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

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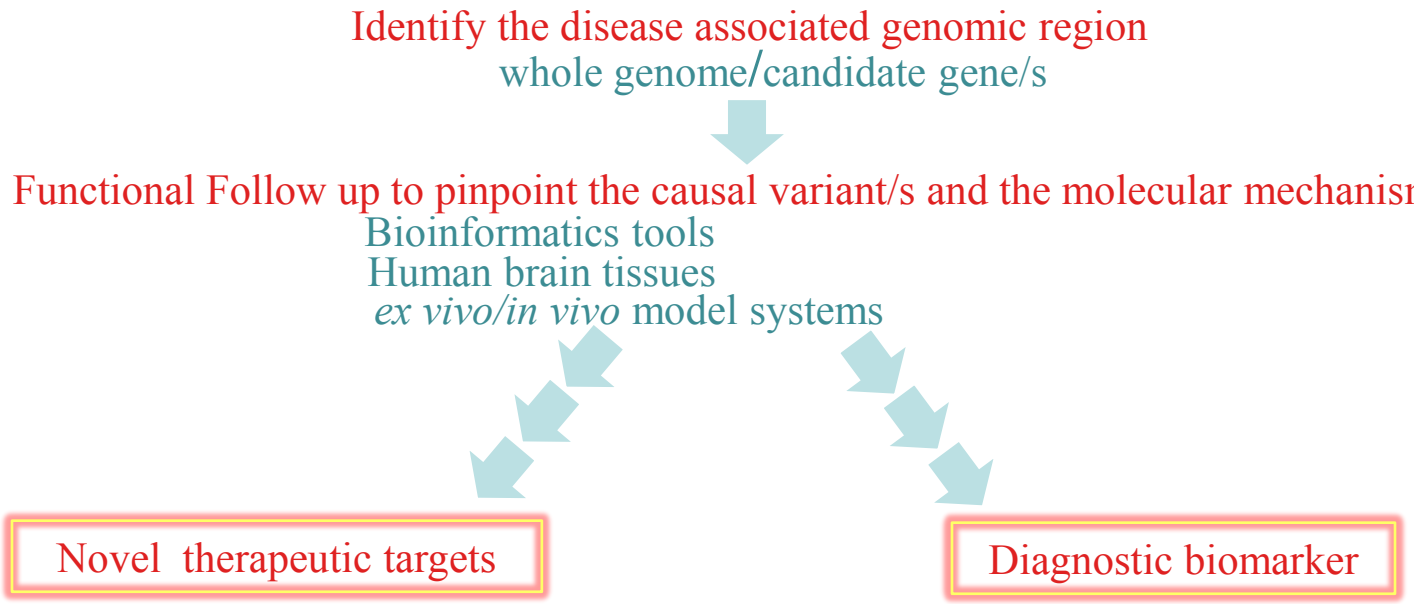
Dementia 2015 Conference
Aug 31st, 2015

The significance and functional consequences of genomic regions/genes associated with neurodegenerative diseases



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

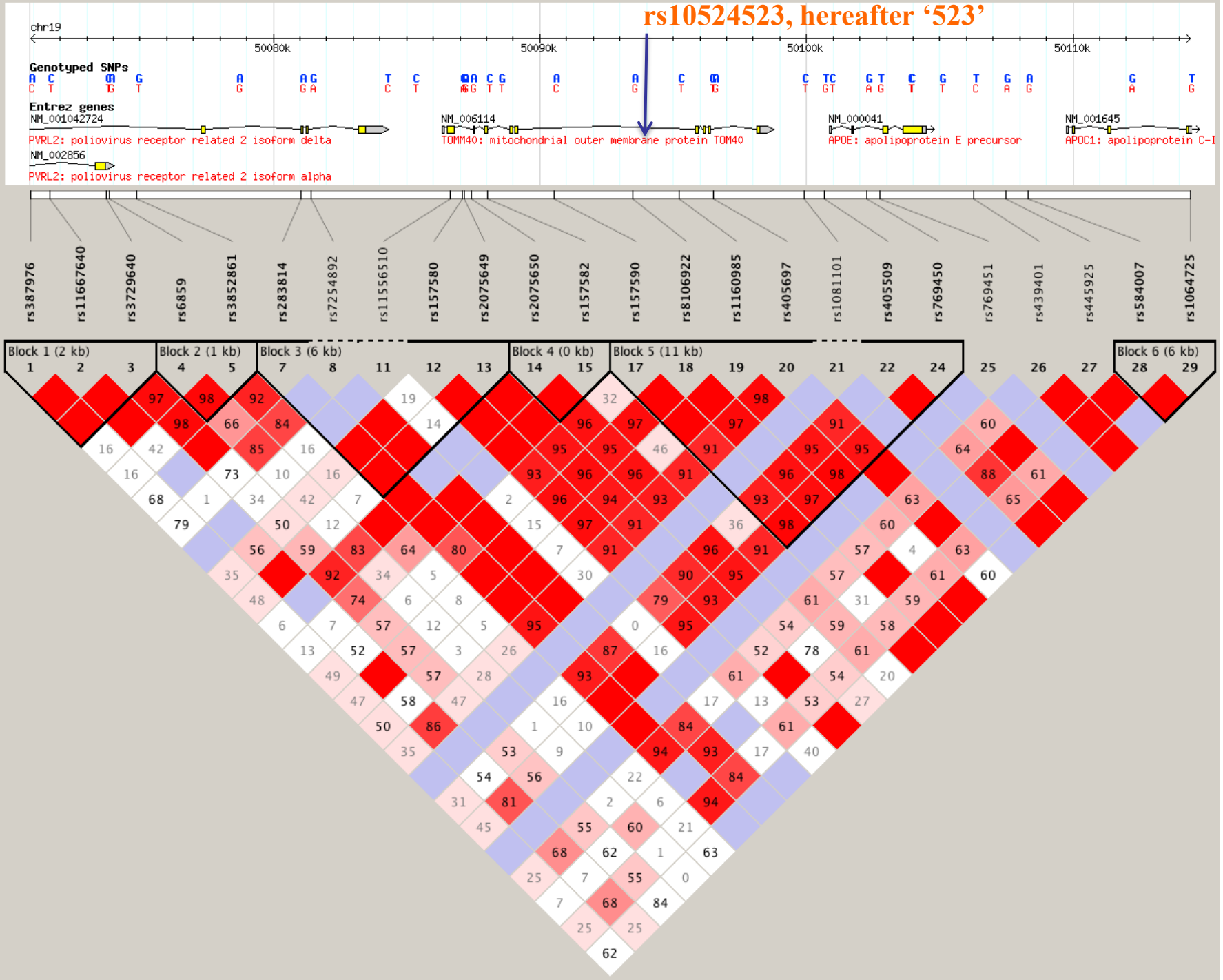
APOE-LD Region and AD-Association Studies

- **The ε4 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.**

- **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)


rs10524523, hereafter '523'
chr19
50080k
50090k
50100k
50110k
Genotyped SNPs
Entrez genes
NM_001042724
PVRL2: poliovirus receptor related 2 isoform delta
NM_002856
PVRL2: poliovirus receptor related 2 isoform alpha
NM_006114
TOMM40: mitochondrial outer membrane protein TOM40
NM_000041
APOE: apolipoprotein E precursor
NM_001645
APOC1: apolipoprotein C-I
rs387976
rs11667640
rs3729640
rs6859
rs3852861
rs283814
rs7254892
rs11556510
rs157580
rs2075649
rs2075650
rs157582
rs157590
rs8106922
rs1160985
rs405697
rs1081101
rs405509
rs769450
rs769451
rs439401
rs445925
rs584007
rs1064725
Block 1 (2 kb)
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Block 3 (6 kb)
Block 4 (0 kb)
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Block 6 (6 kb)

