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Perspective

# The effects of the *TOMM40* poly-T alleles on Alzheimer's disease phenotypes

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**Abstract** The *TOMM40* poly-T is a polymorphism in intron 6 of the *TOMM40* gene, which is adjacent to and in linkage disequilibrium with *APOE*. Roses et al identified the association between the length of *TOMM40* poly-T with the risk and age of onset of late-onset Alzheimer's disease (LOAD). Following the original discovery, additional studies found associations between the *TOMM40* poly-T and LOAD-related phenotypes independent of *APOE* genotypes, while others did not replicate these associations. Furthermore, the identity of the *TOMM40* poly-T risk allele has been controversial between different LOAD-related phenotypes. Here, we propose a framework to address the conflicting findings with respect to the *TOMM40* poly-T allele associations with LOAD phenotypes and their functional effects. The framework is used to interpret previous studies as means to gain insights regarding the nature of the risk allele, very long versus short. We suggest that the identity of the *TOMM40* poly-T risk allele depends on the phenotype being evaluated, the ages of the study subjects at the time of assessment, and the context of the *APOE* genotypes. In concluding remarks, we outline future studies that will inform the mechanistic interpretation of the genetic data.


*Keywords:* *TOMM40* poly-T; *APOE*; Late-onset Alzheimer's disease; Cognitive performance; Brain imaging

The association of genetic variation within the *TOMM40-APOE-C1-2-4* genomic region of Chr:19q13.32 with the risk and age of onset (AOO) of late-onset Alzheimer's disease (LOAD) was established over 20 years ago by linkage analysis of pedigrees [1]. The *APOE* ε4 allele of apolipoprotein E (*APOE*) [1] is the strongest genetic risk factor for LOAD and is associated with lower AOO, and over the ensuing years, these findings have been highly replicated [1–4]. LOAD genome-wide association studies conducted over the last decade have confirmed strong associations with this genomic region [5–10], and no other LOAD association has remotely approached the same level of significance [5,6,11,12]. Although some LOAD genome-wide association studies exclude all variants in this region because of their high linkage disequilibrium (LD) with the coding SNPs that define the *APOE* genotype, other LOAD genetic studies have focused on the association between variants and haplotypes based on the promoter and enhancer regions of genes in this region with LOAD phenotypes [13–20]. However, whether the association signal is attributable to additional variants and haplotypes within this LD region jointly with *APOE* ε4 is an enduring enigma in the field of LOAD genetics.

The *TOMM40* poly-T is a polymorphism in intron 6 of the *TOMM40* gene, which encodes Tom40 protein (translocase of the outer mitochondrial membrane, 40kD) and is adjacent to and in LD with *APOE*. In 2010, Roses et al. reported the association between the length of this highly polymorphic poly-T variant (rs10524523 aka '523) with the risk and AOO of LOAD [21]. The high LD between DNA nucleotide variants in the *APOE* genomic region impedes the assessment of independence by statistical conditional analysis [22]. Therefore, the association analysis in the original report was limited to individuals with the APOE ε3/4

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genotype to reduce genetic heterogeneity and eliminate confounding with the *APOE* SNPs. Subsequently, several studies have supported the hypothesis of an *APOE*-independent association of the *TOMM40* poly-T variant with several phenotypes related to Alzheimer's disease (AD), including hippocampal thinning [23], cognition and gray matter volume [24], cerebrospinal fluid biomarkers for AD [25,26], increased neuritic tangles, a higher likelihood of pathologically diagnosed AD [22], the AOO of mild cognitive impairment due to AD [27], and cognitive performance and rate of decline in nondemented elderly [28–32]. Recently, we tested the association of the *TOMM40* poly-T with cognitive decline, based on longitudinal measures of cognitive function, for example, Montreal Cognitive Assessment obtained from an on-going study at Duke, the Bryan ADRC Prevention Screening Study and Database/Repository. This study population comprises a nondemented sociocultural diverse volunteer cohort from Durham, NC. In unpublished data, we found that the *TOMM40* poly-T was associated with changes in Montreal Cognitive Assessment scores adjusting for different follow-up intervals, sex, age, and for *APOE* genotype.

