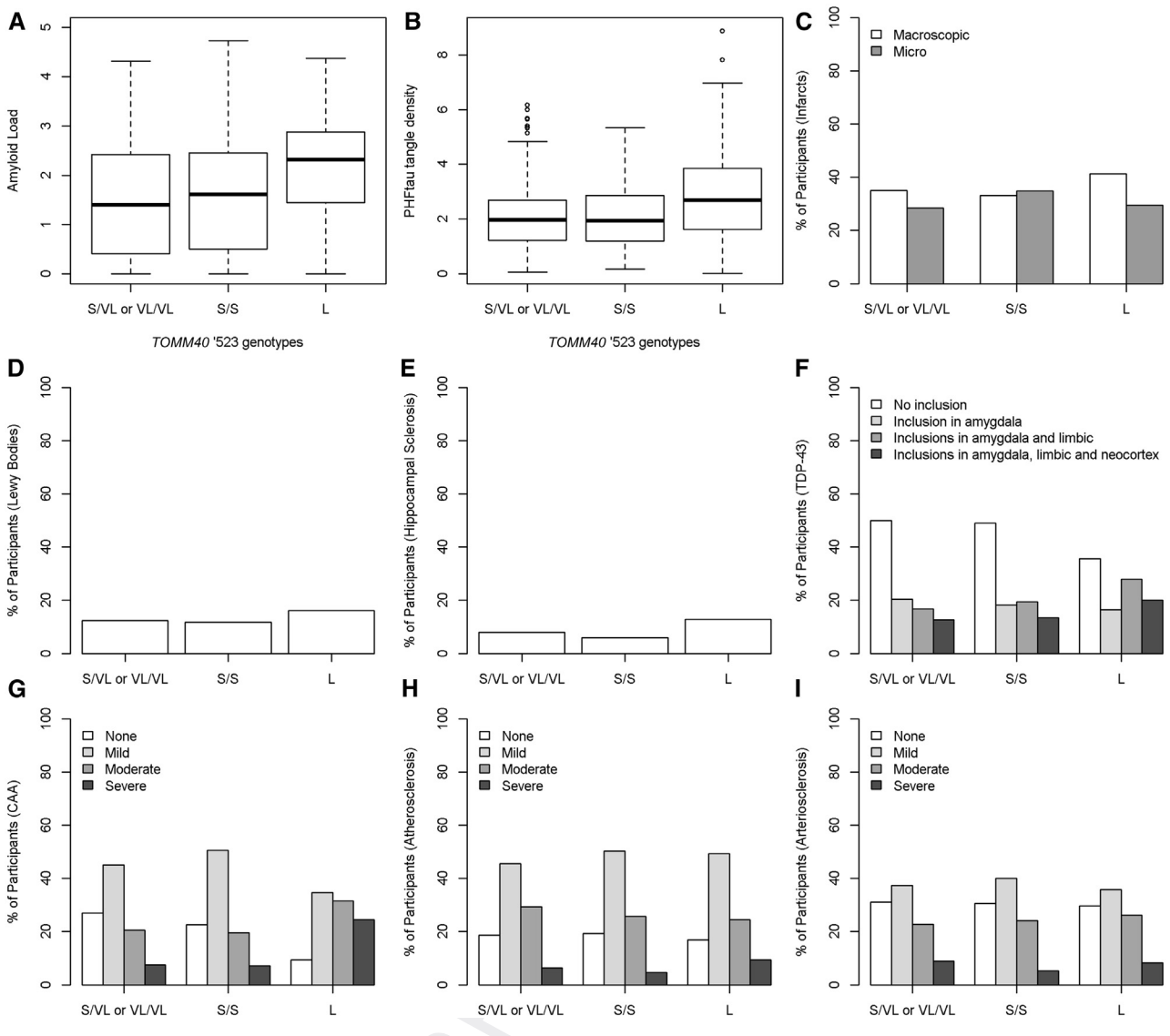

Fig. 1. Distributions of common neuropathologic indices by *TOMM40* '523 genotypes. Panel A: box plot for β amyloid load; Panel B: box plot for PHFtau tangle density; Panel C through Panel I: bar charts for percent participants with cerebral infarcts (C), Lewy bodies (D), hippocampal sclerosis (E), TDP-43 (F), amyloid angiopathy (G), atherosclerosis (H), and arteriolosclerosis (I). Abbreviations: CAA, cerebral amyloid angiopathy; L, long; S, short; VL, very long.

