Published in final edited form as:

Alzheimers Dement. 2013 September ; 9(5): 554–561. doi:10.1016/j.jalz.2012.06.009.

TOMM40 intron 6 poly-T length, age-at-onset and neuropathology of AD in individuals with APOE ε3/ε3

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Abstract

Background—This study investigates the association between *TOMM40* poly-T length, age-at-onset, and neuropathology in Alzheimer's disease (AD) individuals with the *APOE* ε3/ε3 allele.

Methods—Thirty-two *PSEN1* mutation carriers with AD, 27 *PSEN2* mutation carriers with AD, 59 participants with late-onset AD (LOAD), and 168 participants with autopsies from a community-based cohort were genotyped for *TOMM40* intron 6 poly-T (rs10524523) length using short tandem repeat assays.

Results—Among AD patients with *PSEN2* mutations, the presence of a long poly-T was associated with an earlier age-at-onset, whereas there were no such associations for patients with *PSEN1* mutations or LOAD. In community-based participants, the presence of a long poly-T was associated with increased neuritic tangles and a higher likelihood of pathologically diagnosed AD.

Conclusions—*TOMM40* intron 6 poly-T length may explain some of the variation in age-at-onset in *PSEN2* familial AD and may be associated with AD neuropathology in persons with *APOE* ε3/ε3.

Keywords

Alzheimer's disease; age-at-onset; genetic; APOE; TOMM40; PSEN1 mutation; PSEN2 mutation; neuropathology

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Conflict of Interest Disclosure: We have no conflicts of interest or financial disclosures to report.

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1. Introduction

Our previous study showed that participants with familial Alzheimer's disease (FAD) caused by a presenilin 2 (*PSEN2*) mutation (N141I) have a wide variation in age-at-onset that cannot be simply explained by the presence of an apolipoprotein E (*APOE*) ϵ 4 allele [1–2]. A later analysis used Bayesian oligogenic segregation and linkage analysis to simultaneously estimate the effects of *APOE*, *PSEN2*, and additional loci on AD age-at-onset in nine families with *PSEN2* mutations. Although this study found a small modifying effect of *APOE* on age-at-onset in affected participants, it also provided strong evidence that there is at least one additional modifier locus besides *APOE* ϵ 4 allele. A more recent study found that the variable length of a poly-T nucleotide repeat (rs10524523) within intron 6 of the translocase of outer mitochondrial membrane 40 homolog (*TOMM40*) gene is associated with age-at-onset in late-onset AD (LOAD) [3]. A *TOMM40* single nucleotide polymorphism (SNP), rs2075650, located within intron 2, is considered a tagging SNP for the *APOE* ϵ 4 allele and has been associated with both LOAD risk [4–5] and quantitative traits of AD [6–10], such as age-at-onset and A β 42 levels. Our previous study reported that SNPs within the *TOMM40* gene, as well as other SNPs both proximal and distal to *APOE*, are associated with cerebrospinal fluid (CSF) ApoE levels [11] and postmortem ApoE expression in AD hippocampus [12]. These findings suggest that the *TOMM40* region contributes to LOAD phenotypes and that genetic variation within the *TOMM40* gene may actually play a functional role in LOAD risk.

Reports on *APOE* locus linkage disequilibrium (LD) patterns suggest that strong LD with *APOE* ϵ 4 exists both proximally (in a region partially spanning the *TOMM40* gene) and distally to *APOE* [8, 13–14]. Interestingly, the association between *TOMM40* and LOAD risk is not fully explained by LD between *TOMM40* SNPs and the *APOE* SNP (rs429358) that defines ϵ 4 status, which suggests that other *APOE* locus SNPs contribute to this association with LOAD [13].