Overall, the studies summarized in Table 1 determined the *APOE*-independent association of the *TOMM40* poly-T with LOAD-related phenotypes by including *APOE* genotypes as a covariate in the statistical models or by designing the study to include only individuals with the same *APOE* genotypes (e.g., analyzing the association in the *APOE* ε3/3 stratum). It is important to note that other studies have not supported an *APOE*-independent association of the *TOMM40* poly-T variant with LOAD risk or AOO [34–36] (Table 2). These studies included all *APOE* genotypes and adjusted for the *APOE* ε4 allele counts as a covariate in the statistical models, and some repeated the analyses with the *APOE* ε3/3 stratum. Among the two largest size analyses performed for the *APOE* ε3/3 subsets, Jun et al. reported no statistically significant associations of the *TOMM40* poly-T with LOAD risk or age at onset (AAO) [34], while Cruchaga et al. observed statistically significant association with LOAD risk and a trend toward association with AAO [33]. A number of methodological aspects that may account for the contradictory results and requirements for replication studies of genetic associations with AAO were discussed previously [40]; however, there is an interpretation of prior studies that *TOMM40* poly-T associations with LOAD-related phenotypes are simply a consequence of LD with the *APOE* coding SNPs [41].

Three allele groups were defined for the *TOMM40* poly-T polymorphism, based on the modes of the distributions of the number of "T" residues: "short" (S, $T \leq 19$), "long" (L, $20 \leq T \leq 29$), and "very long" (VL, $T \geq 30$) [21]. The VL allele appears to have different effects on LOAD-related phenotypes, depending on whether it forms a haplotype with *APOE* ε3/3 or *APOE* ε3/4. Kaplan-Meier AOO curves showed that in *APOE* ε3/3 individuals, the VL allele was associated with a later AOO of mild cognitive impairment due to AD than S, whereas in *APOE* ε3/4, it was associated with earlier AAO [27], consistent with the original discovery [21]. These observations suggested that although the *TOMM40* poly-T has autonomous associations with LOAD-related phenotypes, as discussed previously, the direction of the effects are influenced by interactions with *APOE*.

We hypothesized that the identity of the *TOMM40* poly-T risk allele depends on the phenotype being evaluated and the ages at assessment of the study subjects. To gain insights into the nature of the *TOMM40* poly-T allele risk pattern, we compared a number of features across several studies that identified significant associations with the *TOMM40* poly-T alleles (Table 1), including study design, the phenotypes evaluated, and the demographic characterization of the study sample. In these studies, the analyses were constrained to *APOE* ε3/3 subjects only and/or adjusted for *APOE* genotypes, so that the *APOE* status did not contribute to the direction of the *TOMM40* poly-T effect. A recent study showed that the VL allele was significantly associated with increased hippocampal thinning relative to the S allele, specifically reduced thickness of the entorhinal cortex [23]. An earlier study found that the VL allele was associated, in a dose-dependent manner, with decreased gray matter volume in regions of the brain affected early in the development of AD in *APOE* ε3/3, cognitively normal, middle-aged people [24]. Together, these studies suggest that the VL allele is deleterious regarding the structural integrity of brain regions affected early in the course of AD. Furthermore, the latter study also reported that the VL/VL group showed lower performance on primacy retrieval from a verbal list learning task, a cognitive deficiency noted in the early manifestation of AD [24]. The VL allele also was associated with lower cognitive measures in several additional community-dwelling studies [28–30,32]. Two cross-sectional design studies in cognitively healthy elderly reported that S homozygotes performed better on measures of memory and executive function, domains that are preferentially affected in early-stage AD [29,30]. Another longitudinal study, in a larger cohort of cognitively normal individuals, found a significant effect of the VL on flattened test-retest improvement only in individuals aged <60 years [28]. The VL allele was also associated with faster rate of cognitive decline, which was significant for vocabulary ability, suggesting that the VL has a risk effect [32]. However, the same study also reported that the S correlated with reduced vocabulary ability [32]. An association analysis using a large *APOE* ε3/3 case-control series found that the VL allele was underrepresented in LOAD cases compared to controls, supporting the opposite direction, that is, the VL exhibited a protective role [33]. The protective effect of the VL allele was further demonstrated in a recent large study of *APOE* ε3/3 elderly individuals who reported statistically significant association of the S allele with faster decline in global cognition, primarily in the domains of episodic and semantic memory [31]. Similarly, in the Prevention Screening Study