3.4. *The role of neuropathologies in* TOMM40 *'523 association with cognitive decline*

To investigate the role of neuropathologies in *TOMM40* '523 association with cognitive decline, we first extended the previous linear mixed model by adding terms for β-amyloid load and PHFtau tangle density (Table 3 Model B). AD pathologies, PHFtau tangle density in particular, were associated with faster cognitive decline. After controlling for AD pathologies, while the association of '523-L remained significant, the effect size was attenuated by about 60% such that the point estimate was reduced from −0.059 to −0.024 (SE = 0.008, P = .003). By contrast, the estimate for '523-S/S only changed about 17% from −0.023 to −0.019 (SE = 0.009, P = .031).

Next, we repeated the model by adding terms for other non-AD pathologic indices (Table 3 Model C). In addition to AD, multiple pathologies including macroscopic infarcts, neocortical Lewy bodies, hippocampal sclerosis, TDP-43, amyloid angiopathy, and atherosclerosis were independently associated with faster decline in cognition. Notably, we observed further attenuation of the '523-L association with cognitive decline from the original −0.059 to −0.011, an 80% reduction such that it no longer reached statistical significance (SE = 0.008, P = .160). By contrast, the estimate for '523-S/S remained almost identical; it was originally −0.023, and after controlling for AD and other pathologies, it was −0.022 (SE = 0.008, P = .007). This strongly suggests that the association of '523-S/S with decline was not attributable to these pathologies. Fig. 2

Table 4
TOMM40 '523 genotypes and neuropathologies

Neuropathologic outcomes	Estimate (SE), *P*
Amyloid load*	
'523-S/S	0.042 (0.098), .669
'523-L	0.674 (0.082), <.001
PHFtau tangle density*	
'523-S/S	0.114 (0.115), .323
'523-L	0.818 (0.096), <.001
Macroscopic infarcts†	
'523-S/S	−0.103 (0.194), .593
'523-L	0.373 (0.158), 0.018
Microinfarcts†	
'523-S/S	0.290 (0.192), .131
'523-L	0.108 (0.166), .513
Lewy bodies†	
'523-S/S	−0.052 (0.276), .852
'523-L	0.331 (0.213), .120
Hippocampal sclerosis†	
'523-S/S	−0.346 (0.370), .350
'523-L	0.615 (0.250), .014
TDP-43†	
'523-S/S	0.079 (0.180), .661
'523-L	0.709 (0.151), <.001
Cerebral amyloid angiopathy†	
'523-S/S	0.049 (0.167), .769
'523-L	1.327 (0.144), <.001
Atherosclerosis†	
'523-S/S	−0.181 (0.166), .276
'523-L	0.054 (0.139), .699
Arteriolosclerosis†	
'523-S/S	−0.084 (0.164), .608
'523-L	0.096 (0.137), .483

Abbreviation: SE, standard error.

NOTE. The estimates show the associations of corresponding '523 genotypes relative to the reference ('523-S/VL or '523-VL/VL). The estimates from logistic regression were log odds ratios of having a neuropathology (or log odds of having more advanced neuropathology) relative to the reference.

*Estimates in each cell were obtained from separate models of analyses of covariance, adjusted for demographics.

†Estimates in each cell were obtained from separate models of logistic regression, adjusted for demographics.

illustrates that the '523-L association with cognitive decline varied before and after controlling for neuropathologies, but '523-S/S did not.

