The functional role of an Alzheimer’s disease-associated poly-T variant in TOMM40 gene

Ornit Chiba-Falek, Ph.D

Dementia 2015 Conference
Aug 31st, 2015
The significance and functional consequences of genomic regions/genes associated with neurodegenerative diseases

Identify the disease associated genomic region
whole genome/candidate gene/s

Functional Follow up to pinpoint the causal variant/s and the molecular mechanism of action
Bioinformatics tools
Human brain tissues
ex vivo/in vivo model systems

Novel therapeutic targets
Diagnostic biomarker

**SNCA**
PD Lewy body related diseases

**SORL1**
AD

**TOMM40-APOE**
AD and cognitive decline in aging
Hypothesis

Changes in expression levels of normal proteins in the brain can lead to neurodegenerative diseases

Regulation of gene expression:
• Genetics

Noncoding Structural Variants
The $\varepsilon 4$ allele of the Apolipoprotein E gene ($APOE$) was the first genetic risk factor identified for sporadic Late onset Alzheimer’s disease (LOAD) [Saunders 1993], and it remains the most reproducible and largest effect size AD genetic risk factor.

An integrated data base of LOAD genetic association studies (alzGene.org) the strongest association signal (by wide margin) has been found again at APOE LD region.

The Largest LOAD GWAs published studies:
- Harold (2009)
- Lambert (2009)
- Hollingworth (2011)
  - Hu (2011)
  - Naj (2011)
- Seshadri (2010)
The Sequence of the \textit{TOMM40-‘523’} Alleles

Poly T

‘S’ Short $T \leq 19$

‘L’ Long $T = 20 - 29$

‘VL’ Very Long $T \geq 30$
## ‘523’ Allele frequencies in Different Ethnicities

### Table 1. ‘523’ Allele frequencies in different ethnicities in the US

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Subjects(N)</th>
<th>S (%)</th>
<th>L (%)</th>
<th>VL (%)</th>
<th>Poly T length (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whites</td>
<td>177</td>
<td>45</td>
<td>11</td>
<td>44</td>
<td>14-39</td>
</tr>
<tr>
<td>African American</td>
<td>370</td>
<td>65</td>
<td>10</td>
<td>25</td>
<td>14-54</td>
</tr>
<tr>
<td>Hispanic</td>
<td>179</td>
<td>43</td>
<td>9</td>
<td>48</td>
<td>14-39</td>
</tr>
</tbody>
</table>

### Table 2. ‘523’ Allele frequencies in non US geographical cohorts (Far Eastern and West Africa)

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Subjects(N)</th>
<th>S (%)</th>
<th>L (%)</th>
<th>VL (%)</th>
<th>Poly T length (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghanaian</td>
<td>40</td>
<td>71</td>
<td>8</td>
<td>21</td>
<td>13-43</td>
</tr>
<tr>
<td>Japanese</td>
<td>60</td>
<td>24</td>
<td>18</td>
<td>58</td>
<td>11-35</td>
</tr>
<tr>
<td>Korean</td>
<td>60</td>
<td>20</td>
<td>8</td>
<td>72</td>
<td>11-38</td>
</tr>
<tr>
<td>Han Chinese</td>
<td>60</td>
<td>38</td>
<td>10</td>
<td>52</td>
<td>12-36</td>
</tr>
</tbody>
</table>

*Genotypes determination was performed by Polymorphic, Inc. using a sequencing based assay.

Linnertz et al. (2012)
Linkage Pattern: **TOMM40-‘523’** and **APOE** Alleles

### Hispanic

- **APOE Genotype**
  - ε3/ε3
  - ε3/ε4
  - ε4/ε4
  - ε2/ε3
  - ε2/ε4
  - ε2/ε2

- **No. of Subjects**

### African American

- **APOE Genotype**

### Caucasian

- **APOE Genotype**

**rs10524523**
- S/S
- S/L
- S/VL
- L/L
- L/VL
- VL/VL

**Hispanic**

**Caucasian**

**African American**

**No. of Subjects**

**Linkage Pattern:**
- **TOMM40-‘523’**
- **APOE** Alleles
✓ In Whites, consistent with previous reports, and Hispanics the L allele is primarily linked to ε4, while the majority of the VL and S alleles are linked to ε3.

