Genomic Instability in Alzheimer's Disease: TOMM40 Poly-T Variations

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Introduction

Late-Onset Alzheimer's Disease (LOAD) accounts for roughly 99% of Alzheimer's cases and is the most common cause of dementia. Age is the greatest known risk factor for LOAD. The next strongest risk factor is genetic background, which accounts for 58-79% of the predisposition to LOAD[1].

Involvement of chromosome 19q13.32 in LOAD:

- Since 1993, the ε4 allele of APOE has been established as the strongest genetic risk factor for LOAD.
- GWAS has detected the strongest LOAD association signal from the 19q13.32 linkage disequilibrium region
- In 2010, the structural variant TOMM40'523 was associated with the age-of-onset of LOAD[2].

In this project, we aimed to further characterize the TOMM40 gene and investigate the functional role of TOMM40'523. Specifically, we were interested in investigating:

1) The regulatory effect of TOMM40'523 on gene expression and splicing.
2) Genomic instability of TOMM40'523.

Background

TOMM40 codes for a pore subunit of the translocase of the outer mitochondrial membrane, a protein complex (*Fig. 1*) facilitates import of nuclear-encoded preproteins into the mitochondria.

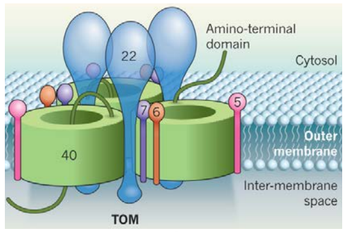


Figure 1. The TOM complex.

The TOMM40'523 structural variant:

- A highly polymorphic, deoxythymidine-homopolymer ("poly-T"); formally named rs10524523 ("523"), located in intron 6 of the TOMM40 gene.
- TOMM40'523 poly-T length has modulatory effects on TOMM40 and/or APOE transcription[4], and is associated with cognitive performance in healthy elderly patients[5]
- A rare alternative splice variant of TOMM40 was recently reported in Genome Browser

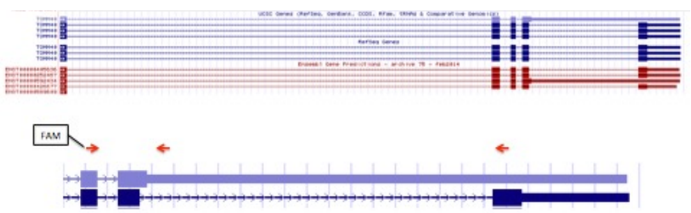


Figure 2. TOMM40 alternative splicing.

Methods

Gene Expression

To examine the regulatory role of TOMM40'523 on gene expression, we first attempted to detect the presence of a rare alternative splice variant within human brain tissue (*Fig. 2*).

We developed an assay to analyze TOMM40 splicing:

- mRNA extracted from fresh frozen human brain tissue
- mRNA converted to cDNA using SuperScript III Reverse Transcriptase
- cDNA samples amplified using optimized PCR settings
- PCR products sent to Eton Bioscience for fragment analysis using capillary electrophoresis
- Sample files analyzed using Peak Scanner to determine peak size and area in base pairs (BP)

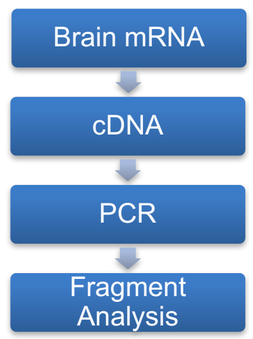


Figure 3. Assay for TOMM40 splice analysis.

Genomic Instability

We examined the stability of the TOMM40'523 genomic locus using two systems to genotype for potential poly-T variations.

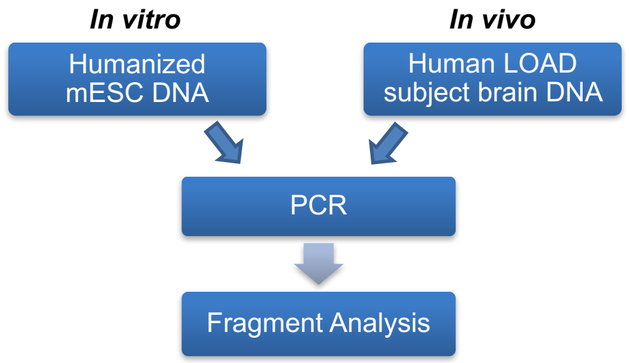


Figure 4. Assays for TOMM40 genomic instability

***In vitro* system**:

- Mouse embryonic stem cells (mESC) were derived from 19q13.32 humanized transgenic mice
- Aliquots of mESC were collected at different division cycles

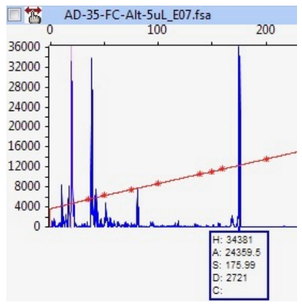
***In vivo* system**:

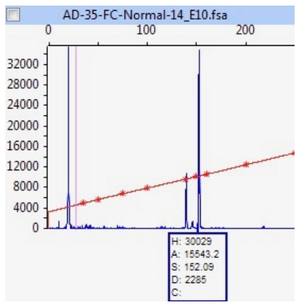
- Two small chunks from each of three brain regions (frontal, occipital, temporal) were embedded from the fresh frozen brain of human donor (M, 86 y.o., Braak Stage 6)
- One 8μm-thick slice was isolated from chunk
- The tip of each slice was collected for DNA extraction using the Epicentre QuickExtract Solution
- Resulting six DNA aliquots sampled a wide area of the brain
- Samples were amplified with 35 cycles of PCR in triplicate to control for potential poly-T variations introduced through increased rounds of amplification

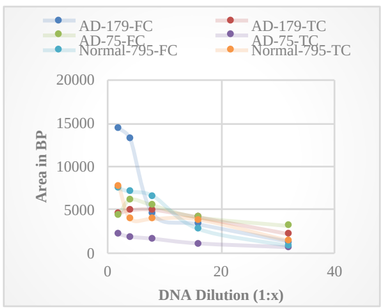
Results

Gene Expression

- Using the splice analysis assay *(Fig. 3)*, we were able to detect a novel TOMM40 alternative splice variant.







- We created a calibration curve such that the TOMM40 alternative splicing assay can be used to estimate the ratios of TOMM40 splice variants

Genomic Instability

We did not detect poly-T instability in either systems (*Fig. 4).*

- Poly-T lengths of mESC remained the same across different division cycles
- Within the one individual studied, poly-T length did not vary across brain regions

Future Directions

We will continue to investigate the role of the TOMM40'523 structural variant in neurodegeneration. Specifically, we aim to:

- Compare the prevalence of the TOMM40 alternative splice variant across brain regions and pathologies
- Test for somatic variations in poly-T length at a finer resolution; single-cell or cell-type using fluorescence activated nuclei sorting (FANS)

References

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