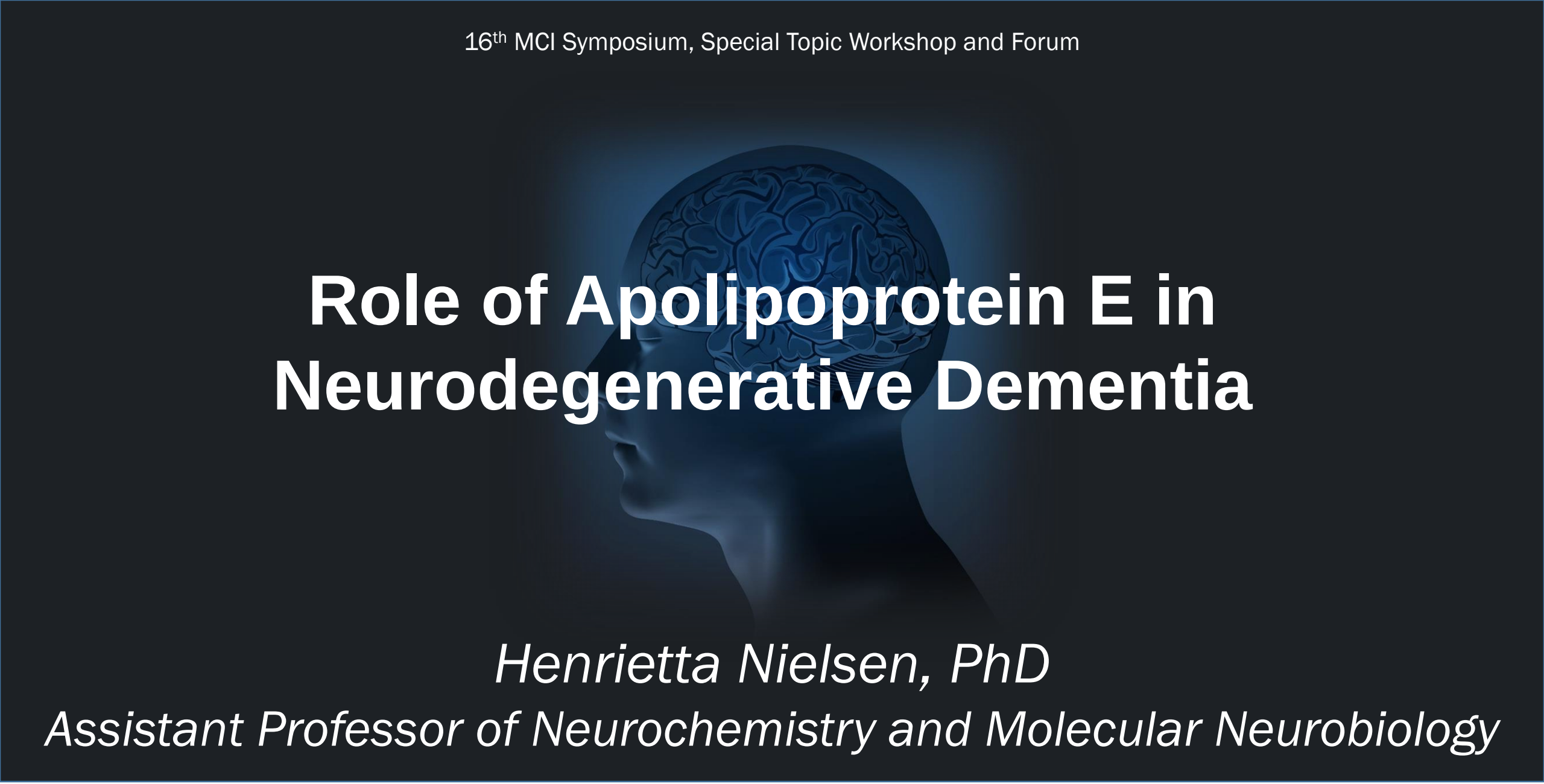

Role of Apolipoprotein E in Neurodegenerative Dementia

Henrietta Nielsen, PhD

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DISCLOSURES

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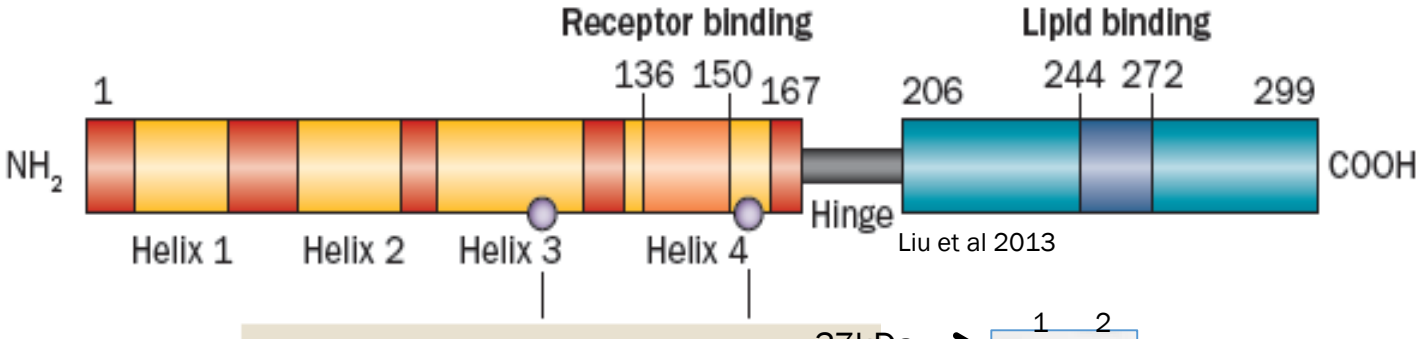
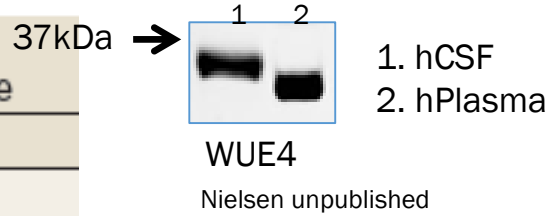
- Alzheimer’s Association New Investigator Award (US)
- Demensfonden (Sweden)
- Åhlen-Foundation (Sweden)
- Marcus Borgström’s Foundation (Sweden)

Apolipoprotein E in humans



Liu et al 2013

	Isoform-specific amino acid difference	
	112	158
Apo-E2	Cys	Cys
Apo-E3	Cys	Arg
Apo-E4	Arg	Arg



Nielsen unpublished

- The *APOE* gene in humans exists in three variants; ε2, ε3, ε4
- 299 amino acids, ≈34kDa
- Important lipid carrier (apoE2>apoE3>apoE4)
- Systemic apoE mainly produced by hepatocytes
- CNS apoE mainly produced by astrocytes
- Does <u>not</u> cross the BBB
- Is a ligand for several receptors of the LDLR-family

APOE and risk of neurodegenerative disease

Stockholm University

APOE allele frequency	General population	Alzheimer's disease (AD)	Dementia with Lewy bodies (DLB)
ε2 (%)[1]	10.6	5.8	5.1
ε3 (%)[1]	75	51.3	62.8
ε4 (%)[1]	14.4	42.9	32.1
ε2 (%)[2]	8.4	ND	3.9
ε3 (%)[2]	77.9	ND	59.4
ε4 (%)[2]	13.7	ND	36.7

OR of disease	AD	DLB
ε2 homozygotes	0.5[1], 0.6[2]	0.4[1]
ε4 homozygotes	15.2[1], 14.9[2]	5.9[1]

1) Berge et al 2014, 2) Farrer et al 1997, ND) not determined

- *APOEε4* is the main genetic risk factor for Alzheimer's disease and dementia with Lewy bodies
- Dose-dependent increase of risk with 15-fold higher risk of getting Alzheimer's disease and 6-fold higher risk of developing dementia with Lewy bodies
- *APOEε2* appears to be protective against both disorders