The *TOMM40* poly-T variant that is found on the *APOE* ϵ 4 chromosomal haplotype is relatively homogeneous, and it ranges between 22 and 29 T nucleotides. In contrast, there are two distinct subgroups of poly-T lengths found on the *APOE* ϵ 3 chromosomal haplotype, the short poly-T length of 11 to 16 repeats and the longer poly-T of 29 to 39 repeats [3]. Thus, the haplotype of the *APOE* ϵ 4 allele with either the short or the long poly-T and the haplotype of the *APOE* ϵ 3 with the intermediate length poly-T are rare. Because of the low frequency of these haplotypes in the general population, the independent effect of the *TOMM40* poly-T on AD risk or the gene-gene interaction could not be fully assessed using the conventional method of adjusting the *APOE* genotype or testing an interaction term of *APOE* x *TOMM40* poly-T in a multiple regression model. To discern the effect of the *APOE* ϵ 4 allele on age-at-onset and risk of AD, we restricted our analysis to participants with the *APOE* ϵ 3/ ϵ 3 genotype only. We first compared age-at-onset in AD patients with and without long poly-T lengths (≥ 20 T nucleotides) in three patient samples: (1) participants recruited from the University of Washington (UW) Alzheimer's Disease Research Center (ADRC) with either the presenilin 1 (*PSEN1*) mutation or the *PSEN2* mutation (FAD cohort); (2) participants from the UW ADRC clinic with LOAD ($\geq$ age 60 years) who may or may not have a family history of AD (LOAD cohort); and (3) participants from a Seattle-area, community-based cohort study, the Adult Changes in Thought (ACT) study, with AD (ACT cohort). We next examined the associations between the presence of a long poly-T and the time-to-onset of AD using survival analysis. Finally, we examined the association between poly-T length and neuropathological changes of AD in the ACT autopsy cohort.

2. Methods

2.1. Participants

To differentiate the effect of *TOMM40* from that of *APOE* on age-at-onset, we selected study participants with the *APOE* $\epsilon 3/\epsilon 3$ genotype from three different cohorts.

2.1.1. FAD cohort—*PSEN1* and *PSEN2* mutation carriers were ascertained and assessed by the UW ADRC genetic core. Thirty-one *PSEN2* mutation carriers were recruited from 9 pedigrees of Volga German descent [15] that ranged from 1 to 7 family members in size. Of the 31 *PSEN2* mutation carriers from these families, 27 were diagnosed with AD and 4 were unaffected but at risk for AD at the time of the study (that is, at the censoring age of 46, 48, 51, and 79 years, which were later than the earliest onset age of 41). The *PSEN1* mutation carriers ($n = 33$) were recruited from 14 pedigrees that ranged from 1 to 6 family members in size. Thirty-two *PSEN1* mutation carriers were affected with FAD, and one was unaffected (age = 40 years). Unlike the *PSEN2* mutations, the *PSEN1* mutations differed by family. Both *PSEN1* and *PSEN2* mutation carriers were ascertained and assessed using the methods described in our previous report [1]. Diagnosis of AD was made based on a clinical examination and/or a detailed history that was provided by informants. Study participants met criteria for “probable AD” based on the National Institute of Neurologic and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [16], except one case with “possible AD” based on history and clinical examination. In addition, 10 of the 27 FAD patients with a *PSEN2* mutation and 20 of the 32 FAD patients with a *PSEN1* mutation also received a neuropathological diagnosis of AD. Age-at-onset was determined to be the age at which informant reports and medical records agreed that individuals first began showing signs of memory loss or behavioral changes.

2.1.2. LOAD cohort—Fifty-nine participants with a clinical diagnosis of probable AD and an age-at-onset of 60 years or greater were recruited from the UW ADRC. The clinical diagnosis and age-at-onset for these participants were determined using the methods described above. Of the 59 participants with a clinical diagnosis of AD, 45 were confirmed by autopsy.

2.1.3. ACT cohort—One hundred sixty-eight participants with autopsy exam were recruited from the ACT study, a community-based cohort. The ACT study design and methods have been described in detail elsewhere [17]. Beginning in 1994, cognitively normal individuals age 65 and older who resided in the Seattle area and enrolled in Group Health Cooperative (GHC), a large integrated delivery system, underwent a baseline cognitive examination using the Cognitive Abilities Screening Instrument (CASI; total score 0–100) [18] and were then followed biennially for dementia. ACT participants with poor cognition as demonstrated by a CASI score < 86 underwent a complete dementia evaluation, including a neuropsychological and clinical test battery and a physical/neurologic examination by a geriatrician or neurologist. Diagnoses were assigned at a consensus diagnosis conference using the Diagnostic and Statistical Manual of Mental Disorders-IV [19] for all-cause dementia and NINCDS-ADRDA criteria for probable and possible AD [16]. Neuropathological examinations were performed in those deceased ACT participants who consented to brain autopsy.

