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Featured Article

Hippocampal thinning linked to longer *TOMM40* poly-T variant lengths in the absence of the *APOE* ε4 variant

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Abstract

Introduction: The translocase of outer mitochondrial membrane 40 (*TOMM40*), which lies in linkage disequilibrium with apolipoprotein E (*APOE*), has received attention more recently as a promising gene in Alzheimer's disease (AD) risk. *TOMM40* influences AD pathology through mitochondrial neurotoxicity, and the medial temporal lobe (MTL) is the most likely brain region for identifying early manifestations of AD-related morphology changes.
Methods: In this study, we examined the effects of *TOMM40* using high-resolution magnetic resonance imaging in 65 healthy, older subjects with and without the *APOE* ε4 AD-risk variant.
Results: Examining individual subregions within the MTL, we found a significant relationship between increasing poly-T lengths of the *TOMM40* variant and thickness of the entorhinal cortex only in subjects who did not carry the *APOE* ε4 allele.
Discussion: Our data provide support for *TOMM40* variant repeat length as an important contributor to AD-like MTL pathology in the absence of *APOE* ε4.
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Keywords: *APOE*; *TOMM40*; Hippocampus; Alzheimer's disease; MRI; Entorhinal cortex

1. Introduction

For more than two decades, the apolipoprotein E (*APOE*) gene has been consistently identified as the primary risk gene for late-onset Alzheimer's disease, accounting for approximately 50% of the genetic risk for AD [1–3]. Despite the strength of the *APOE* ε4 risk variant in predicting AD, population studies of *APOE* allele frequency among AD patients indicate that 36%–50% of patients do not carry the ε4 variant [4], and that other significant genetic contributions to disease risk and pathological progression remain unidentified or uncharacterized for their role in AD [5]. Possession of the ε4 variant of the *APOE* gene does not provide sufficient sensitivity, selectivity, or power to be used as a predictive tool for AD diagnosis [6], and much of the last decade of genetics research in AD has focused on identifying other genetic markers related to disease risk and age of onset in the hopes of identifying those more likely to experience future cognitive decline.

Several studies implicate *APOE*'s neighbor on chromosome 19, the translocase of outer mitochondrial membrane 40 (*TOMM40*) homolog gene, in risk for AD [4–7]. The variant (rs10524523, "523") in intron 6 of the *TOMM40* gene is a variable length poly-T sequence with lengths classified as short (14–20 repeats; i.e., "S"), long (21–29 repeats, i.e., "L"), or very long (>29 repeats, i.e., "VL"). The number

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of T residues in the homopolymer, "N," is 35, and the specific variation described by rs10524523 is a 19-base pair deletion, making the homopolymer T16 (N = 16 T residues) the variant allele [8,9]. One of the earliest studies of this variant concluded that the longer length poly-T allele increases risk for AD and decreases age of onset [10]; this finding was replicated in an independent study a few years later [11]. Linkage disequilibrium between *TOMM40* and *APOE* genes ensures that the L poly-T repeat in the *TOMM40* gene is almost always (with rare exceptions) inherited with the *APOE* ε4 allele. However, the ε3 allele is most commonly with either a VL or an S poly-T variant, again, with rare exceptions [10,12]. In individuals homozygous for the ε3 variant, the VL variant was found to be associated with a higher risk and earlier age of onset for AD, whereas S variant carriers had a later age of onset [10]. A review of the *APOE-TOMM40* phylogenetic field suggests that the discovery of the polymorphism in *TOMM40* may improve AD-risk prediction [13].

Recent results suggest that the protein encoded by the *TOMM40* gene may affect the development of AD via mitochondrial function [12,14]. The protein that *TOMM40* encodes, TOM40, is a mitochondrial import channel protein that facilitates the transport of amyloid-β protein precursor (APP) and amyloid-β (Aβ) transport to the mitochondria [15,16]. The TOM40 protein acts as a chaperone, expediting the movement of preproteins through the channel and assembling them posttranslationally in the mitochondrion [16]. Because *APOE* ε4, Aβ, and APP have been found to influence function and motility of mitochondria, it has been postulated that *APOE* and *TOMM40* genes might mediate disease risk, and lower age of onset, through mitochondrial dysfunction [10,17–19]. Mitochondrial dysfunction is a well-documented factor in the pathogenesis of several age-related diseases, including Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, and AD [20–23].