The Sequence of the *TOMM40*-‘523’ Alleles

Poly T

‘S’ Short T≤19

‘L’ Long T=20-29

‘VL’ Very Long T≥30

'523' Allele frequencies in Different Ethnicities

Table 1. ***'523' Allele frequencies in different ethnicities in the US***

Ethnicity	Subjects(N)	S (%)	L(%)	VL(%)	Poly T length (range)
Whites	177	45	11	44	14-39
African American	370	65	10	25	14-54
Hispanic	179	43	9	48	14-39

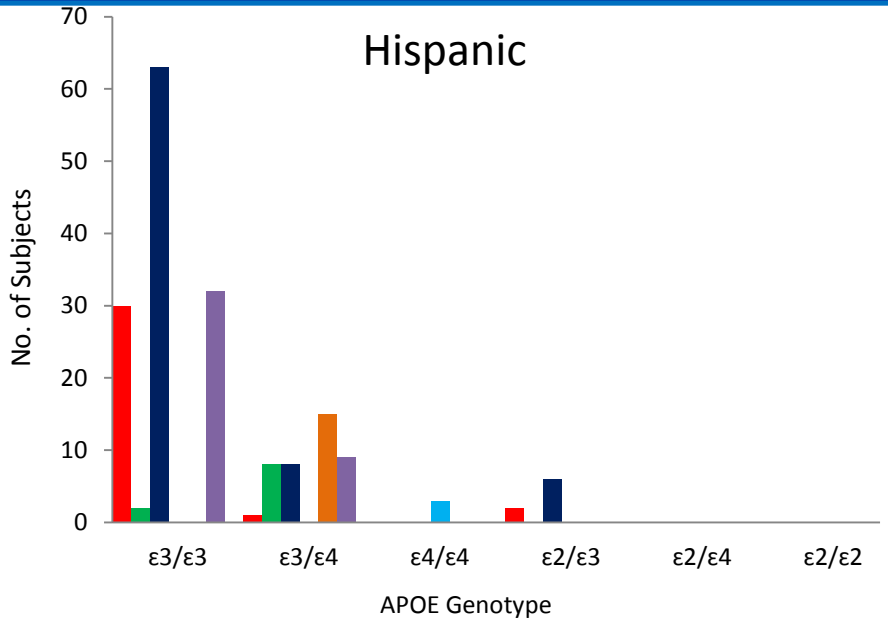
Table 2. ***'523' Allele frequencies in non US geographical cohorts (Far Eastern and West Africa)***

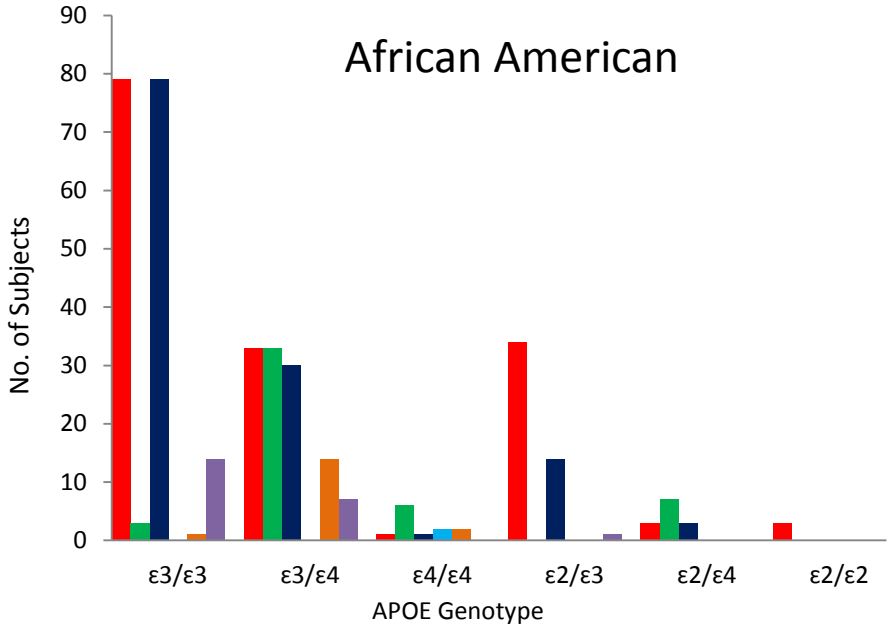
Ethnicity	Subjects(N)	S (%)	L(%)	VL(%)	Poly T length (range)
Ghanaian	40	71	8	21	13-43
Japanese	60	24	18	58	11-35
Korean	60	20	8	72	11-38
Han Chinese	60	38	10	52	12-36

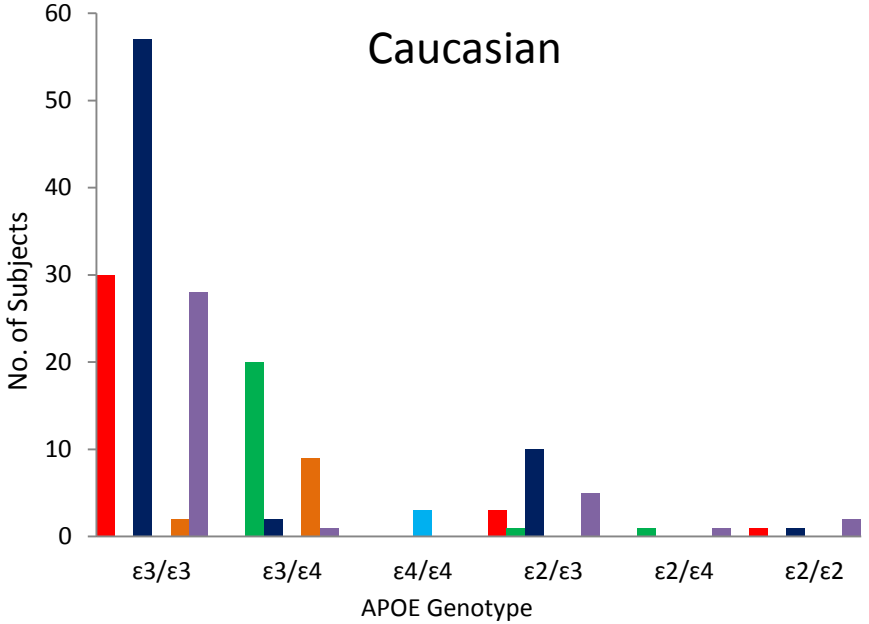
*Genotypes determination was performed by Polymorphic, Inc. using a sequencing based assay.

Linnertz *et al.* (2012)