2.2. Neuropathological examinations

Neuropathological examinations were performed in the UW Division of Neuropathology and the UW ADRC Neuropathology Core. A detailed description of the neuropathological examination methods has been reported elsewhere [20–21]. Neuritic plaques were scored

according to Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [22–23], and neurofibrillary tangles were scored according to the methods of Braak and Braak [24–25]. Neuropathological AD was defined as a Braak stage V and a CERAD rating of moderate or higher. Amyloid angiopathy was scored according to the method of Vonsattel [26]. We also assessed the severity of atherosclerosis of large vessels, the number of cystic infarcts on gross inspection of the brain, and the presence of microvascular lesions of 2 mm or smaller (in their greatest dimension) that were identified by way of microscopic examination and that were defined as encephalomalacic lesions. Lewy body pathology was assessed using immunohistochemistry for α -synuclein in both subcortical and cortical regions [20–21].

2.3. Genotyping TOMM40 intron 6 poly-T and APOE

For the *TOMM40* intron 6 poly-T length, we used a short tandem repeat genotyping-based assay developed in Dr. Yu's laboratory at VA Puget Sound Health Care System, Seattle, WA. This assay used a custom PCR primer set (F: Vic-GCTGACCTCAAGCTGTCCTC, R: GGAGGGACAGGGAAAGAAAA) that has been labeled with VIC fluorescent dye to amplify a 247-bp genomic section; the assay is based on the human genome reference sequence and represents the poly-T marker. The precise length of the amplified fragments from each participant was acquired through an ABI 3130xl genetic analyzer and processed using ABI Genemapper v4.0 software (Applied Biosystems, CA). Alleles (or poly-T count) were then determined via manual inspection and cross-checking of the acquired data. Individual samples were reprocessed in instances where initial data produced unreliable reads. Poly-T length was dichotomized into short (< 20) and long (≥ 20) in the analysis based on a bimodal distribution of the number of T on the *APOE* $\epsilon 3$ chromosomal haplotype [3]. The vast majority of the alleles that were considered long *TOMM40* poly-T had 34 or more T-repeats (98% of all alleles in this study); there were only two alleles with 22 T-repeats and two alleles with 29 T-repeat that were within the intermediate length which is associated with both *APOE* $\epsilon 3$ and $\epsilon 4$ alleles and that were classified as the long in this study.

The $\epsilon 2/\epsilon 3/\epsilon 4$ status of *APOE* was determined by the traditional approach of PCR amplification followed by digestion with the restriction enzyme *HhaI* [27], as reported previously [13, 28].

2.4. Statistical analysis

For continuous variables, differences by 2-category poly-T genotype (short/short vs. any long) were assessed using two sample *t*-tests, and differences by 3-category poly-T genotype (short/short, short/long, or long/long) were assessed using linear regression. Fisher's exact test was used to test for differences by poly-T genotype in categorical variables. For the FAD cohort, differences in age-at-onset by poly-T genotype (short/short vs. any long) were assessed using linear regression with robust standard errors to account for clustering of participants within families. In addition, to compare mean age-at-onset by *TOMM40* poly-T genotype, we also examined the relationship between *TOMM40* poly-T genotype and time-to-onset of AD using survival analysis. Parametric survival models were used to model time-to-onset of AD with the assumption that the effect of having a poly-T long allele is proportional to survival time for those with a short/short genotype (i.e., an accelerated failure time assumption). Results were illustrated using Kaplan-Meier curves and presented as survival ratios and 95% confidence intervals (CIs) based on robust standard errors to account for clustering by family. Significance was assessed using likelihood ratio (LR) tests.

Logistic regression was used to determine the association between neuropathology and poly-T genotype in the ACT cohort adjusting for age-at-death and gender. Analyses were

conducted using R.2.11.1 [29], and more specifically, the Design [30] and Survival packages [31] were used to carry out the survival analyses.