In homozygous *APOE* ε3/ε3 subjects, a phylogenetic experiment suggested that possession of the VL poly-T repeat was associated with increased disease risk and earlier age of onset [10]. A separate study revealed similar findings; possession of the VL poly-T variant in subjects homozygous for ε3 was linked to developing AD at a higher rate when they were ≥79 years old [11]. The authors conclude that in the absence of ε4, longer poly-T variants increase the likelihood of developing AD, where the ε3 allele may be linked to either an S or a VL poly-T repeat [10].

To pinpoint the biologically relevant endophenotypes that relate to the *TOMM40* gene, it is crucial to investigate healthy individuals using sensitive metrics to assess the very earliest manifestations of pathophysiological changes in the brain, before the onset of clinical symptoms. Although hippocampal volume is a hallmark brain imaging phenotype in AD, substantial work has shown that the first brain changes in AD begin in entorhinal cortex (ERC) [24]. Additionally, subregional analysis of the medial temporal lobe (MTL), especially in ERC, can be more sensitive to possible preclinical morphology differences in both nondemented *APOE* ε4 subjects [25] and MCI patients [26] than volumetric measures. Our group [27–29] and others [30,31] have used high-resolution MRI combined with a cortical unfolding technique that improves visibility of the MTL to investigate subregional changes in this area, even in nondemented, cognitively intact subjects who carry the at-risk ε4 variant [25].

The aim of the present study was to use high-resolution imaging combined with subregional data analysis techniques in nondemented, older subjects to investigate the impact of rs10524523 poly-T alleles on the MTL in vivo, which is the site of the very earliest structural changes in AD [32]. We focused analyses on subjects who did not carry the ε4 risk variant of *APOE*, to investigate the contribution of the poly-T variant length in the absence of other known genetic risk attributable to the *APOE* gene.

2. Methods

2.1. Participants

The study was conducted with the approval of the University of California, Los Angeles Institutional Review Board; all subjects signed informed consent forms before participation. Participants were drawn from a larger study of predictors of cognitive decline by the UCLA Longevity Center [33,34]. Briefly, volunteers from the local community were recruited through local advertisements. Subjects were screened over the phone by research staff of the Longevity Center. However, subjects meeting criteria for AD or any other dementia were excluded from the study [35]. Subjects were also excluded for any history of substance abuse, head trauma or other major systemic disease affecting brain function, a history of neurological or psychiatric disorders, and hypertension or cardiovascular disease.

During their visit to the Longevity Center, subjects underwent neuropsychological testing and a clinical interview, in addition to a medical examination and laboratory screening including blood tests, to rule out medical conditions that could affect cognitive performance. The present study was conducted on a subset of 65 of these participants (see Table 1) who had successfully completed genotyping for both *APOE* and *TOMM40* and cognitive and imaging procedures.

2.2. Neuropsychological testing

Neuropsychiatric test scores were divided into the following domains of cognitive function: processing speed (trail making test, part A; Stroop test, word reading speed; Weschler adult intelligence scale-III digit symbol), memory encoding (Buschke-Fuld selective reminding test, consistent long-term retrieval; Weschler memory scale-II, logical memory I and verbal paired associates I), delayed memory (Weschler memory scale: logical memory II and verbal

Table 1
Demographic and clinical characteristics of study participants

	APOE ε4 noncarriers			
	S/S group	S/L* group	L*/L* group	*APOE* ε4 carriers
	Summed *TOMM40* poly-T length <35	35 < Summed *TOMM40* poly-T length <65	Summed *TOMM40* poly-T length >65	35 < *TOMM40* poly-T length <67
Cohort	n = 10	n = 18	n = 13	n = 24
Mini-Mental State Examination score	28.8 ± 0.8	29.1 ± 1.3	28.5 ± 1.4	28.7 ± 1.0
Age, y	66.1 ± 11.0	62.27 ± 7.7	62.2 ± 9.0	64.4 ± 9.9
Educational achievement, y	15.6 ± 3.2	16.2 ± 1.5	16.7 ± 2.6	16.7 ± 3.1
Female sex, n (%)	6 (60)	15 (83.3)	9 (69.2)	13 (54.2)
Family history of dementia, n (%)	6 (60)	10 (55.5)	11 (84.6)	13 (54.2)
Hamilton Depression Scale score	1.2 ± 1.9	2.4 ± 3.2	1.8 ± 3.4	2.5 ± 3.5
Ethnicity (no. of African-American [%], Caucasian [%], Asian [%], Latino [%])	3 (30), 0 (0), 7 (70), 0 (0)	1 (6), 0 (0), 17 (94), 0 (0)	0 (0), 1 (8), 11 (85), 1 (8)	1 (4), 1 (4), 20 (83), 2 (8)

Abbreviations: *APOE*, apolipoprotein E; *TOMM40*, translocase of outer mitochondrial membrane 40.