Linkage Pattern: *TOMM40*-'523' and *APOE* Alleles





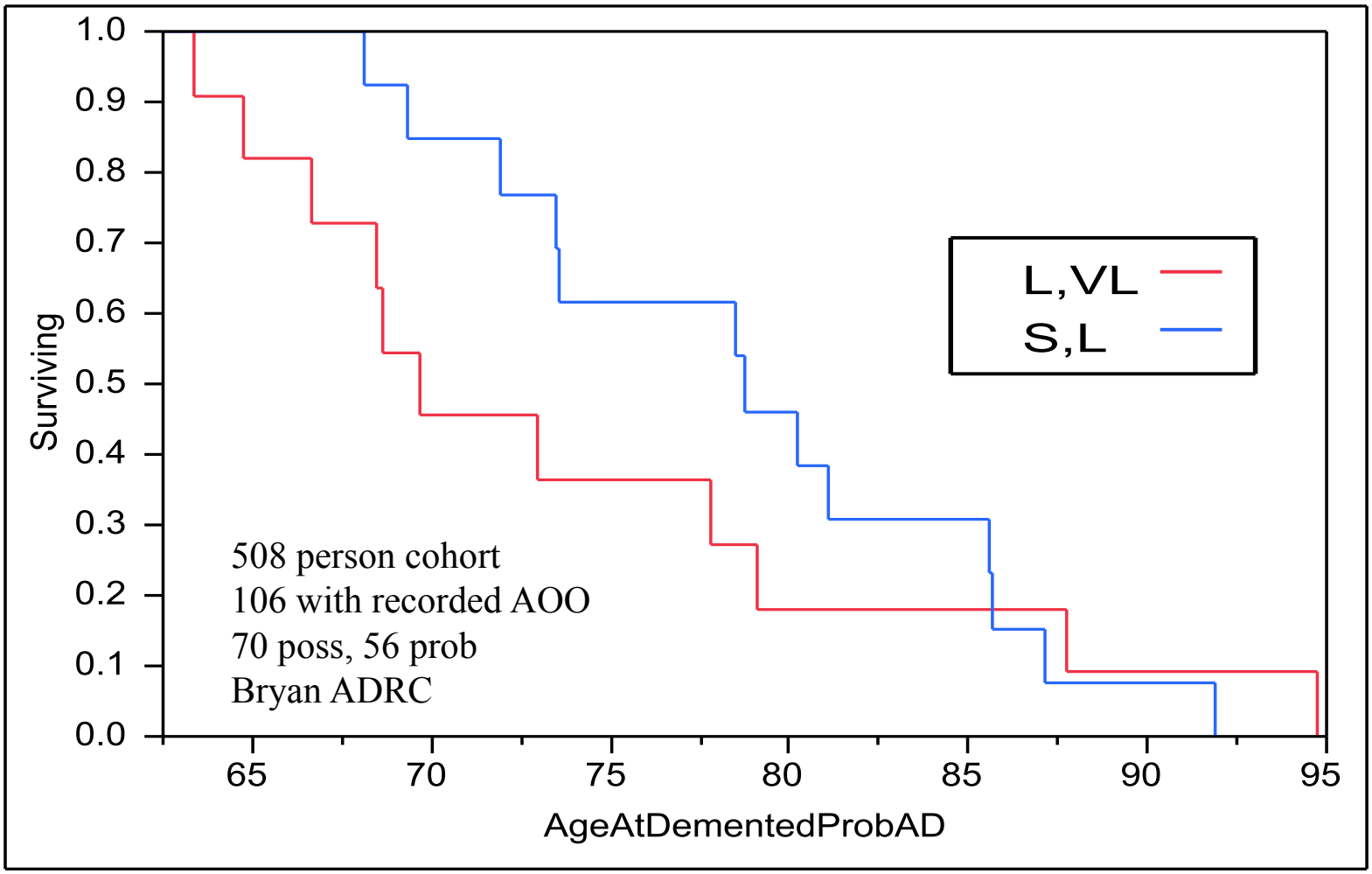


Linkage Pattern: *TOMM40*-'523' and *APOE* Alleles

- ✓ **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)

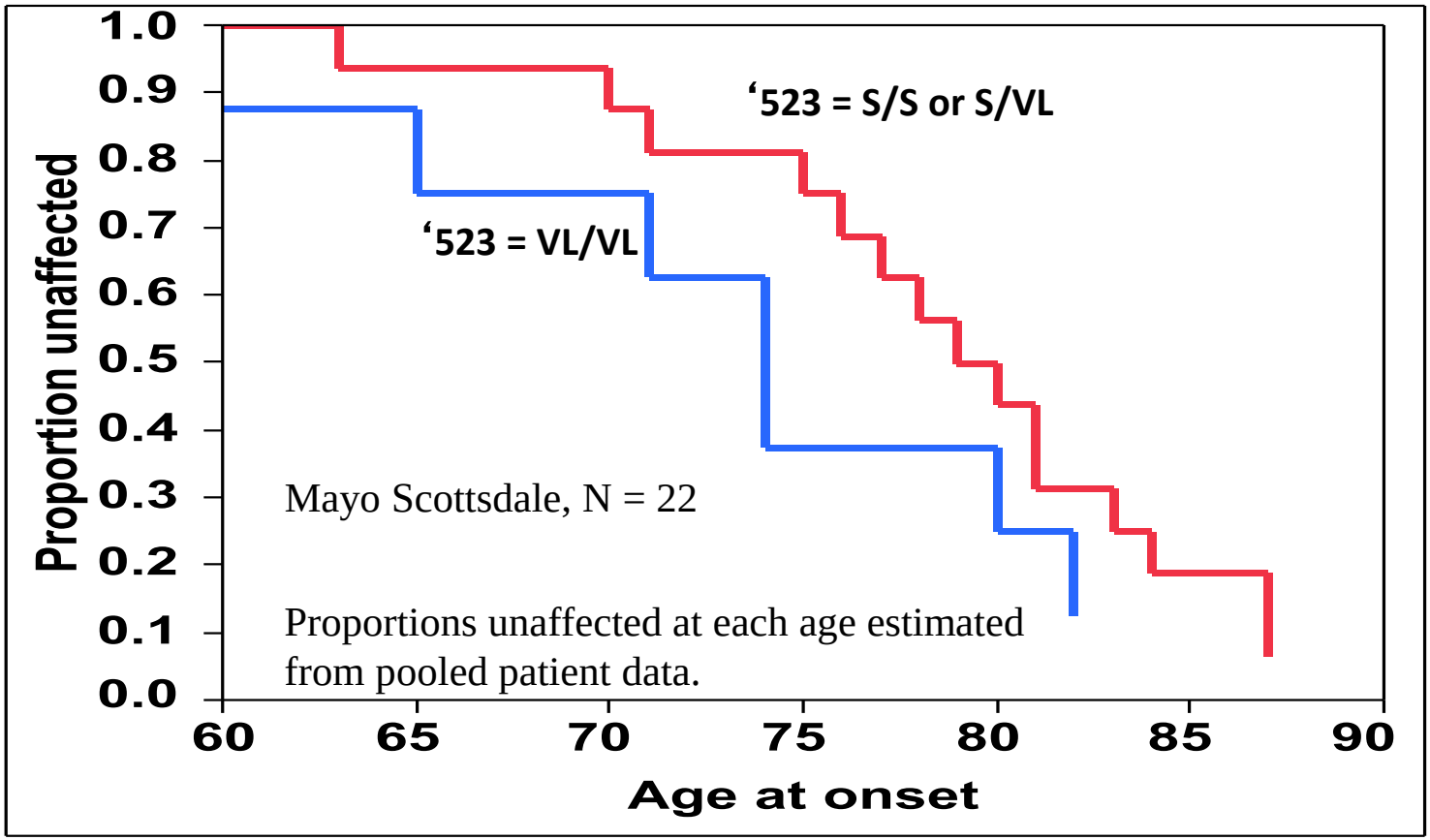
AOO for Poss/Prob AD – APOEε3/4



Roses *et al.* (2009)

AOO for AD – APOEε3/3

Prospective Arizona Cohort



Caselli *et al.* (2010)