✓ African Americans, Ghanaians and Japanese, there is an increased frequency of the ‘523’ S-APOE ε4 haplotype.

Linnertz *et al.* (2012)
AOO for Poss/Prob AD – APOEε3/4

Roses et al. (2009)
Proportions unaffected at each age estimated from pooled patient data.

AOO for AD – APOEε3/3

Prospective Arizona Cohort

Mayo Scottsdale, N = 22

Proportions unaffected at each age estimated from pooled patient data.

Caselli et al. (2010)
Normal cognitive aging
An outreach project in the local retirement communities of independent livings.

Data collected:
- Personal and demographic details
- Life style (habits and hobbies)
- Cognitive performance: MoCA, CANTAB, 11 individual memory tests.

Biological samples:
- Saliva DNA
- BLOOD RNA, Protein and Plasma (subset group).
The Triangle Cognitively Normal Retirees Cohort

Table 1. Demographic Characteristics n=127

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Range</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean(SD)</td>
<td>64-93</td>
<td>80.6 (6.0)</td>
</tr>
<tr>
<td>Sex, female</td>
<td></td>
<td>87 (68.5)</td>
</tr>
<tr>
<td>Education, mean(SD)</td>
<td>12-20</td>
<td>16.8 (2.3)</td>
</tr>
<tr>
<td>English as 1st language</td>
<td></td>
<td>120 (94.5)</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td>126 (99.2)</td>
</tr>
<tr>
<td>APOE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 ε4 alleles</td>
<td></td>
<td>101 (79.5)</td>
</tr>
<tr>
<td>1 ε4 allele</td>
<td></td>
<td>25 (19.7)</td>
</tr>
<tr>
<td>2 ε4 alleles</td>
<td></td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>TOMM40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td></td>
<td>25 (19.7)</td>
</tr>
<tr>
<td>S/L, S/VL</td>
<td></td>
<td>63 (49.6)</td>
</tr>
<tr>
<td>L/L, L/VL, VL/ VL</td>
<td></td>
<td>39 (30.7)</td>
</tr>
<tr>
<td>MoCA</td>
<td></td>
<td>27.4 (2.4)</td>
</tr>
<tr>
<td>BDI-II</td>
<td></td>
<td>4.9 (4.1)</td>
</tr>
</tbody>
</table>

Abbreviations: SD=Standard deviation; MoCA=Montreal Cognitive Assessment; BDI-II=Beck Depression Inventory, 2nd Edition.
Values are number (%) unless indicated as mean (SD).

Hayden et al. (2012)
Retirees Cohort: Allele and Genotype Distribution

Hayden et al. (2012)
TOMM40 ‘523’ Associated with Cognitive Performance

Subsample APOE ε3/ε3 (N=82)

The S/S group performed significantly better than the other genotype groups on measures of specific cognitive domains of memory and executive control that are preferentially affected in early-stage Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Memory</td>
<td>PAL Mean Errors to Success</td>
<td>0.0204</td>
</tr>
<tr>
<td></td>
<td>PAL Mean Trials to Success</td>
<td>0.0163</td>
</tr>
<tr>
<td></td>
<td>VRM Free Recall Items Correct</td>
<td>0.0325</td>
</tr>
<tr>
<td>Attention</td>
<td>RVP Latency</td>
<td>0.0475</td>
</tr>
<tr>
<td>Executive</td>
<td>Digit Data</td>
<td>0.0349</td>
</tr>
<tr>
<td></td>
<td>IED Ratio Errors/Trials</td>
<td>0.0771</td>
</tr>
<tr>
<td></td>
<td>IED Total Errors</td>
<td>0.0905</td>
</tr>
</tbody>
</table>

*Models are adjusted for age, sex, years of education, and Beck Depression Inventory-II
**Abbreviations: IED= Intra-Extra Dimensional set shift; PAL=Paired Associates Learning; RVP= Rapid Visual Information Processing; VRM=Verbal Recognition Memory
### Association of Gene Expression with Cognitive Performance