3. Results

As shown in Table 1, the distribution of *TOMM40* intron 6 poly-T genotype among *APOE* $\epsilon 3/\epsilon 3$ AD patients with *PSEN1* mutations, AD patients with *PSEN2* mutation, and LOAD patients was similar. The average age-at-onset of AD patients with the *PSEN1* mutation was almost a decade earlier than the age-at-onset of AD patients with the *PSEN2* mutation.

3.1. Association between *TOMM40* intron 6 poly-T genotype and age-at-onset in AD

The age-at-onset of AD was an average of seven years earlier for *PSEN2* mutation carriers with a long poly-T allele than for those with a short/short poly-T genotype ($t = 1.94$, $df = 25$, $p = .06$). The age-at-onset of AD did not differ between *PSEN1* mutation carriers with a short/short poly-T genotype and those with a long poly-T genotype (Table 1; $t = -0.25$, $df = 30$, $p = .81$). In the ADRC LOAD cases, the average age-at-onset was 3 years earlier for cases with a long poly-T than for those with a short/short poly-T, but the difference did not reach statistical significance ($t = 1.45$, $df = 57$, $p = .15$).

3.2. Time-to-onset of AD and *TOMM40* intron 6 poly-T

Parametric survival models were used to compare the time-to-onset of AD for *PSEN2* or *PSEN1* mutation carriers with at least one long poly-T allele to the time-to-onset of AD for those carriers with a short/short poly-T genotype. These models included four *PSEN2* mutation carriers who remained unaffected by clinical AD at ages (46, 48, 51, and 79 years) that were later than the earliest age-at-onset (age 41 years) and one *PSEN1* mutation carrier who was unaffected by clinical AD (age 40). Survival time was modeled as log-logistic. As shown in the left panel of Figure 1, *PSEN2* mutation carriers with a long poly-T allele developed AD earlier than those with a short/short poly-T genotype (survival ratio 0.84, 95% CI [0.72, 0.97], LR test $p < .01$). In other words, on average, time-to-onset for *PSEN2* mutation carriers with a poly-T long allele was shortened by 16%. The median age-at-onset for *PSEN2* mutation carriers with a poly-T short/short genotype was 60.8 years; the median for those with a poly-T long allele was 50.9 years. There was no evidence that time to AD differed by poly-T genotype in AD patients who carried the *PSEN1* mutation (Figure 1 middle panel, survival ratio 0.95, 95% CI [0.77, 1.17], LR test $p = .51$) or in patients from the LOAD sample (Figure 1 right panel, survival ratio 0.96, 95% CI [0.90, 1.06], LR test $p = .25$).

3.3. *TOMM40* intron 6 poly-T, age-at-onset and risk of AD in the ACT cohort

Of the 168 autopsied ACT participants with *APOE* $\epsilon 3/\epsilon 3$, 29% ($n = 49$) had a *TOMM40* intron 6 poly-T short/short genotype, 51% ($n = 85$) had a poly-T short/long genotype, and 20% ($n = 34$) had a poly-T long/long genotype. The *TOMM40* poly-T genotype did not differ by gender, age-at-enrollment to ACT, age-at-death, or follow-up time (Table 2). During an average 7.1 year follow-up, 51 participants (30.4%) developed dementia, with a mean age-at-onset of 85.5 years. Out of these 51 participants who developed dementia, 42 were classified as probable AD ($n = 25$) and possible AD ($n = 17$) based on NINCDS-ADRDA criteria. The age-at-onset of dementia or AD did not differ by presence of a long poly-T (Table 2). The survival curves of clinically diagnosed AD did not differ by presence of a long poly-T (LR test = 0.90, $p = .34$).

3.4. *TOMM40* intron 6 poly-T and brain pathologies

As shown in Table 3, individuals with a higher number of poly-T alleles (0 vs. 1 vs. 2) were found to exhibit a high level of neuritic tangle (Braak stage of V–VI) and a higher frequency

of pathologically defined AD (Braak stage V and CERAD moderate). In a logistic regression model of pathologically diagnosed AD that adjusted for age-at-death and gender, pathologically diagnosed AD was associated with an increasing number of poly-T long alleles (LR test $p = .04$). Using no long poly-T (short/short) as the reference group, the odds ratios (OR) for 1 and 2 alleles were 2.96, 95% CI (0.80, 11.0), and 5.66, 95% CI (1.40, 22.9), respectively. However, there were no significant differences between participants with and without a long poly-T for CERAD score of neuritic plaques or the presence of amyloid angiopathy (Table 3). There also were no associations between the presence of a long poly-T and cerebrovascular damage or Lewy body pathology in the exploratory analyses.