The Genetic of Cognitive Changes in Elderly

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

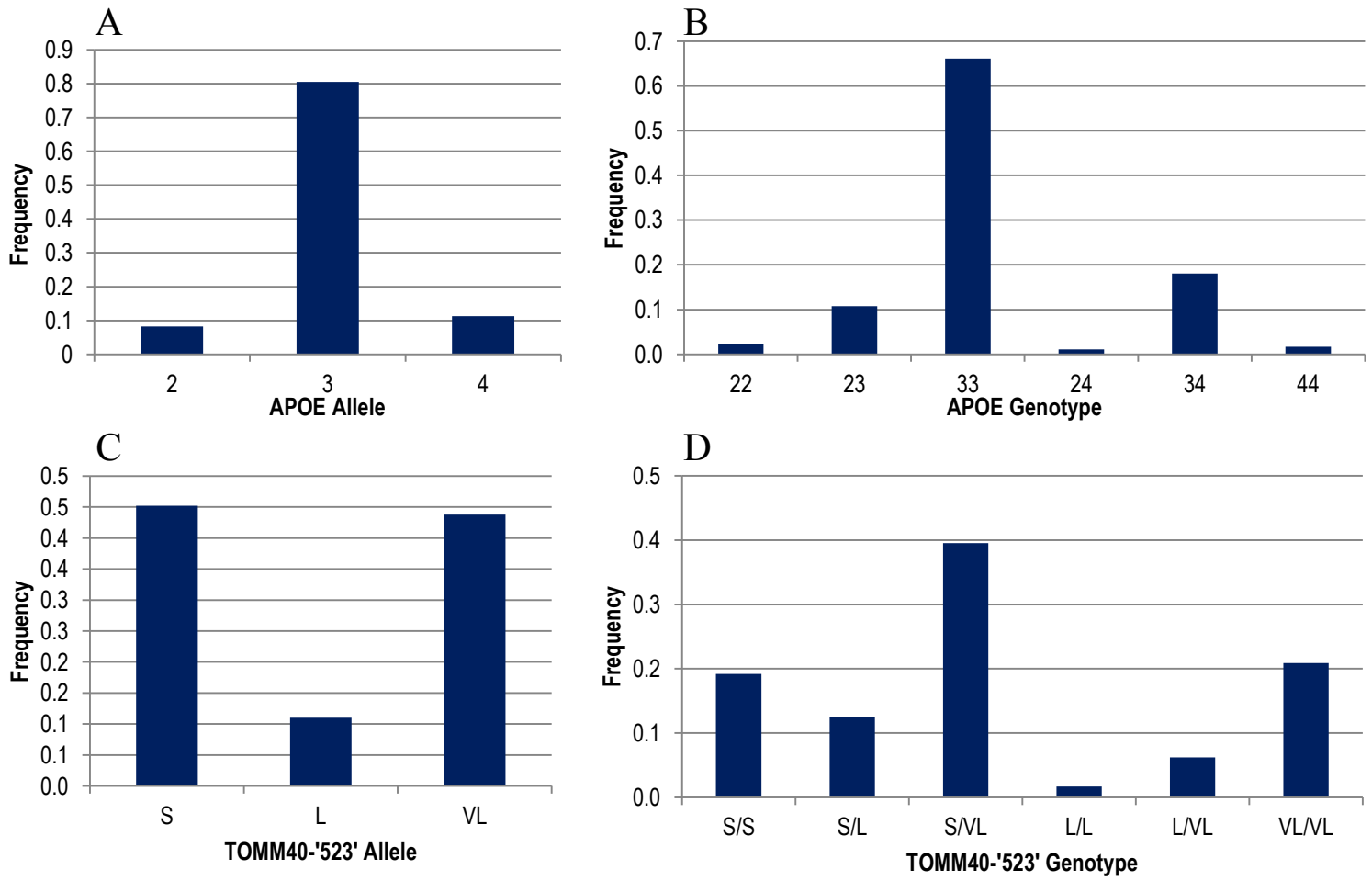
Table 1. Demographic Characteristics n=127

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

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 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.

Cognitive Domain Test	***p*-value**
Memory	
PAL Mean Errors to Success	**0.0204**
PAL Mean Trials to Success	**0.0163**
VRM Free Recall Items Correct	**0.0325**
Attention	
RVP Latency	**0.0475**
Executive	
Digit Data	**0.0349**
IED Ratio Errors/Trials	0.0771
IED Total Errors	0.0905

*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

Hayden *et al.* (2012)

Association of Gene Expression with Cognitive Performance

Blood PaxGene RNA (N=66)

Cognitive Domain Test	***APOE-mRNA*** ***p*-value**	***APOC1-mRNA*** ***p*-value**
General Screening		
MoCA	0.06	0.01
Attention		
RVP Latency	0.01	--
Executive		
Digit Data	0.009	0.03