NOTE. Subjects were divided into ε4 carriers and noncarriers to begin with, and then noncarriers were subdivided according to the combined length of both *TOMM40* variants. There were no significant differences across the three *TOMM40* variant length groups, or between *APOE* ε4 carrier and noncarrier groups, according to the characteristics listed in the table.

paired associates II; Rey-Osterrieth complex figure, delayed recall; Buschke-Fuld selective reminding test, delayed recall), and executive functioning (trail making test part B; verbal fluency FAS and animal naming tests; Stroop test, interference). Studies using these domains have been reported elsewhere [36–40]. We converted the raw scores to *Z* scores (*Z* = [raw score − mean]/standard deviation) and created a domain *Z* score by averaging the *Z* scores belonging to the cognitive tests in each domain.

2.3. DNA sampling and genotyping

DNA samples were aliquoted on 96-well plates for determination of both *APOE* and *TOMM40* genotypes. Genotyping for the *APOE* gene was done by the UCLA Center for Neurobehavioral Genetics (principal investigator, D. Geshwind, MD, PhD) using standard methods [41]. Genotyping for *TOMM40* using the rs10524523 ("523") allele was completed at Polymorphic DNA Technologies (Alameda, CA, USA, http://www.polymorphicdna.com). *TOMM40* polymorphisms were analyzed using polymerase chain reaction and bidirectional direct Sanger sequencing of the DNA templates on an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Inc., Carlsbad, CA, USA) followed by sequence data analysis. This polymorphism, 523, is a homopolymer length polymorphism (poly-T) located in an intronic region of *TOMM40*. The poly-T lengths for each chromosome were converted into the S, L, and VL standard labeling [10].

2.4. MRI acquisition

MRI scans were performed on a Siemens 3T Allegra head-only scanner. Two scans were acquired: (1) sagittal T1-weighted magnetization-prepared rapid acquisition gradient echo volumetric scans were acquired to serve as a guide in sulcal visualization during segmentation procedures in the same way an atlas is used as a visual reference (repetition time: 2300 ms, echo time: 2.93 ms, slice thickness: 1 mm, 160 slices, in-plane voxel size 1.3 × 1.3 mm, field of view [FOV] 256 mm); (2) high-resolution oblique coronal T2-weighted fast-spin echo sequence scan (repetition time: 5200 ms, echo time: 105 ms, slice thickness: 3 mm, skip 0, 19 slices, in-plane voxel size: 0.39 × 0.39 mm, FOV: 200 mm).

2.5. Whole-brain structural imaging

To calculate intracranial volume (ICV) estimates to normalize subregional hippocampal thickness values, we used FreeSurfer [42] on whole-brain T1-weighted scans. This software suite uses tissue contrast to determine the boundary between gray matter (GM), white matter (WM), and the pial surfaces of the brain to calculate the difference between vertices plotted as a mesh surface for each of the layers across the entire cortex. After the automated portion of the FreeSurfer pipeline is complete, each subject's scan is visually checked for accuracy. Minimal manual edits were completed by a single individual when necessary (TMH). ICV values from FreeSurfer were used to normalize hippocampal complex thickness as detailed subsequently.

2.6. High-resolution hippocampal structural imaging

Cortical unfolding is used to improve visualization of the convoluted MTL cortex by flatting the entire three-dimensional (3D) volume into a 2D flat map [25,27,29]. We use a technique that maximizes resolution in-plane (0.39 × 0.39 mm), where there is significant variability in subregional structure, and increases signal-to-noise ratio by using thicker slices in the long axis where there is less variability in structure. We acquired T2 images

perpendicular to the long axis of the hippocampus to minimize the variability in slice-to-slice changes across the images. Thus, we maximize in-plane resolution and recover signal by increasing thickness in the invariant longitudinal axes, creating maximally resolved anisotropic voxels, while minimizing variability from slice to slice [27–29]. We begin by manually defining WM and cerebral spinal fluid (CSF) on the in-plane oblique coronal images. To maximize segmentation, these original images are interpolated by a factor of 7. Then, up to 18 continuous layers of GM are grown out from the boundary of WM, using a region-expansion algorithm to cover all pixels of GM between WM and CSF space (Fig. 1A). Boundary demarcations divided the following subregions encompassed by GM: cornu ammonis (CA) fields 1, 2, and 3, the dentate gyrus (DG), subiculum (sub), ERC, perirhinal cortex, parahippocampal cortex (PHC), and the fusiform gyrus (FUS) (Fig. 1B and C). Because of limits in resolution in CA fields 2 and 3 and DG, we treat these regions as a single entity (CA23DG). This strip of GM is used as the input for the unfolding procedure, an iterative algorithm based on multidimensional scaling (http://ccn.ucla.edu/wiki/index.php/Unfolding). Boundary delineations were projected to their corresponding coordinates in flat map space (Fig. 1D).