4. Discussion

Our study found an association between the *TOMM40* intron 6 poly-T variant and an earlier age-at-onset in FAD patients with the *APOE* $\epsilon 3/\epsilon 3$ genotype who carry *PSEN2a* mutation, but we observed no significant association between the *TOMM40* poly-T and age-at-onset in families with a *PSEN1* mutation or in participants with LOAD. In a community-based autopsy cohort who had the *APOE* $\epsilon 3/\epsilon 3$ genotype and who were initially free of dementia, we found that the presence of a long *TOMM40* intron 6 poly-T was associated with a higher level of neuritic tangles and with increased risk of pathologically diagnosed AD but not with increased risk of clinical AD during life. The association between long poly-T and earlier penetrance of AD in the *PSEN2* mutation carriers, as well as with increased neuropathological changes in AD, suggests that the *TOMM40* intron 6 poly-T locus is either an effect modifier itself or a surrogate marker for effect modifier of AD.

The association between the *TOMM40* poly-T variant and an earlier age-at-onset has been inconsistent. The association between the *TOMM40* poly-T variant and an earlier age-at-onset in FAD patients with the *APOE* $\epsilon 3/\epsilon 3$ genotype and *PSEN2* mutation is in line with finding from Roses and colleagues who found that the *TOMM40* long poly-T polymorphism was associated with an earlier age-at-onset of LOAD [3]. However, in a much larger sample of research participants with LOAD, Cruchaga and colleagues were unable to replicate this finding [32]; moreover, they found that the poly-T polymorphism was associated with a decreased risk of LOAD. In another study, Johnson and colleagues found that homozygosity of the long poly-T allele was associated with poorer cognitive functioning and a decrease in the volume of gray matter in the ventral precuneus, a brain region that is affected early in AD, in middle-aged nondemented adults with the *APOE* $\epsilon 3/\epsilon 3$ genotype [33]. Despite these controversial findings, several lines of additional evidence have suggested that *TOMM40* may have an independent role in the AD disease process. The *TOMM40* gene encodes a translocase of the outer mitochondrial membrane protein, and mitochondrial function plays an important role in the aging processes and has been implicated in the pathogenesis of AD [34–36]. In a pathway analysis using genome-wide association data from a large French population, Hong and colleagues [37] found a highly significant enrichment of genes involved in intracellular protein transmembrane transport, including several mitochondrial proteins and nucleoporins that are associated with AD. When they further analyzed how the identified protein transport pathway was related to the *APOE* locus, which contains four genes that cannot be readily discerned due to LD (i.e., *APOE*, *TOMM40*, *PVRL2*, and *BCL3*), only *TOMM40* was closely connected to the set of 18 protein transport genes but not to *APOE*. In addition, our group has recently reported that in contrast to the *TOMM40* short poly-T, the *TOMM40* long poly-T is associated with a decrease in *TOMM40* promoter activity [38]. This finding suggests that poly-T length may functionally contribute to *TOMM40* expression, which implicates a decrease in *TOMM40* expression as a contributing factor in AD pathogenesis through the disruption of mitochondrial function [38]. Together with our observations in the current study, this suggests that it is plausible that accelerated

onset in persons with a long *TOMM40* poly-T might be due to the decreased mitochondrial function that has been associated with advancing age [37].