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

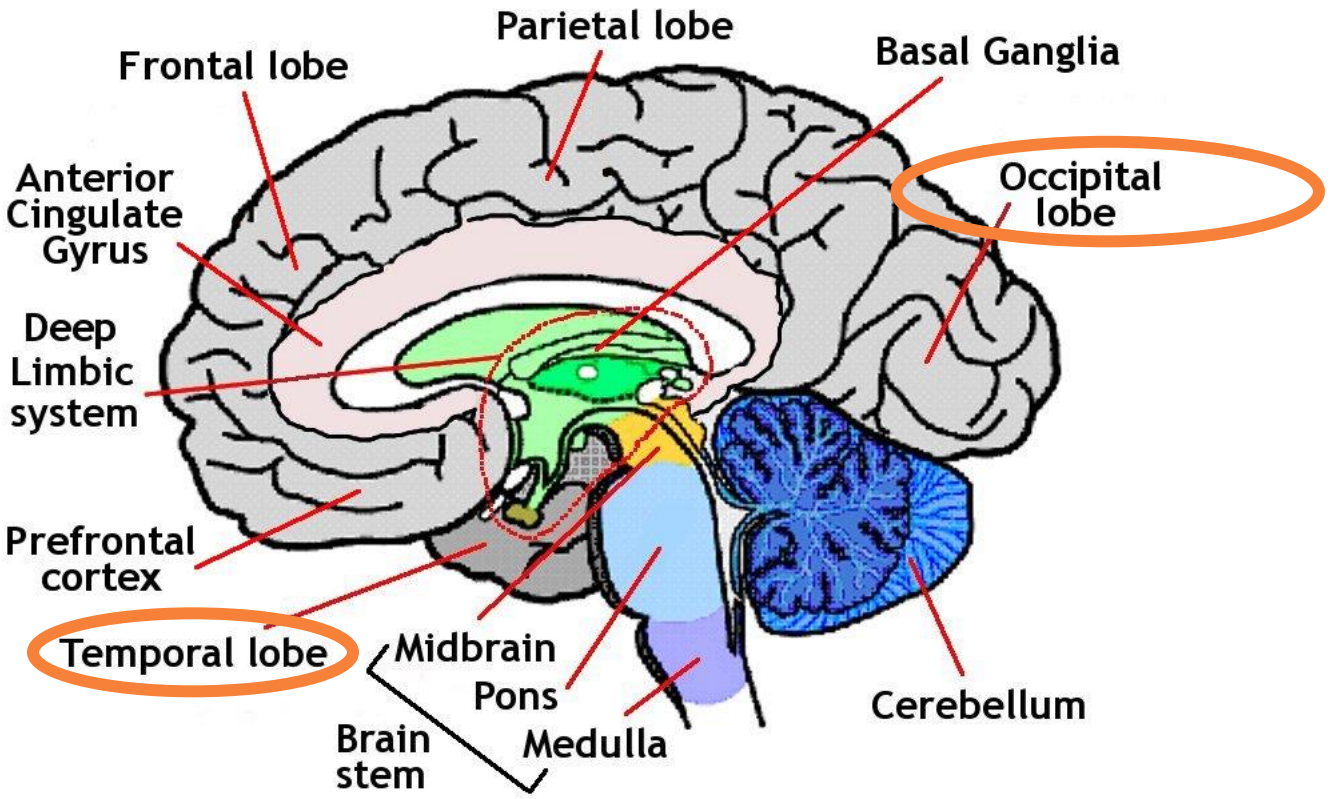
Literature Support

- **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



Brain Sample: Demographic Description

Table 1. Demographic description of the brain samples

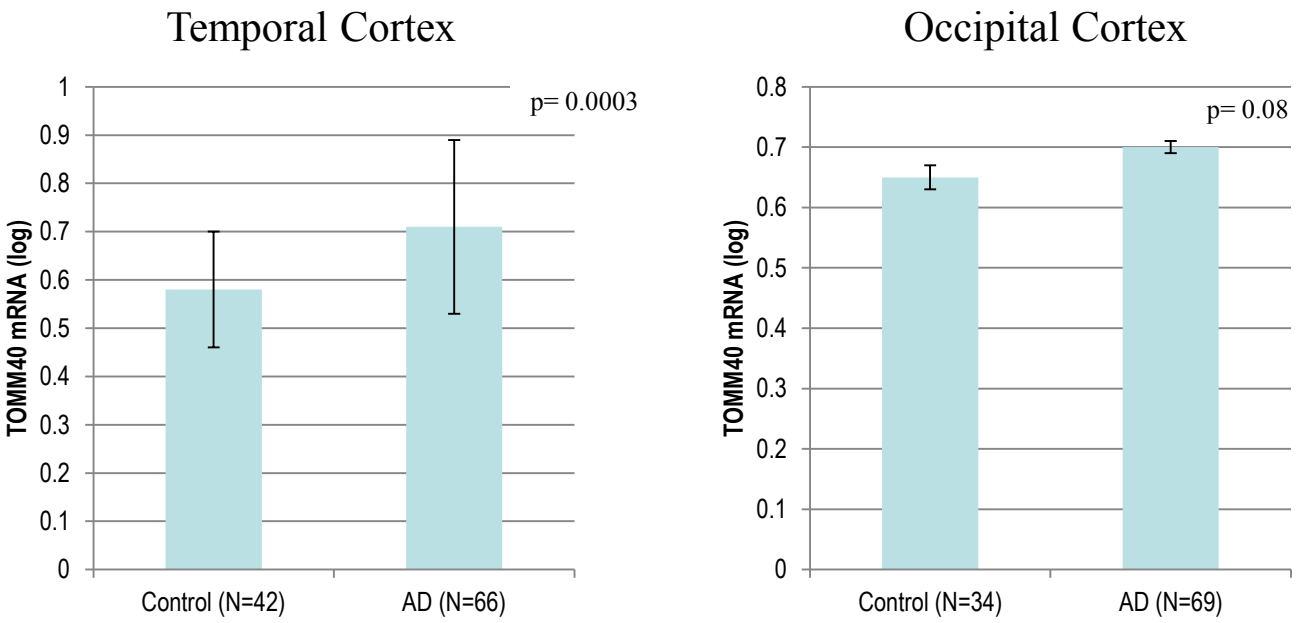
	LOAD	Normal
Total subjects (N)	69	42
†TC (N)	66	42
‡OCC (N)	69	34
Male %	40	50
Age (yr) mean±SD	76.9±13.3	78.2±15.1
§PMI (hr) mean±SD	12.3±12.1	11.4±7.7
Caucasians %	100	100

†TC- temporal cortex, ‡OCC- occipital cortex, §PMI- post mortem interval

Linnertz *et al.* (2014)

Normal & Disease Brains: *TOMM40*-mRNA Expression

Caucasians *APOE* ε3/ε3

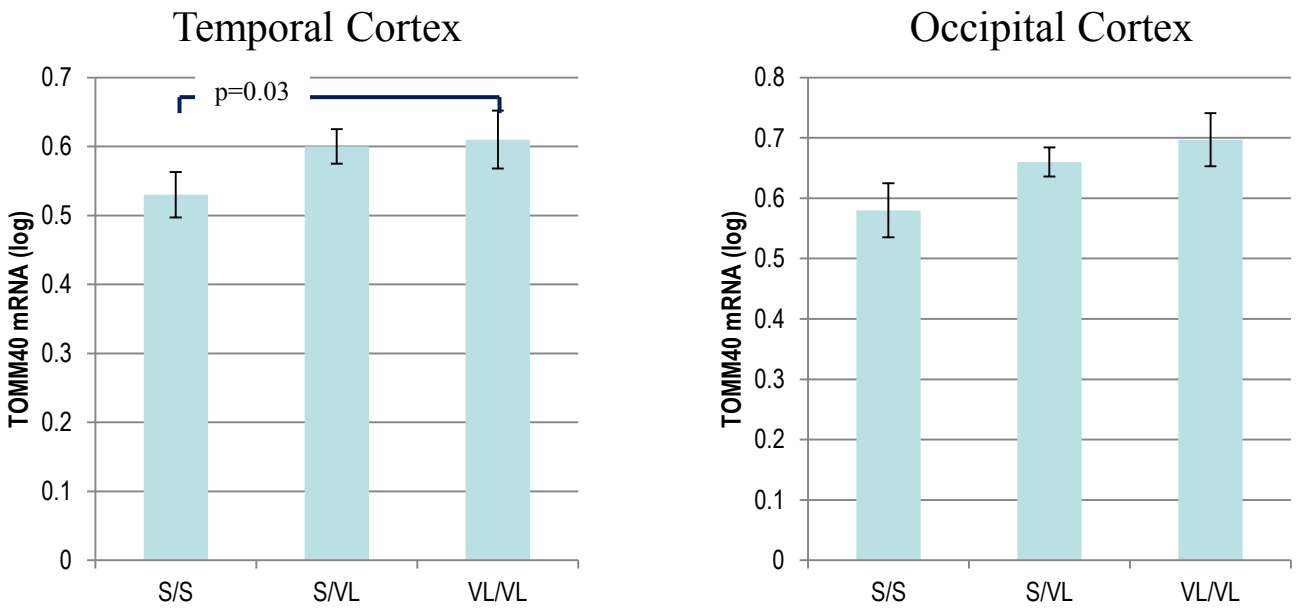


Means± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz *et al.* (2014)

Normal Brains: *TOMM40*-mRNA Expression

Caucasians *APOE* ε3/ε3

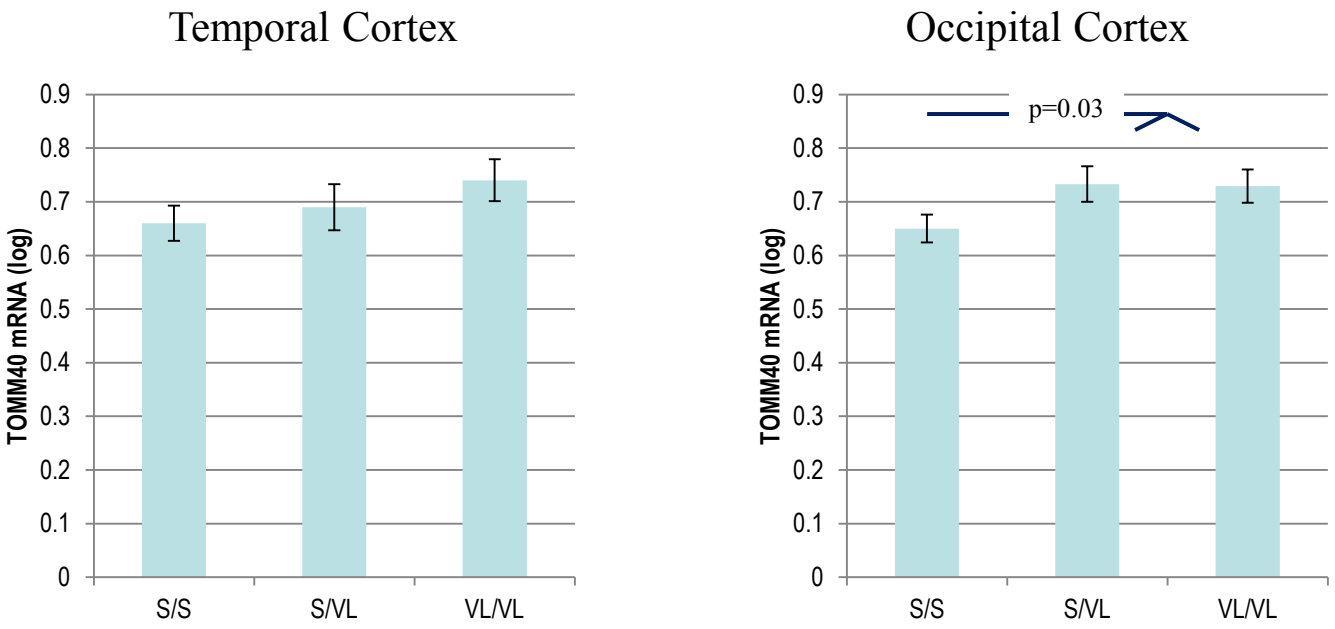


Means± SE corrected for: sex, age, PMI

Linnertz *et al.* (2014)