To calculate thickness, we computed the distance for each voxel in in-plane space to the nearest non-GM voxel, we took the maximum distance value in 2D voxels of the corresponding 3D voxels across all layers and multiplied by two and calculated the mean thickness in subregions by averaging thickness of all 2D voxels (Fig. 1E). Cortical thickness within subregions was averaged over both hemispheres.

We corrected for differences in head size across subjects by normalizing hippocampal thickness values to ICV estimates. The following formula was used to normalize thickness values: ICV-corrected thickness = ([thickness in mm/ICV in mm^3] $\times$ 10^6). Multiplying by 10^6 results in values at the same order of magnitude as original thickness estimates.

2.7. Statistical analyses

Statistical models were used to investigate the effect of *TOMM40* genetic variant lengths on subregional MTL thickness in the absence of the *APOE* ε4 variant. As has been done previously in the literature to condense the largest number of potential genotype combinations into subgroups, the L and VL alleles were pooled into an L* group; participants with the S/S genotype were compared with those carrying only

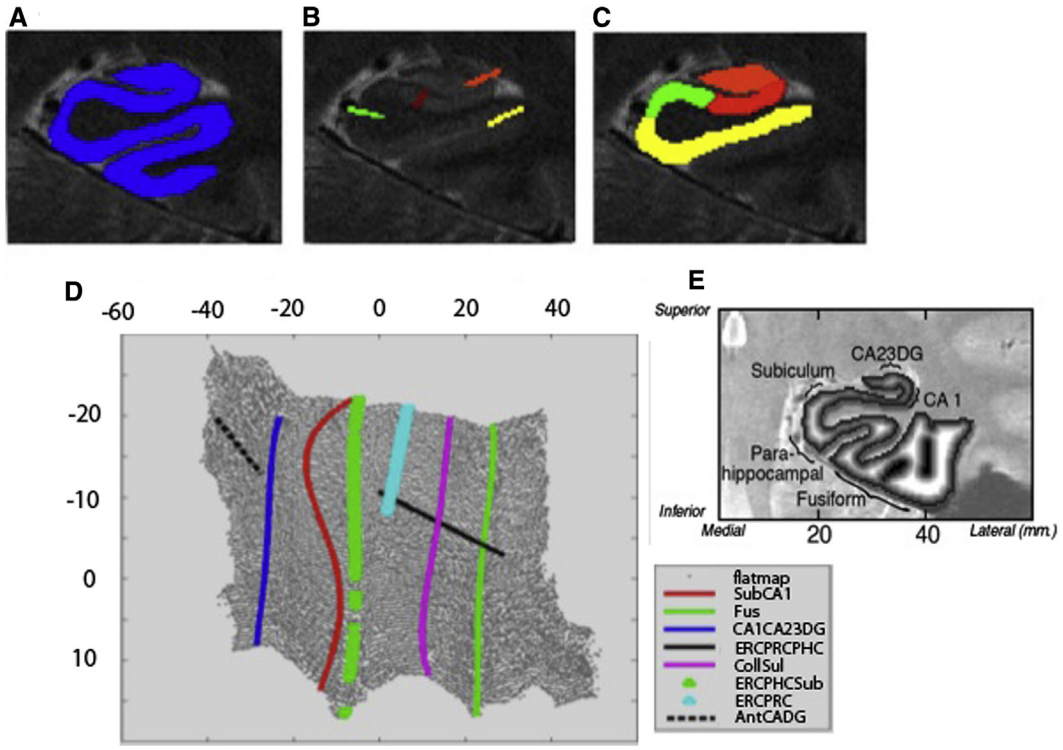

Fig. 1. High-resolution hippocampal image processing and thickness calculations. (A) The goal of high-resolution hippocampal image processing is to isolate the strip of gray matter (GM) in the medial temporal lobe that encompasses the subregions of the hippocampus proper and surrounding neocortex, shown in (A) in blue. This is done by manually segmenting cerebrospinal fluid and white matter (WM) and growing sequential layers of GM from the edge of WM until the layer reach the cerebrospinal fluid boundary. (B) The boundaries between medial temporal lobe subregions are marked according to anatomical landmarks. Demarcations shown here include CA23DG | CA1 (orange), CA1 | subiculum (sub) (red), parahippocampal gyrus | sub (light green), and fusiform gyrus (yellow). (C) Each subregion is considered separately for cortical thickness calculations. (D) Demarcations are projected from in-plane space to the corresponding location in two-dimensional flat map space and then extended for form complete and smooth boundaries between subregions. (E) Cortical thickness is visualized in in-plane space as a gray-scale map of thickness values between maximum (white) and minimum (black) values.