In this study we found no relationship between the *TOMM40* poly-T and age-at-onset of AD in families with the *PSEN1* mutation. The reason for this lack of a relationship is not entirely clear. However, in contrast to the single *PSEN2* mutation (N141I) that appears to cause AD in our sample of *PSEN2* mutation carriers, there are multiple *PSEN1* mutations that are known to cause AD. Therefore, it is plausible that the heterogeneity of *PSEN1* mutations results in a larger variation in age-at-onset and that this variation decreases precision in detecting modifying effects of *TOMM40*. Because of our relatively small sample size, the possibility of a poly-T effect on age-at-onset in *PSEN1* cases cannot be excluded, but if such an effect exists, its magnitude seems to be much smaller than the magnitude of the effect that was observed in our *PSEN2* cases. A large family study with multiple affected AD cases who share the same *PSEN1* mutation will be useful to answer this question. The lack of association between the poly-T and age-at-onset in *PSEN1* families could also be related to age-at-onset itself; compared to *PSEN2* cases, *PSEN1* cases had a much earlier age-at-onset, with a mean age of around 44, and this may suggest that *PSEN1* mutations had a stronger effect in determining age-at-onset than the *PSEN2* mutation. It is also possible that *TOMM40* functionally interacts with *PSEN1* and *PSEN2* differently. Presenilin mutations have been shown to sensitize cells to apoptosis by mechanisms that impair mitochondrial function [39]. A study that investigated the impact of presenilin/ γ -secretase on mitochondrial function using mouse embryonal fibroblasts derived from wild-type, PS1^{-/-}, PS2^{-/-}, and PS double knock-out embryos showed a specific role of presenilin2/ γ -secretase activity for proper mitochondrial function but no role for PS1/ γ -secretase [40].

Our study also found that the *TOMM40* intron 6 poly-T was associated with neuropathological changes of AD but not with a clinical diagnosis of AD. We suspect that this finding is due to the heterogeneity of neuropathological changes that underlie the clinical diagnosis of AD, which in our study included both probable and possible AD by NINCDS-ADRDA criteria, and due to variability in the classification of study outcome. Although AD pathologies are the most common cause of dementia, other late-life pathological changes, such as cerebrovascular damage, cortical Lewy bodies, and hippocampal sclerosis, also contribute to cognitive impairment and dementia diagnosis [20]. For example, of the 23 cases in our study with a clinical diagnosis of AD who did not meet criteria for pathological AD, 15 (65.2%) had a Braak stage of III–IV. Of these 15 cases with intermediate Braak stages, 13 had comorbid microvascular lesions, cystic infarcts, and/or Lewy bodies, and only 2 had no comorbid pathology identified. Similarly, 7 cases with a low Braak stage had comorbid pathologies. These additional dementia-causing brain pathologies likely explain the differences we observed between clinical and pathological diagnoses in this study. If these comorbid non-AD brain pathologies contribute to an accelerated clinical manifestation of AD-like symptoms or to the clinical diagnoses of AD but are not associated with *TOMM40*, the strength of the association between *TOMM40* and clinical diagnosis of AD, or age-at-onset, would be weakened. In a previous clinicopathological study of genetic risk factors and AD [41], people with the *APOE* ϵ 3/ ϵ 4 genotype were at greater risk for clinical AD than people with the *APOE* ϵ 3/ ϵ 3 genotype (OR 3.4, 95% CI 3.0–3.8), and their risk for pathologically diagnosed AD was even higher (OR 5.5, 95% CI 4.2–7.2). These findings suggest that increasing diagnostic precision through the use of neuropathological phenotypes [42] and diagnostic surrogate biomarkers, such as CSF A β _{1–42}, tau [9–10, 32], and neuroimaging [7], could potentially increase our statistical power to discover or test a susceptibility gene for AD, especially for those genes with moderate risk.