***APOEε2*: protective**
***APOEε4*: risk factor**

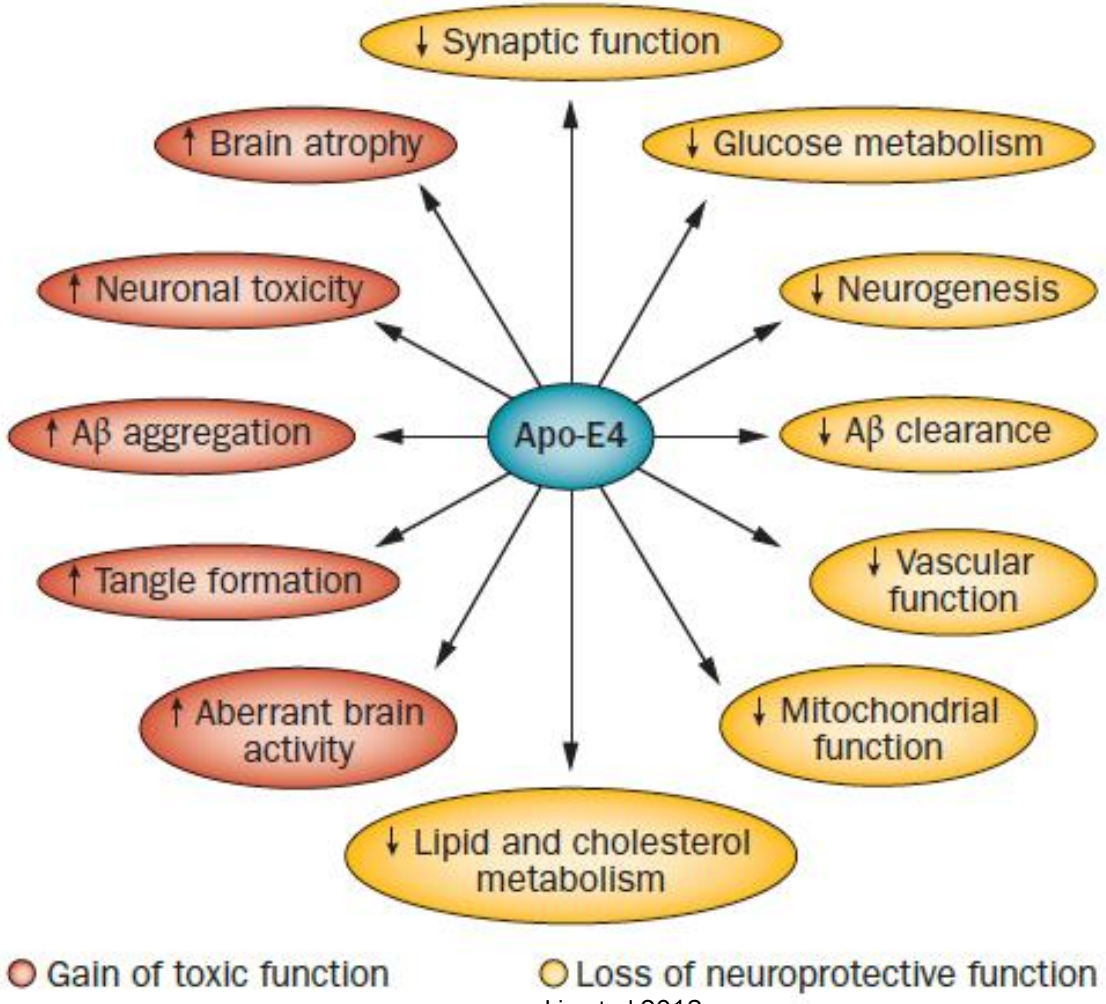
Molecular mechanisms linking apoE to Alzheimer's disease

Amyloid-beta dependent pathways

- Potently catalyze formation of amyloid filaments/fibrils
- Promote APP transcription and Aβ production
- Hamper cellular clearance of Aβ
- Interfere with Aβ clearance across the BBB
- Enhance neurotoxic effects of Aβ