AD Disease Brains: *TOMM40*-mRNA Expression

Caucasians *APOE* ε3/ε3

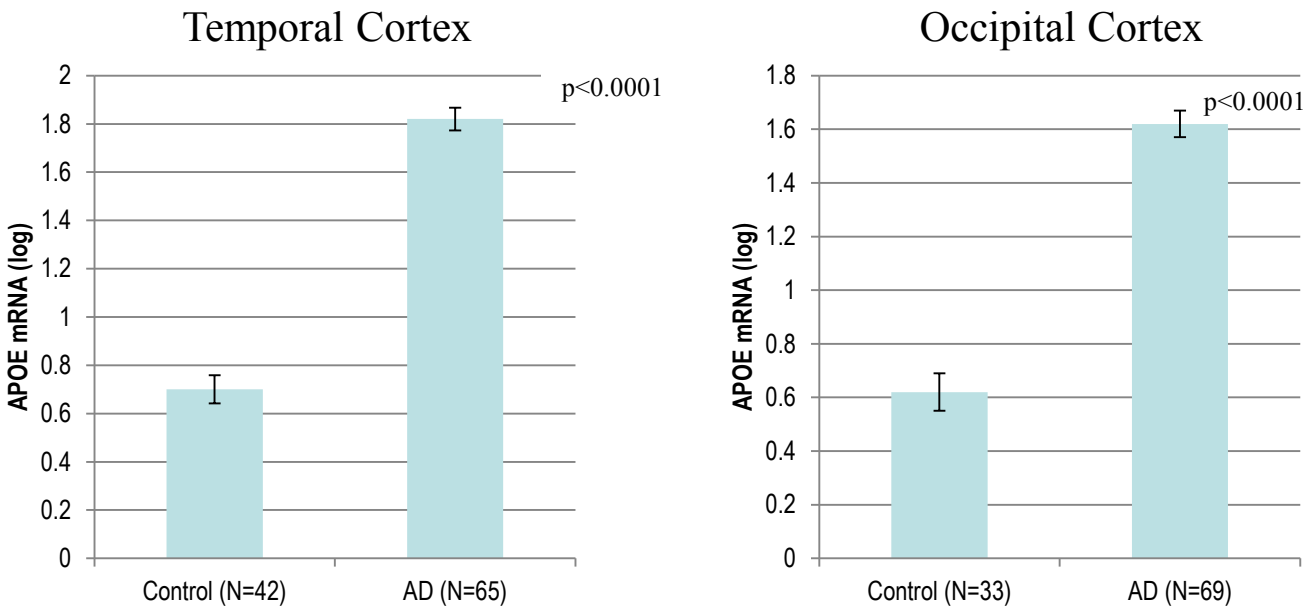


Means± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz *et al.* (2014)

Normal & Disease Brains: *APOE*-mRNA Expression

Caucasians *APOE* ε3/ε3

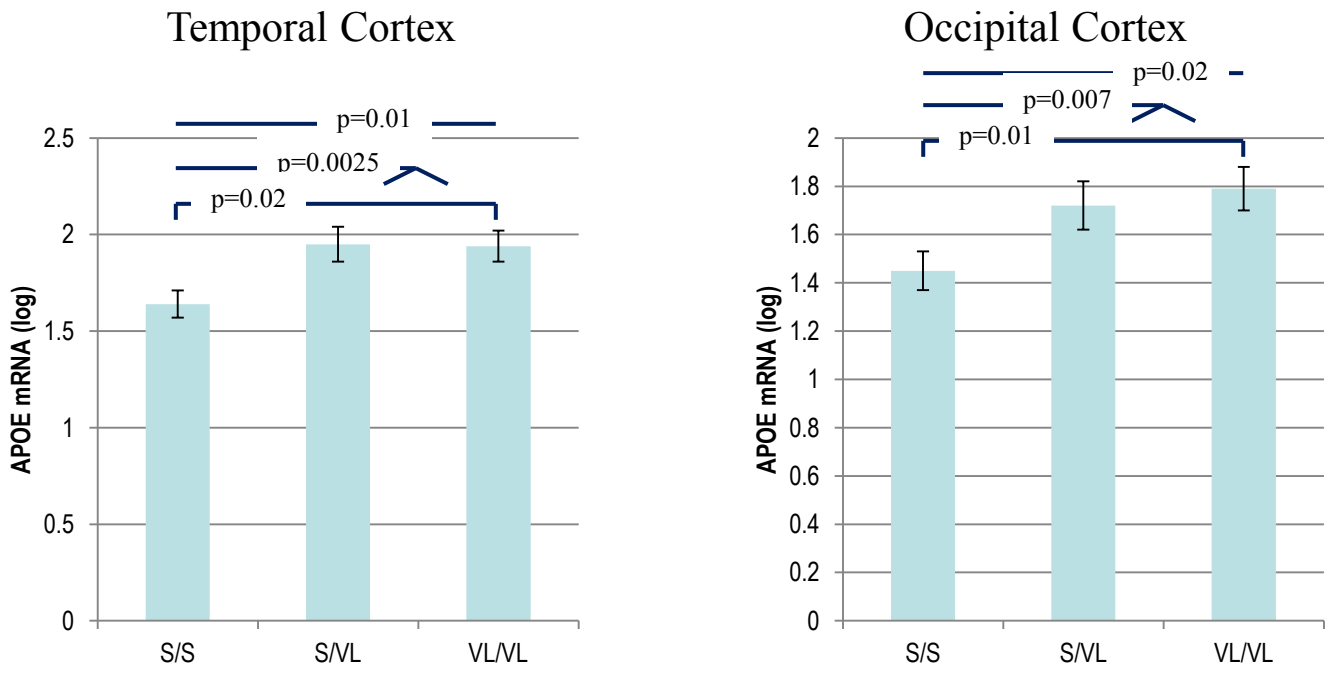


Means± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz *et al.* (2014)

AD Disease Brains: *APOE*-mRNA Expression

Caucasians *APOE* ε3/ε3



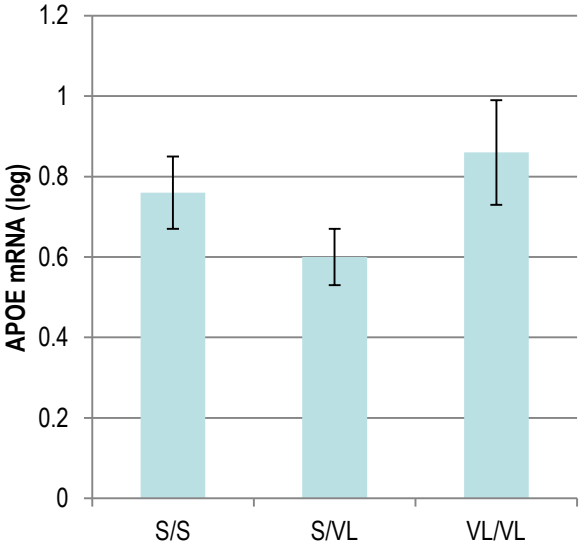
Means± SE corrected for: sex, age, PMI, Braak&Braak stage

Linnertz *et al.* (2014)