**Blood PaxGene RNA (N=66)**

<table>
<thead>
<tr>
<th>Cognitive Domain/ Test</th>
<th>APOE-mRNA p-value</th>
<th>APOC1-mRNA p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MoCA</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Attention</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVP Latency</td>
<td>0.01</td>
<td>--</td>
</tr>
<tr>
<td><strong>Executive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit Data</td>
<td>0.009</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Models adjusted for: sex, age, and APOE genotype
Molecular Mechanism of Action?
Hypothesis

The *TOMM40* ‘523’ polyT tract has a regulatory function and modulates the expression of genes in the *TOMM40-APOE* LD region, thereby impacting the pathways, in which these proteins participate, and mediates LOAD pathogenesis.
polyT acts as an enhancer element regulating transcription, via nucleosome organization (Anderson et al., Molecular and cellular biology 2001; Segal et al., Curr Opin Struct Biol 2009)

An extended haplotype upstream from *APOE* which encompasses ‘523’ modulates *APOE* expression levels in both cerebrospinal fluid and *postmortem* brain suggesting that *cis*-regulation of *APOE* expression extends far upstream of *APOE* basic promoter. (Bekris et al., J Alzheimers Dis. 2008 and Am J Med Genet B Neuropsychiatr Genet. 2010)

A synthetic construct containing the 523 locus acts as an enhancer/silencer of *TOMM40* promoter activity in cultured neuronal, but not hepatocyte, cell lines. The report suggested a complex transcriptional regulatory region for *TOMM40* and *APOE* expression that extends throughout both genes and is influenced by multiple polymorphisms including the 523 locus (Bekris et al., J Hum Genet. 2012)
mRNA Analysis of Human Brain
### Table 1. Demographic description of the brain samples

<table>
<thead>
<tr>
<th></th>
<th>LOAD</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects (N)</td>
<td>69</td>
<td>42</td>
</tr>
<tr>
<td>†TC (N)</td>
<td>66</td>
<td>42</td>
</tr>
<tr>
<td>‡OCC (N)</td>
<td>69</td>
<td>34</td>
</tr>
<tr>
<td>Male %</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Age (yr) mean±SD</td>
<td>76.9±13.3</td>
<td>78.2±15.1</td>
</tr>
<tr>
<td>§PMI (hr) mean±SD</td>
<td>12.3±12.1</td>
<td>11.4±7.7</td>
</tr>
<tr>
<td>Caucasians %</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

†TC- temporal cortex, ‡OCC- occipital cortex, §PMI- post mortem interval
Normal & Disease Brains: \textit{TOMM40}-mRNA Expression

Caucasians \textit{APOE} $\varepsilon3/\varepsilon3$

\begin{itemize}
  \item \textbf{Temporal Cortex} \hspace{2cm} \textbf{Occipital Cortex}
  \begin{itemize}
    \item Control (N=42) \hspace{1cm} AD (N=66)
    \item Control (N=34) \hspace{1cm} AD (N=69)
  \end{itemize}
  \begin{itemize}
    \item TOMM40 mRNA (log)
    \item p = 0.0003 \hspace{2cm} p = 0.08
  \end{itemize}
\end{itemize}

Means± SE corrected for: sex, age, PMI, Braak&Braak stage

\textit{Linnertz et al.} (2014)
Normal Brains: *TOMM40*-mRNA Expression

**Caucasians APOE ε3/ε3**

- **Temporal Cortex**
  - **S/S**
  - **S/VL**
  - **VL/VL**

- **Occipital Cortex**
  - **S/S**
  - **S/VL**
  - **VL/VL**

Means± SE corrected for: sex, age, PMI

Linnertz *et al.* (2014)
AD Disease Brains: *TOMM40*-mRNA Expression

Caucasians *APOE* ε3/ε3

Temporal Cortex

Occipital Cortex

Means± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz et al. (2014)
Normal & Disease Brains: *APOE*-mRNA Expression

Caucasians *APOE* ε3/ε3

Means ± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz *et al.* (2014)
AD Disease Brains: *APOE*-mRNA Expression