The major strength of this study is its use of highly homogeneous samples. These samples provide great statistical power to detect a moderate association and offer a unique opportunity to understand the potential mechanisms of *TOMM40*, including the way that *TOMM40* interacts with *PSEN1* and *PSEN2*. Moreover, our autopsy study enabled us to examine the relationship between *TOMM40* and specific brain pathologies of AD. However, the study also has several shortcomings. First, because the sample size of each cohort is relatively small, the positive associations of *TOMM40* poly-T variant with age-at-onset in the *PSEN2* families and with the increased neuropathologies of AD could be spurious and the lack of a significant association between *TOMM40* and age-at-onset in the participants with *PSEN1* mutations or in the participants with LOAD could be due to our limited statistical power to detect a moderate association. These findings need to be further investigated in a larger sample. Second, the definition and determination of age-at-onset may vary across studies. For example, our determination of age-at-onset in AD in the clinical case series is primarily based on the recollection of informants, and such recollections could be largely subjective and prone to recall bias. In the ACT study, which included biennial follow-up appointments, onset was determined as halfway between the visit in which participants received a diagnosis and the previous visit. Third, because of the considerable discrepancy between clinical and pathological classifications of AD and the potential selection bias associated with autopsy [43], our findings of an association between *TOMM40* intron 6 poly-T length and pathological AD should be extrapolated to clinical AD with the greatest caution, if at all. Associations of *TOMM40* poly-T with neuritic plaques and other brain pathologies need to be further investigated in an independent study. Finally, we should emphasize that the association between the *TOMM40* intron 6 poly-T variant, age-at-onset, and AD neuropathological changes is limited to those participants with *APOE* $\epsilon 3/\epsilon 3$ and should not be extrapolated to persons with other *APOE* genotypes, especially the $\epsilon 4$ allele, because it is conceivable that *APOE* $\epsilon 4$ interacts differently with the *TOMM40* intron 6 poly-T in terms of the moderation of age-at-onset or the pathogenesis of AD. Whether the *TOMM40* poly-T length has an independent effect on the risk of AD or a synergistic effect with *APOE* $\epsilon 4$ needs to be further investigated. However, the interaction between *APOE* and *TOMM40* poly-T length could not be tested by simply using joint analysis in multiple regression models because the *APOE* $\epsilon 4$ allele is almost exclusively associated with an intermediate length of the *TOMM40* poly-T, whereas the $\epsilon 3$ allele is almost exclusively associated with either short or long poly-T but not intermediate poly-T.

In conclusion, *TOMM40* intron 6 poly-T length may explain some of the variation in age-at-onset in *PSEN2* familial AD and may be associated with AD neuropathology in persons with *APOE* $\epsilon 3/\epsilon 3$. If these findings are confirmed in independent studies, the risk of AD could be further stratified in those “risk-neutral” people with *APOE* $\epsilon 3/\epsilon 3$. Furthermore, our results, together with previous contrasting findings [3, 32–33], suggest that it is important for future studies to use a biological markers, such as CSF biomarkers or neuroimaging results, to increase the precision of measuring phenotypes and to thereby increase the power to detect a moderate genetic risk factor in AD.

Acknowledgments

This study is supported by grants AG033693, AG006781, AG05136, and AG034214 from the National Institute of Aging and by the US Department of Veterans Affairs, Office of Research and Development, Biomedical Laboratory Research Program. The study sponsors had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript.

We thank Andrew David for editing the manuscript.

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Figure 1.

Kaplan-Meier survival curve estimates of AD by *TOMM40* intron 6 poly-T genotype for the FAD participants with PSEN2 (left panel) and PSEN1 (middle panel) mutations, and for the LOAD participants (right panel)

Table 1

Characteristics of participants with AD from the UW ADRC

	<i>PSEN1</i> mutation carriers (N=32)	<i>PSEN2</i> mutation carriers (n=27)	LOAD Participants (n=59)
Gender, male (%)	20 (62.5)	15 (55.6)	28 (47.5)
Race, white (%)	26 (81.3)	27 (100.0)	55 (94.8) ^a
<i>TOMM40</i> intron 6 poly-T genotype, n (%)			
Short/short	9 (28.1)	9 (33.3)	14 (23.7)
Short/long	11 (34.4)	15 (55.6)	31 (52.5)
Long/long	12 (37.5)	3 (11.1)	14 (23.7)
Age-at-onset, years, mean (SD, range)	44.9 (9.6, 31–79)	54.1 (8.8, 41–75)	74.1 (7.9, 60–88)
Short/short	45.6 (7.5, 36–56)	59.1 (9.5, 45–72)	76.8 (7.8, 67–88)
Short/long or long/long	44.7 (10.5, 31–79)	51.7 (7.5, 41–75)	73.3 (7.9, 60–85)

^a 1 case race unknown.