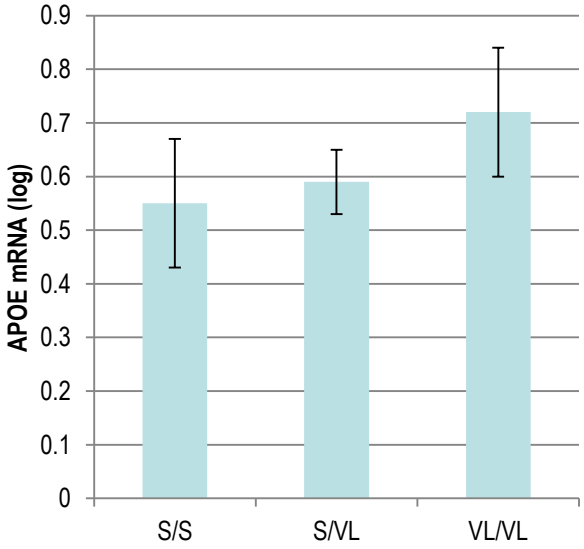
Normal Brains: *APOE*-mRNA Expression

Caucasians *APOE* ε3/ε3

Temporal Cortex



Occipital Cortex



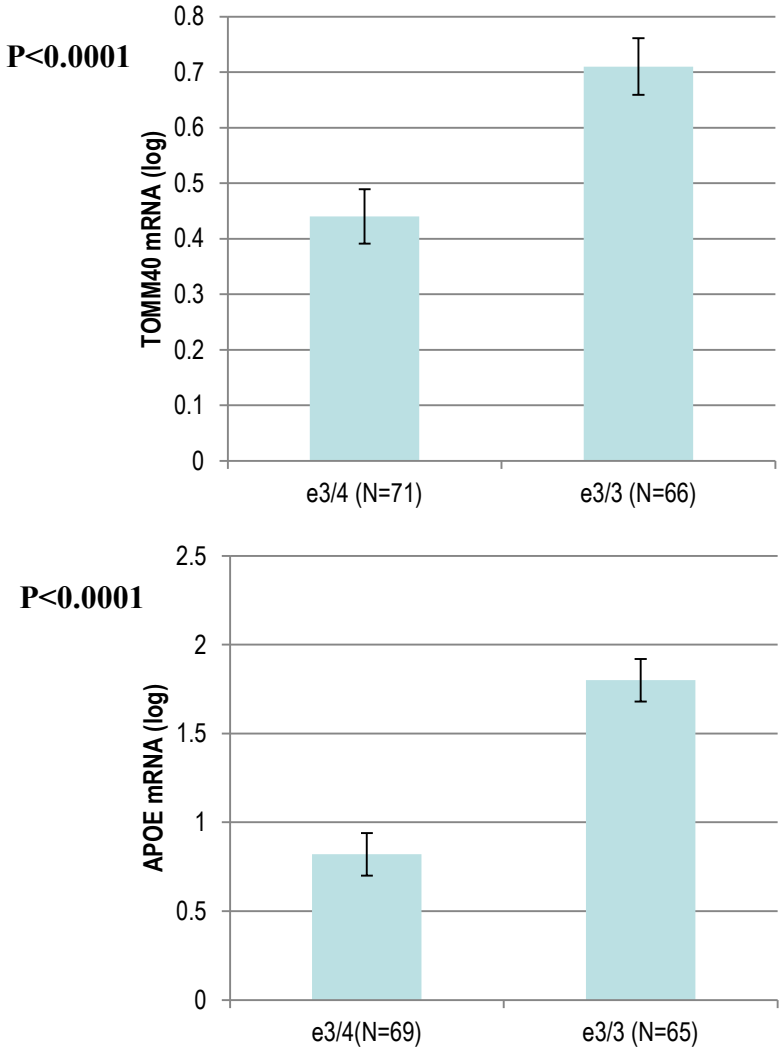
Means± SE corrected for: sex, age, PMI

Linnertz *et al.* (2014)

AD Disease Brains: mRNA Expression

Caucasians *APOE* ε3/ε3 vs. ε3/ε4

Temporal Cortex



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

AD Disease Brains: mRNA Expression

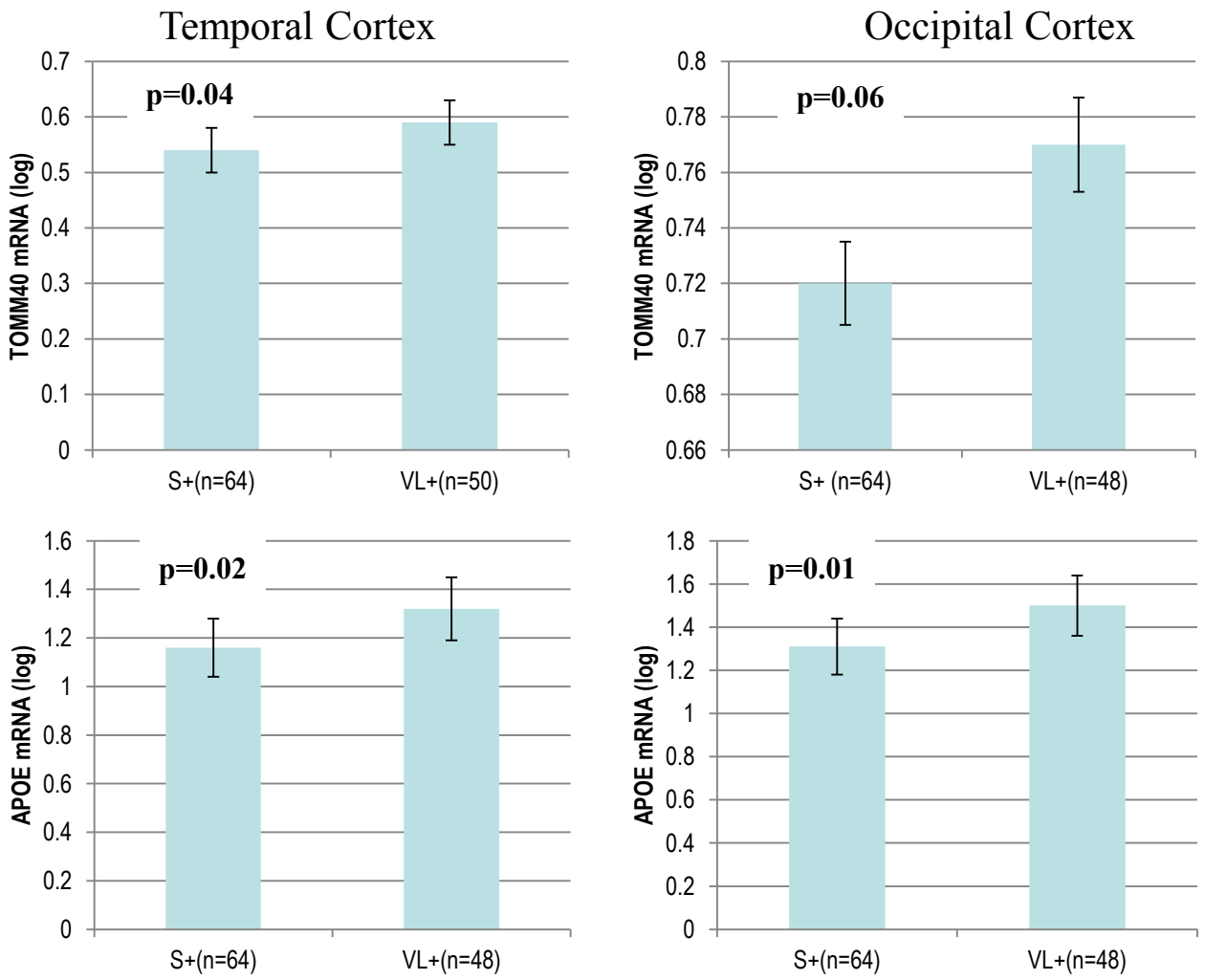
Caucasians *APOE* ε3/ε4

***TOMM*-mRNA and *APOE*-mRNA**

S/L<L/VL n.s.