4. Discussion

There is evidence that multiple genetic variations in the *APOE* haplotype block are implicated in AD dementia susceptibility [3–7]. Using data from more than 1000 community-dwelling older persons who had died and undergone brain autopsy, we confirmed two distinct association signals at the *TOMM40* '523 locus in relation to late-life cognitive decline, the clinical hallmark of AD. We investigated the extent to which AD and other common age-related neuropathologies contribute to these relationships. We found '523-L carriers had higher burden of neuropathologies including AD and other common non-AD neuropathologies. By contrast, we did not observe difference in neuropathologies for ε3/3 homozygotes with '523-S/S. Notably, the '523-L association with cognitive decline is mediated through common neuropathologies. This is expected due to its strong LD with *APOE* ε4. On the other hand, the '523-S/S association among *APOE* ε3/3 homozygotes is not explained by these pathologies, indicating a separate association signal. These findings offer new insights into the neuropathologic basis underlying the association between *TOMM40* '523 and late-life cognitive decline and provide strong evidence that a haplotype within *TOMM40* is associated with AD independent of *APOE* ε4. Because of the strong linkage between *TOMM40* '523-L and *APOE* ε4 [10,34], '523-L carriers almost exclusively have the ε4 allele and vice versa, and less than 3% of 1114 individuals included in this study are discordant cases. Consequently, we expect that the relationship between '523-L and neuropathologies as well as downstream cognitive decline would highly mimic that of ε4. Indeed, we found that '523-L was strongly associated with multiple neuropathologies including AD. Similar associations have been widely reported for *APOE* ε4 [32,35–38]. Furthermore, we found that '523-L carriers had faster cognitive decline and the association diminished after accounting for neuropathologies. This is highly consistent with our previous observation that the association of ε4 with cognition and cognitive decline is also largely attributable to AD and other non-AD pathologies [16,17]. Taken together, these findings suggest that '523-L and ε4 share a common neuropathologic footprint in relation to cognitive decline. However, our study does not inform the extent to which the *APOE* ε4–'523-L haplotype associations with neuropathologies and cognitive decline results from *APOE* ε4, '523-L, or the complete haplotype.

In contrast to *APOE* ε4, three major *TOMM40* '523 genotypes are present in *APOE* ε3/3 homozygotes. Previous studies show that the risk of clinical diagnosis of AD and age at onset differ by these '523 genotypes, though findings are inconsistent [10,11,20,39,40]. Using data from the entire ROS and MAP cohorts, both dead and alive, we previously reported an association of *TOMM40* '523-S/S with faster cognitive decline among *APOE* ε3/3 homozygotes. Here, we expand on prior work in an important way. After confirming that the same association exists among the autopsied subgroup, we examine its relation to multiple neuropathologies. We show that unlike '523-L, none of these pathologic indices differ by '523-S/S status. Consequently, the association of '523-S/S with cognitive decline is not affected by any of these pathologies, including AD. Our findings implicate that '523-S/S represents a risk factor for cognitive decline separate from '523-L or *APOE* ε4; furthermore, the neuropathologic basis of this association also