Abbreviations: AD, Alzheimer's disease; LOAD, late-onset Alzheimer's disease; *PSEN1*, presenilin 1; *PSEN2*, presenilin 2; SD, standard deviation; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog; UW ADRC, University of Washington Alzheimer's Disease Research Center

Table 2

Characteristics of participants by *TOMM40* intron 6 poly-T genotype in the ACT cohort

	<i>TOMM40</i> intron 6 poly-T genotype				
	Short/short (n = 49)	Short/long (n = 85)	Long/long (n = 34)	Total (n = 168)	p-value
Gender, male, n (%)	24 (49.0)	36 (42.4)	10 (29.4)	70 (41.7)	.20
Race, Caucasian, n (%)	48 (98.0)	81 (95.3)	34 (100)	163 (97.0)	.60
Clinical diagnosis of dementia, n (%)	16 (32.7)	22 (25.9)	13 (38.2)	51 (30.4)	.38
Clinical AD (probable + possible), n (%)	15 (30.6)	15 (17.6)	12 (35.3)	42 (25.0)	.07
Age-at-baseline, mean (SD, range)	79.4 (5.3, 68–93)	78.5 (6.5, 66–92)	80.6 (7.6, 65–90)	79.2 (6.4, 65–93)	.26
Age-at-death, mean (SD, range)	88.1 (5.3, 78–99)	87.3 (7.1, 68–102)	88.1 (7.7, 72–100)	87.7 (6.7, 68–102)	.72
Year of follow-up, mean (SD, range) ^a	7.3 (3.0, 2.0–13.3)	7.3 (3.2, 1.8–14.2)	6.2 (3.3, 1.8–13.0)	7.1 (3.2, 1.8–14.2)	.28
Age-at-onset, years (SD, range)					
Dementia (n = 51)	84.8 (3.7, 74–90)	84.5 (6.2, 73–96)	88.2 (4.6, 80–94)	85.5 (5.3, 73–96)	.10
Clinical AD (n = 42)	85.1 (3.7, 74–90)	84.1 (7.1, 73–96)	88.8 (4.4, 80–94)	85.8 (5.6, 73–96)	.08

^a 1 case excluded with no clinical follow-up before death.

Abbreviations: ACT, Adult Changes in Thought; AD, Alzheimer's disease; SD, standard deviation; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog

Table 3

Brain pathologies by *TOMM40* intron 6 poly-T genotype in ACT study participants with *APOE* ε3/ε3

	<i>TOMM40</i> intron 6 poly-T				
	Short/short n = 49	Short/long n = 85	Long/long n = 34	Total n = 168	p-value
Brain weight, mean (SD)	1209.7 (158.3)	1218.5 (123.8)	1202.6 (158.6)	1212.8 (140.9)	.85
AD pathology, n (%)					
Braak stage V-VI	3 (6.1)	17 (20.0)	10 (29.4)	30 (17.9)	.01
CERAD score moderate	22 (44.9)	33 (38.8)	14 (41.2)	69 (41.1)	.80
Pathologically diagnosed AD	3 (6.1)	14 (16.5)	10 (29.4)	27 (16.1)	.02
Amyloid angiopathy (moderate)	2 (4.2)	9 (10.6)	2 (5.9)	13 (7.8)	.44
Cerebrovascular pathology, n (%)					
Any microvascular lesions	17 (35.4)	42 (49.4)	18 (52.9)	77 (46.1)	.21
Any cystic infarcts	13 (27.7)	20 (24.4)	10 (29.4)	43 (26.4)	.82
Atherosclerosis (moderate)	31 (66.0)	49 (59.0)	14 (43.8)	94 (58.0)	.14
Lewy body pathology, n (%)					
In cortex	6 (12.2)	4 (4.7)	2 (5.9)	12 (7.1)	.29
In any region	12 (26.1)	9 (11.4)	5 (16.7)	26 (16.8)	.11

NOTE. Number of missing cases: 2 in the brain weight row, 1 in the microvascular lesions row, 1 in the amyloid angiopathy row, 5 in the cystic infarcts row, 6 in the atherosclerosis row, and 13 in Lewy body pathology in any region row.

Abbreviations: ACT, Adult Changes in Thought; AD, Alzheimer's disease; *APOE*, apolipoprotein E; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; SD, standard deviation; *TOMM40*, translocase of outer mitochondrial membrane 40 homolog.