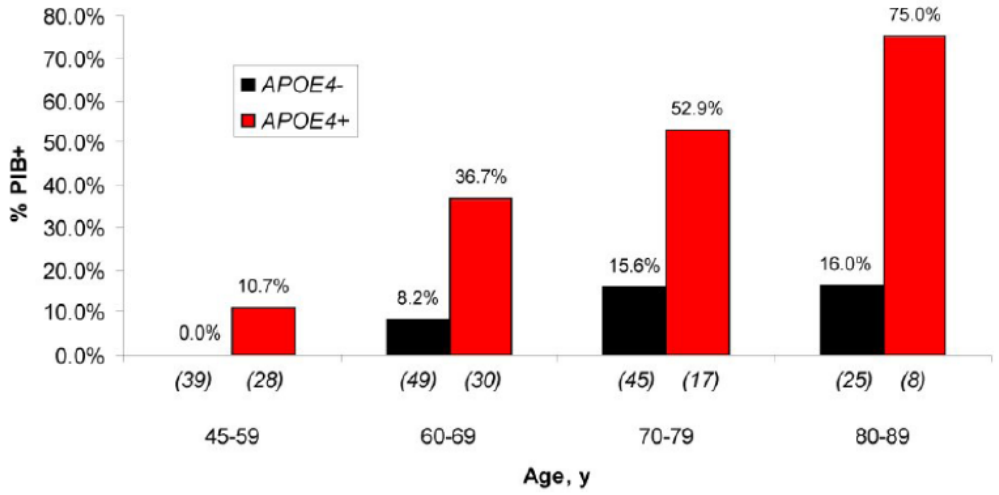
Amyloid-beta independent pathways

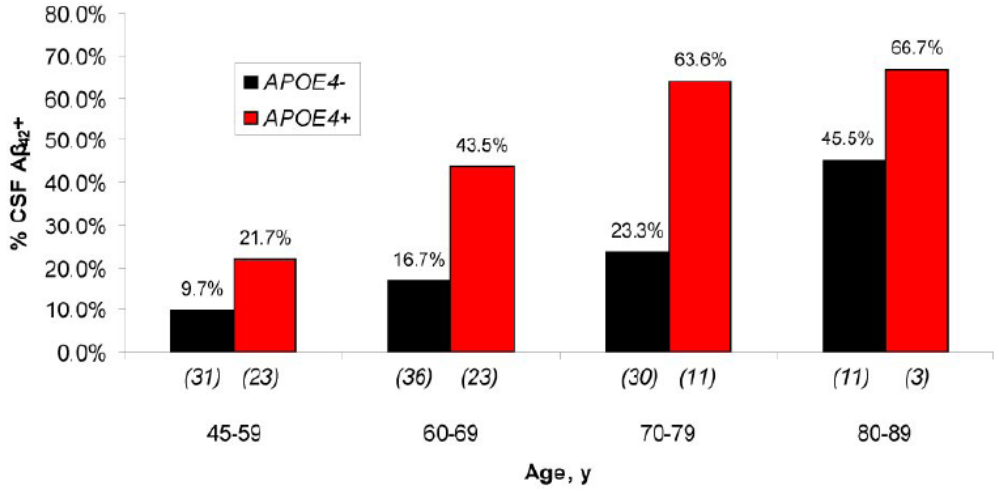
- Decrease neurite outgrowth
- Reduce spine density
- Induce tau pathology through effects of neurotoxic apoE fragments
- Promote neuroinflammation and the shift to neurotoxic phenotypes in microglia