one S allele (pooled S/L and S/VL, hereinafter S/L*) and also compared with participants carrying no S alleles (pooled L/L, L/VL, and VL/VL, hereinafter L*/L* [9]). To assess whether varying *TOMM40* poly-T lengths were associated with thinner hippocampal cortex in individual subregions in the absence of *APOE* ε4, we computed a multivariate analysis of covariance (MANCOVA) with thicknesses of all subregions as the dependent variables, categorical groups of additive poly-T lengths as the predictors (S/S, S/L*, and L*/L*), and age, education, sex, and MMSE as covariates. To assess whether varying *TOMM40* poly-T lengths were associated with cognitive performance in the absence of *APOE* ε4, we computed a MANCOVA with *Z*-scaled cognitive performance scores as dependent variables, poly-T lengths as predictors (S/S, S/L*, and L*/L*) and age, education, sex, and MMSE as covariates.

3. Results

Of the 65 subjects enrolled, 24 subjects carried at least one copy of the ε4 variant for the *APOE* gene and 41 were non-ε4 carriers. Among the *APOE* ε4 noncarriers, the *APOE* genotype was as follows: 1 *APOE* ε2/ε2, 3 *APOE* ε2/ε3, and 37 *APOE* ε3/ε3 subjects. There were no differences in clinical and demographic variables across the groups (Table 1); however, we also included age, education, sex, and MMSE score as covariates in the multivariate analysis. Ethnicity is also reported in Table 1; however, the number of subjects enrolled in each category was too small to study the effect of ethnicity on *TOMM40* poly-T lengths and hippocampal thickness separately. Fig. 2 shows the breakdown of *TOMM40* variant lengths among subjects by *APOE* genotype.

The MANCOVA revealed a significant relationship between longer poly-T lengths and thickness ($F(14,54) = 3.61$, $P = .0003$, excluding the ε2s: $F(14,46) = 3.29$, $P = .001$) in non-ε4 carriers. Follow-up univariate analyses indicated that ERC thickness was significantly associated with longer poly-T lengths ($F(2,33) = 16.21$, $P < .0001$; excluding the ε2s: $F(2,30) = 14.67$, $P < .0001$), with the L*/L* group showing significantly reduced thickness compared with both the S/L* and S/S groups (both $P < .0001$) (Fig. 3). In addition, PHC thickness was marginally associated with increasing poly-T lengths ($F(2,33) = 3.22$, $P = .054$; excluding the ε2s: $F(2,30) = 3.28$, $P = .051$). No other subregions showed significant differences between increasing poly-T variant lengths in either ε4-carriers or noncarriers (Fig. 4). We did not find a significant relationship between *TOMM40* poly-T lengths and *Z*-scaled cognitive score in any of the five domains of cognitive function.

4. Discussion

We show here that in older, normal control subjects who do not carry the *APOE* ε4 variant, longer *TOMM40* poly-T lengths are significantly associated with thinner ERC. Our