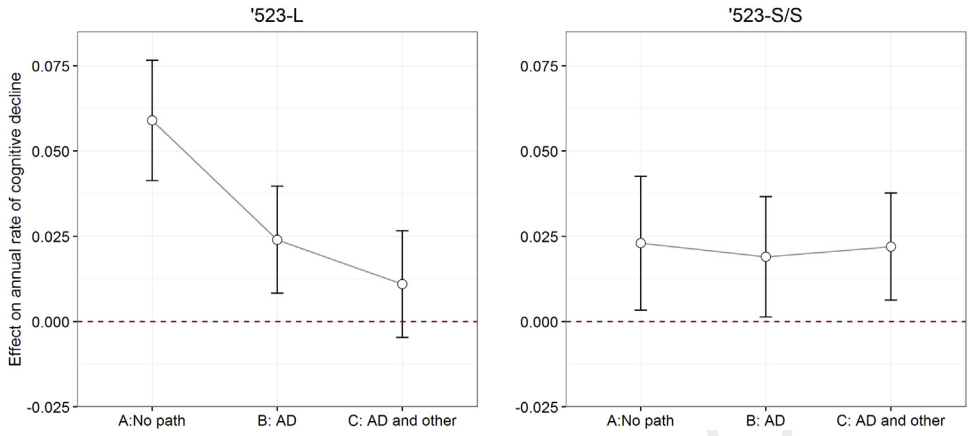

Fig. 2. The effects of *TOMM40* '523 genotypes on annual rate of cognitive decline before and after the adjustment for neuropathologies. On the x-axis, "A: No path" refers to the effects estimated from the model adjusted only for demographics; "B: AD" refers to the effect estimated from the model adjusted for demographics and AD pathologies only; "C: AD and other pathologies" refers to the effect estimated from the full model adjusted for demographics, AD, and other non-AD pathologies. Mean estimates +/−1.96 standard error for the effects of '523-L and '523-S/S are shown on the y-axis. It is evident that the association of '523-L with cognitive decline was attenuated after controlling AD pathology and became not significant after further controlling for other non-AD pathologies (left panel). By contrast, the association of '523-S/S remained essentially unchanged, such that the estimates with and without controlling for pathologies are similar and all are significantly above zero (right panel). Abbreviation: AD, Alzheimer's disease.

differs from that of '523-L or ε4. These results are somewhat unexpected. However, emerging evidence from large clinical pathologic studies suggests that while common neuropathologic burdens such as Alzheimer's, cerebrovascular, or Lewy body diseases account for a majority of person-specific variation in late-life cognitive decline, an appreciable amount of variation remains unexplained (i.e., residual cognitive decline) [21]. The independent association of '523-S/S with cognitive decline reported here suggests that it accounts for some of this residual decline.

The molecular effects of *APOE–TOMM40* '523 haplotypes remain unclear. Proteins encoded by both genes have been functionally implicated in AD and other neurodegenerative diseases. Although the involvement of APOE in β-amyloid accumulation and clearance has been well established [14,41,42], mitochondrial dysfunction is also shown to increase the risk for AD [43,44]. The mitochondrial protein encoded by *TOMM40* is essential in transporting protein precursors into mitochondria [45,46]. Alterations of *TOMM40* expression have been reported in AD, but with conflicting results [47,48]. Notably, several studies have shown a cis-eQTL where '523-S acts as a repressor to reduce the gene expression [48,49]. This regulatory function of the '523 variant has also been reported in human cell culture, where the study demonstrates that '523 is a putative regulatory element that influences the *TOMM40* promoter activity in vitro [50].

To our knowledge, this is the largest study to interrogate the relationship between *TOMM40* '523 with postmortem neuropathologies. Comprehensive postmortem evaluations quantified multiple neuropathologies that are observed in aging brain. Annual uniform cognitive assessments up to 22 years help to capture person-specific trajectories of cognitive change with a high level of fidelity. Limitations are noted. The present study is restricted to older persons of European ancestry. The linkage patterns of *APOE* and *TOMM40* '523 are known to differ in African Americans; therefore, the extent to which these findings are generalizable to other population is unknown. In addition, both ROS and MAP are voluntary cohorts, and the findings await replications from other longitudinal clinical pathologic studies.

In conclusion, through investigating the role of Alzheimer and other common neuropathologies in the relationship between *TOMM40* '523 and late-life cognitive decline, the study revealed two distinct association signals. The association of *TOMM40* '523-L with cognitive decline is primarily mediated by common neuropathologies. By contrast, the association of *TOMM40* '523-S/S is relatively independent of these pathologies.

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RESEARCH IN CONTEXT

1. Systematic review: Literature reviews via PubMed search suggest that *TOMM40* '523 variant is associated with late-life cognitive decline and the association is not fully attributable to the linkage disequilibrium with *APOE* variant. Yet, the underlying neuropathologic correlates remain unclear.
2. Interpretation: Through investigating the role of AD and other common neuropathologies in the relationship between *TOMM40* '523 and longitudinal cognitive decline, this study reveals two association signals. The '523 long allele, in linkage with *APOE* ε4, primarily acts through common neuropathologies, whereas the '523 short/short genotype among *APOE* ε3/3 homozygotes represents a separate risk factor that operates through a different mechanism.
3. Future directions: This study is restricted to older persons of European ancestry, and the generalizability of our findings to other populations awaits investigation. Future studies are warranted to determine the neurobiology that drives the association of *TOMM40* '523 with cognitive decline that are not due to common age-related neuropathologies.

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