AD Disease Brains: mRNA Expression

Caucasians *APOE* ε3/ε3 and ε3/ε4



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

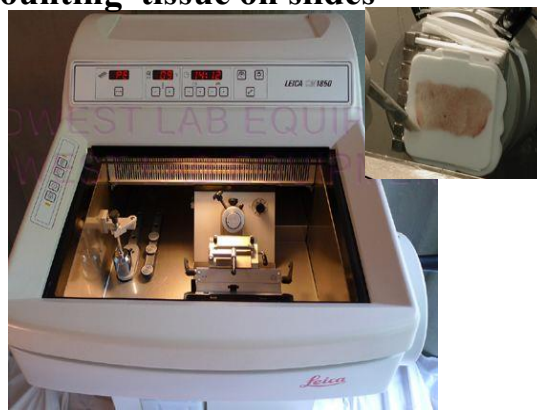
<u>Single cell-type:</u>

- **Neurons**
- **Astrocyte**
- **Microglia**

Laser Capture Microdissection (LCM)

Frozen Brain

Cryostat Embedding, Sectioning & Mounting tissue on slides

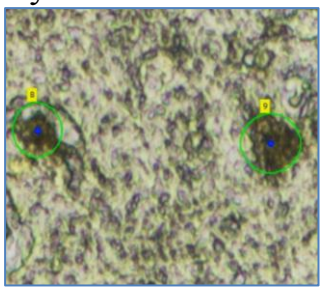
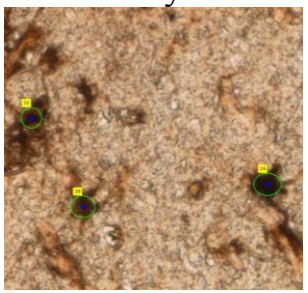
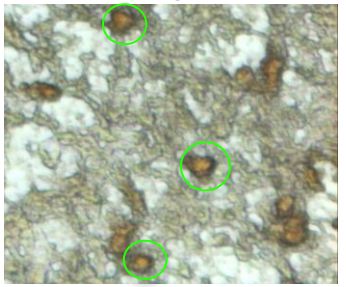
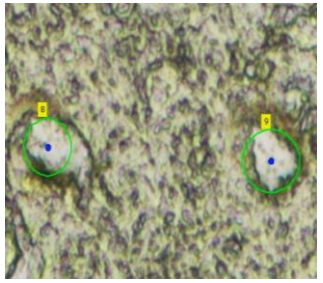
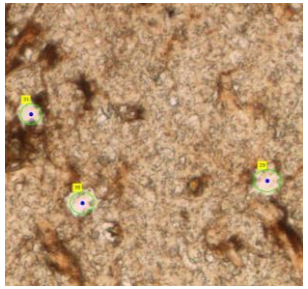
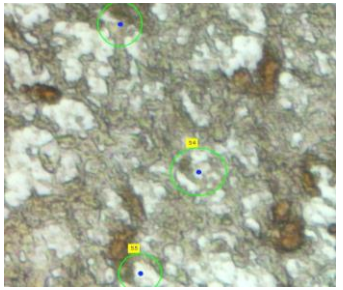


Staining

SMI-32
GFAP
Iba1

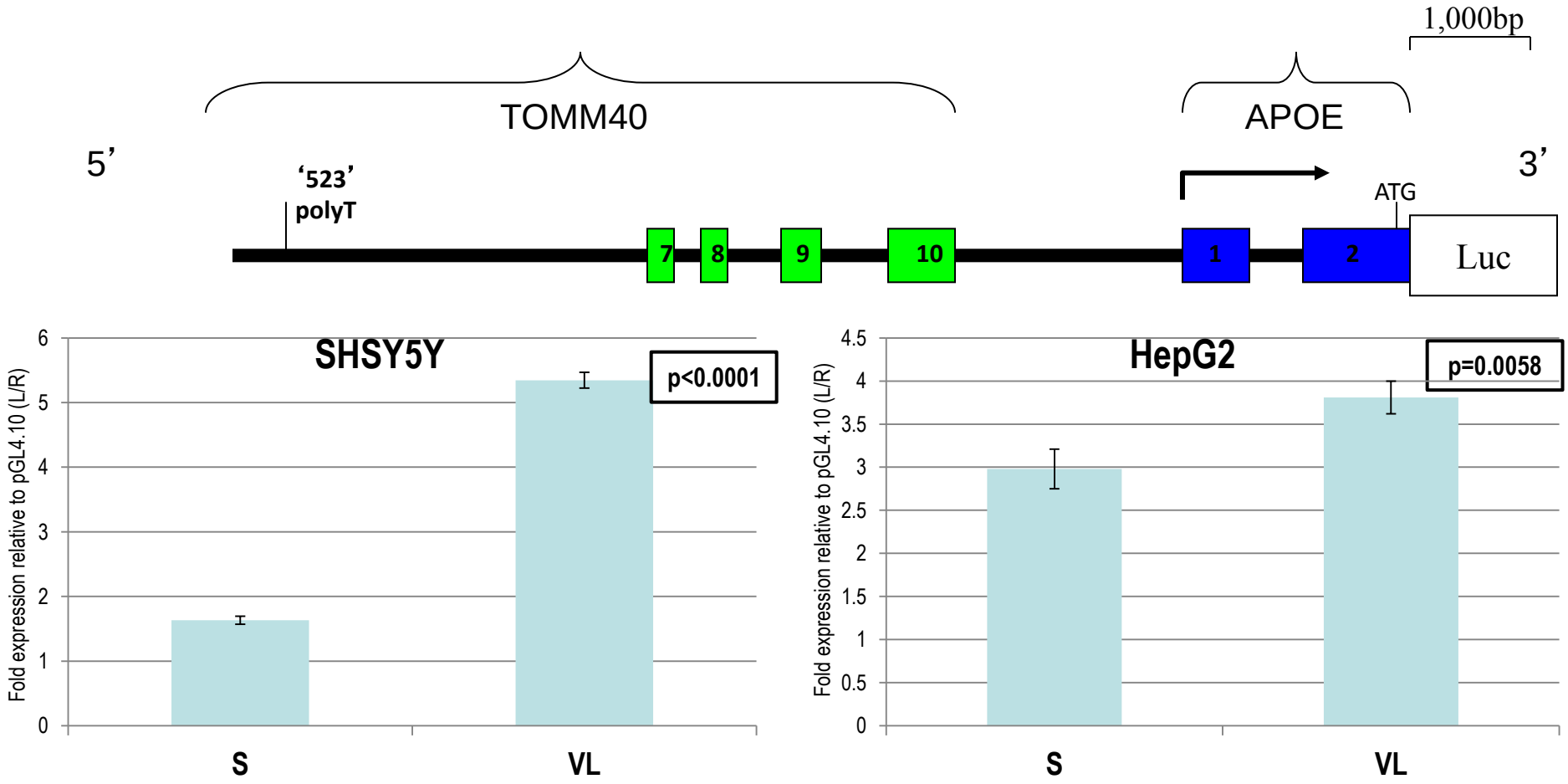
ZEISS Palm System

Selecting, cutting & collecting cells

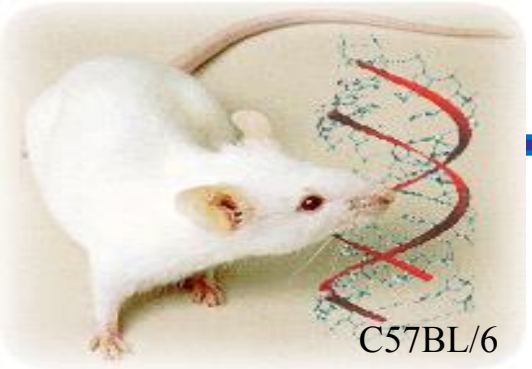
Pyramidal Neurons	Astrocytes	Microglias	
			before
			after

RNA Extraction → RNA Amplification → nCounter single-cells expression assay (NanoString)

Luciferase Reporter Assay: '523' polyT Alleles



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

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