**Caucasians** *APOE* ε3/ε3

**Temporal Cortex**

- **S/S**
- **S/VL**
- **VL/ VL**

**Occipital Cortex**

- **S/S**
- **S/ VL**
- **VL/VL**

Means ± SE corrected for: sex, age, PMI, Braak & Braak stage

*Linnertz et al.* (2014)
Normal Brains: APOE-mRNA Expression

Caucasians APOE ε3/ε3

Means± SE corrected for: sex, age, PMI

Linnertz et al. (2014)
Temporal Cortex

Caucasians *APOE* ε3/ε3 vs. ε3/ε4

**Means± SE corrected for: sex, age, PMI, Braak&Braak stage**

![Graph showing mRNA expression for TOMM40 and APOE for ε3/ε3 vs. ε3/ε4 in AD disease brains.](image-url)
AD Disease Brains: mRNA Expression

Caucasians $APOE\,\varepsilon3/\varepsilon4$

$TOMM$-mRNA and $APOE$-mRNA

$S/L<L/VL\,\text{n.s.}$
AD Disease Brains: mRNA Expression

Caucasians $APOE\varepsilon3/\varepsilon3$ and $\varepsilon3/\varepsilon4$

Means± SE corrected for: sex, age, PMI, Braak&Braak stage, APOE genotype
S+, S/S S/L; VL+, L/VL VL/VL
What cell type is responsible for the expression change?

Expression analysis of homogenous pool of cells vs. whole tissue

**Single cell-type:**
- Neurons
- Astrocyte
- Microglia
Laser Capture Microdissection (LCM)

Frozen Brain

ZEISS Palm System
Selecting, cutting & collecting cells

Cryostat Embedding, Sectioning & Mounting tissue on slides

Staining
SMI-32 GFAP Iba1

Pyramidal Neurons
Astrocytes
Microglias

before
after

RNA Extraction

RNA Amplification
nCounter single-cells expression assay (NanoString)
Luciferase Reporter Assay: ‘523’ polyT Alleles

SHSY5Y

Fold expression relative to pGL4.10 (L/R)

HepG2

Fold expression relative to pGL4.10 (L/R)

Linnertz et al. (2014)
Humanized Mice

Replacement with BAC that contains ~40Kb from *PVRL2* 3’ through *APOC1* 3’ (19q13.32)

- Allele VL  risk

- Allele S
Summary

• **TOMM40 523** associated with LOAD AOO and risk (Roses).
  • Expression of genes in the **TOMM40-APOE** locus is associated with AD status.

• **TOMM40 523** associated with cognitive performance in normal aging.
  • Association trends of the locus’ genes expression in blood with cognitive performance in normal aging.

• Expression of genes in the **TOMM40-APOE** locus in healthy and disease brains is associated with 523 genotype.

523 acts as a regional regulator of **TOMM40** and **APOE** genes expression.

➢ Molecular mechanisms for the genetic association of 523 with AD and cognition.
## Acknowledgements

<table>
<thead>
<tr>
<th>Chiba-Falek lab</th>
<th>Duke/Neurology &amp; Bryan ADRC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colton Linnertz</td>
<td>Allen Roses</td>
</tr>
<tr>
<td>Lidia Tagliafierro</td>
<td>Mike Lutz</td>
</tr>
<tr>
<td>Omolara-Chinue Glenn</td>
<td>William Gottschalk</td>
</tr>
<tr>
<td>Laura Saucier</td>
<td>Mirta Mihovilovic</td>
</tr>
<tr>
<td>Sunita Saith</td>
<td>Kathleen A. Welsh-Bohmer</td>
</tr>
<tr>
<td>Jawara Allen</td>
<td>James R. Burke</td>
</tr>
<tr>
<td>Natalie Miller</td>
<td>Kathleen Hayden</td>
</tr>
<tr>
<td>Christina He</td>
<td>John Ervin</td>
</tr>
<tr>
<td>Taylor Novice</td>
<td></td>
</tr>
<tr>
<td>HayLee Bergstorm</td>
<td></td>
</tr>
<tr>
<td>Shobana Subramanian</td>
<td></td>
</tr>
<tr>
<td>Kirsten Bonawitz</td>
<td></td>
</tr>
</tbody>
</table>