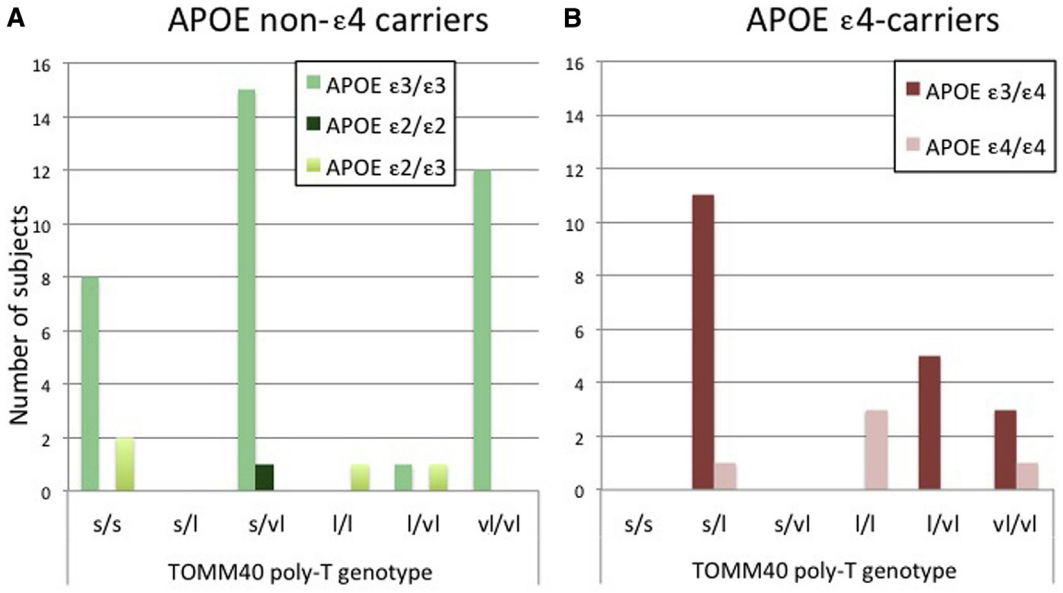

Fig. 2. Distribution of translocase of outer mitochondrial membrane 40 (*TOMM40*) variant lengths by apolipoprotein E (*APOE*) group. Subjects were categorized first by whether they were *APOE* ε4 carriers or noncarriers, then grouped according to the combined length of both *TOMM40* poly-T length variants. The distribution of non-ε4 carrier subjects is shown in panel A, whereas ε4 carriers are shown in panel B. Similar to the previous reports [7,43], most poly-T lengths in the ε3/ε3 cohort were either S (<21) or VL (≥30). In agreement with the previous reports that the ε4 variant is typically bound by linkage disequilibrium to L *TOMM40* variants [10], most ε4 carriers (n = 20) possessed as least one L *TOMM40* variant, although four subjects were homozygous for VL (B). Non-ε4 carriers showed a distribution (A) where most subjects possessed two short S copies of the *TOMM40* variant (S/S, n = 10), two copies of the very long VL variant (n = 12), or a heterozygous combination of S/VL (n = 16). As demonstrated, there were no non-*APOE* ε4 subjects. However, for consistency's sake, we chose to continue the nomenclature for S/L and S/VL carriers to be pooled into an S/L* group much like participants carrying no S alleles (L/L, L/VL, and VL/VL) were pooled into an L*/L* group.

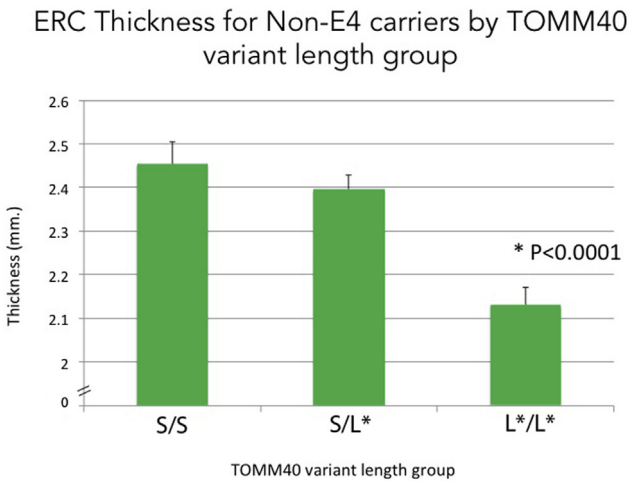

Fig. 3. Entorhinal cortex (ERC) thickness for ε4 non-carriers by translocase of outer mitochondrial membrane 40 (*TOMM40*) variant length. Averaged ERC thickness for subjects in each of the *TOMM40* summed variant length group (S/S group: 2.45 mm, S/L* group: 2.40 mm, and L*/L* group: 2.13 mm). Univariate analyses indicated that ERC thickness was significantly associated with increasing poly-T lengths ($F(2,33) = 16.21$, $P < .0001$), with the VL group showing a significantly reduced thickness compared with both the L and S groups (both $P < .0001$).

results demonstrate an association between the *TOMM40* poly-T variant and subregional morphological differences in the MTL only in subjects who do not carry the *APOE* ε4 risk variant. The pattern of cortical thinning in subjects with low *APOE* risk, but elevated *TOMM40* risk, closely resembles that seen in healthy, older *APOE* ε4 carriers. To our knowledge, this is the first investigation to investigate the effect of the *TOMM40* gene on brain morphology in MTL subregions. These data highlight the importance of assessing multiple risk variants to detect morphological differences in healthy, older adults before the possible onset of clinical symptoms. Our results also underscore the importance of investigations that assess the integrity of the ERC in healthy, older adults when assessing risk for AD and highlight the utility of imaging tools that isolate individual subregions of the MTL, including the ERC. We suggest that, given the growth of automated hippocampal segmentation tools in recent years [44], assessing the structural integrity of the ERC plays a crucial role in assessing risk for AD, even in nondemented healthy, older control subjects.

Novel brain imaging techniques reveal structural changes that may be phenotypic in prodromal AD. For decades, the most prominent genetic marker for preclinical manifestations of the disease has been the *APOE* ε4 variant on chromosome 19. *TOMM40*'s location in proximity to the *APOE* gene has prompted queries for whether the effects noted for this

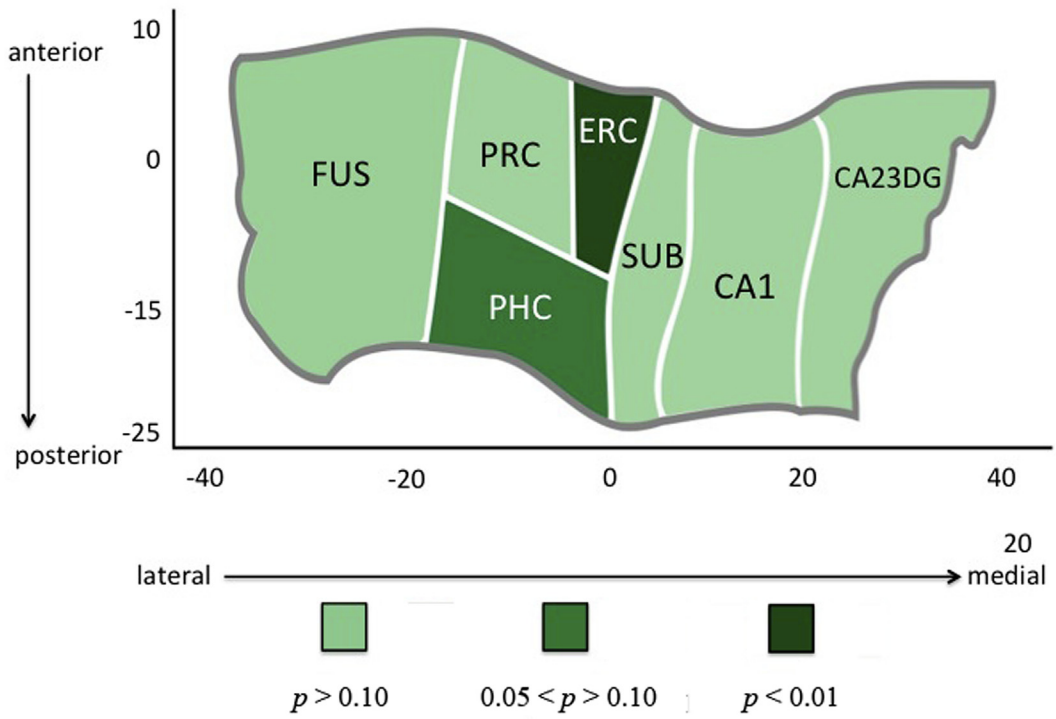

Fig. 4. Hippocampal complex unfolding reveals subregional relationship with increasing length of translocase of outer mitochondrial membrane 40 (*TOMM40*) poly-T variants. A cortical unfolding procedure was used to produce a flat map of the hippocampal complex. Regions are color coded according to the strength of the statistical association between *TOMM40* poly-T variant length and cortical thickness in individual subregions within the hippocampus and surrounding neocortex. Results revealed that ERC thickness was significantly associated with increasing poly-T lengths ($P < .0001$), with the VL group showing a significantly reduced thickness compared with both the L and S groups (both $P < .0001$). In addition, parahippocampal cortex thickness was almost significantly associated with increasing poly-T lengths ($P = .054$). No other subregions showed significant differences between increasing poly-T variant lengths in either ε4 carriers or noncarriers.

newly discovered gene are working in concert with, independently of, or instead of *APOE*'s effects. Because *TOMM40* is in strong linkage disequilibrium with *APOE* on chromosome 19, the ε4 allele of *APOE* is almost exclusively linked to the L poly-T variant. As suggested in an article detailing the full impact of the TOM40-mediated mitochondrial protein import mechanism in aging [15], the effects of the VL/VL genotype may be associated with presymptomatic events in younger people that are masked by later pathology in advanced AD. Several studies have suggested that SNPs within *TOMM40* are associated with increased risk for AD [45–55] or associated endophenotypes of the disease, including cognitive performance [56] and hippocampal atrophy [57] independently of *APOE*. However, other studies failed to replicate the association between *TOMM40* variants and risk for AD [58,59]. These results underscore the importance of further investigations into the relationship between these multiple risk variants and suggest the need for investigations in younger, healthy subjects before the appearance of more widespread brain changes in later disease stages.

The nonspecific nature of *APOE*'s effects in AD was the original impetus leading investigators to postulate that other genes/proteins in the chromosomal interval containing *APOE* might be responsible for the wide variation in genetic risk associated with AD [10,45]. The ε4 risk variant is neither necessary nor sufficient for disease onset, and genetics research alone is unlikely to definitively diagnose AD [60]. However, identifying which markers have the greatest sensitivity and specificity among those identified as markers for AD risk will allow us to assess and follow subjects with the greatest likelihood of cognitive decline related to genetic risk for AD-onset over time, thereby strengthening the likelihood of maximizing the effect of current therapeutic interventions and testing novel therapies as they are developed. Additionally, within this analysis, the four subjects who carried at least one copy of the *APOE* ε2 variant were analyzed in the non-*APOE* ε4 group as the intention was to investigate the effect of *TOMM40* poly-T length in the absence of the *APOE* ε4 variant. The data here are too small to analyze *APOE* ε2 subjects separately, but we suggest that in future, larger datasets, the question of whether *TOMM40*-associated morphology differences exist in *APOE* ε2 carriers is worthy of investigating.

It is noteworthy that the genotype and family history of AD distribution in the population studied here differs from that found in a random sampling of the general population. Typically, 20%–25% of the general public carries at least one copy of the ε4 variant [61–63], whereas in the present study, 37% carried at least one *APOE* ε4 copy. Additionally, depending on genotype, 54%–85% of subjects in this study reported a family history of dementia compared with 10% in the general population.

Our recruitment method yielded a sample of highly motivated, physically healthy subjects concerned about age-related memory problems and resulted in a sample enriched for possession of the ε4 risk variant. Although the sample may not be representative of the general population, having a higher concentration of subjects with the *APOE* ε4 variant does not explain the cortical thickness differences in ERC between the genetic groups. Additionally, as mentioned in the statistical methods section, family history was used as a covariate, ensuring any effect of that factor was not responsible for the morphology results. Finally, genotype and family history percentages reported here are similar to those reported in our laboratory [25,64,65] and others [66,67].

Additional limitations must also be acknowledged. The sample size is small, and, unfortunately, the ethnic breakdown across the groups resulted in limited diversity for statistical analysis. The analytical method of cortical unfolding reported here is an extremely time-consuming technique. However, advances in imaging methodology, both in image acquisition and in data analysis, are expected to make more rapid analysis possible in the near future. Larger analyses should address race and ethnicity given that they are known to vary with dementia risk [68] and are also suspected to vary with *TOMM40* variant length [69]. Finally, we also acknowledge that future studies will be more powerful in detecting differences in morphology associated with genetic risk using longitudinal assessment as opposed to the cross-sectional analysis we report here [70].

These results demonstrate specific subregional morphological changes within the MTL related to the *TOMM40* gene in the absence of the *APOE* ε4 risk factor. Identifying relationships between gene-brain risk metrics in the absence of the *APOE* ε4 allele promises to shed light on the question of which ε4 negative subjects are at greater risk for AD progression. As clinical trials of novel AD treatments continue, identifying biomarkers that isolate subjects at greater risk for AD than the general population will enhance our ability to identify subjects likely to benefit from these interventions and demonstrate results from effective treatments.

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RESEARCH IN CONTEXT

1. Systematic review: Several studies implicate apolipoprotein E (*APOE*)'s neighbor on chromosome 19, translocase of outer mitochondrial membrane 40 (*TOMM40*), in Alzheimer's disease (AD) risk. In homozygous *APOE* ε3 subjects, a phylogenetic experiment suggested that possession of longer-length poly-T repeats was associated with increased disease risk and earlier age of onset, but further structural magnetic resonance imaging studies have shown mixed results.
2. Interpretation: To our knowledge, this is the first study to investigate the effect of *TOMM40* on morphology in medial temporal lobe subregions and show thinner entorhinal cortex (ERC) in older, heterogeneous *APOE* ε3 control subjects with longer *TOMM40* poly-T lengths. We focused on subjects who do not carry the ε4 risk variant of *APOE*, to investigate the contribution of the poly-T variant length in the absence of *APOE* risk.
3. Future directions: Our results underscore the importance of assessing the ERC in healthy, older adults when investigating risk for AD and highlight the importance of identifying gene-brain risk metrics in the absence of *APOE* ε4.

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