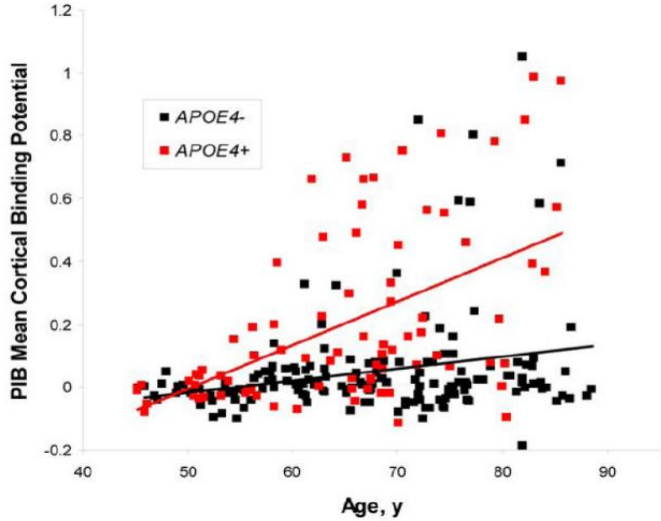


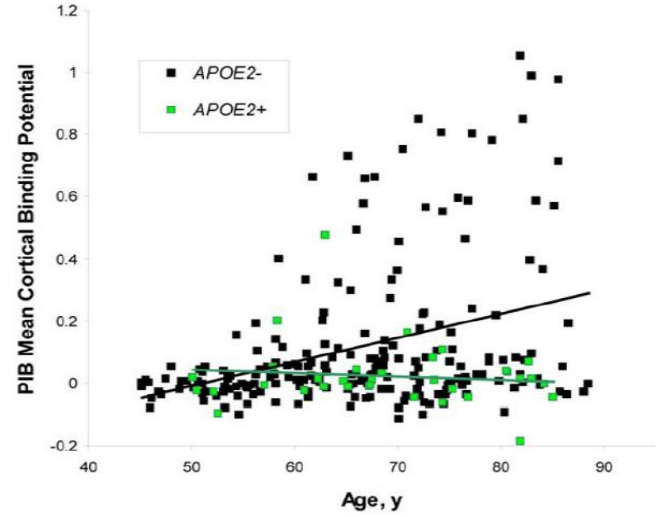
Liu et al 2013

APOEε4 promotes amyloid-deposition at an early age



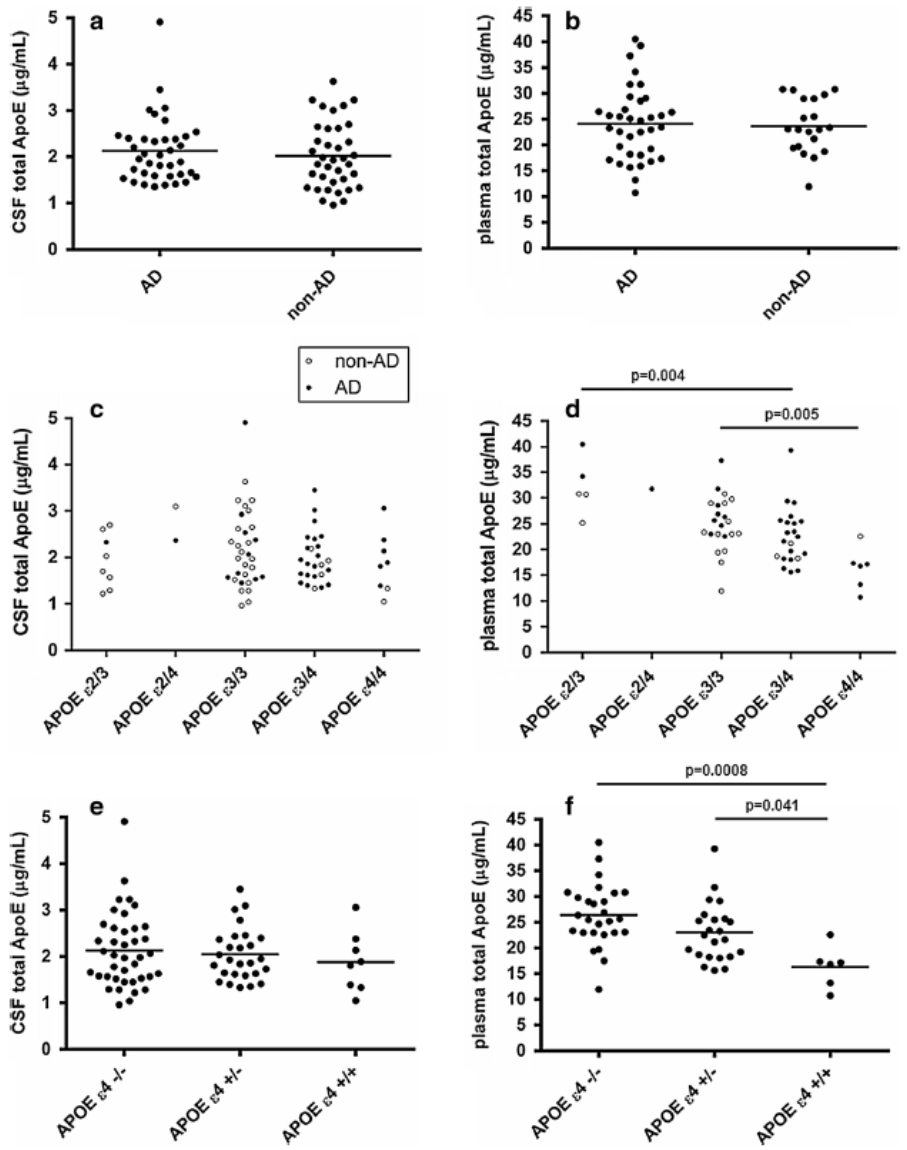


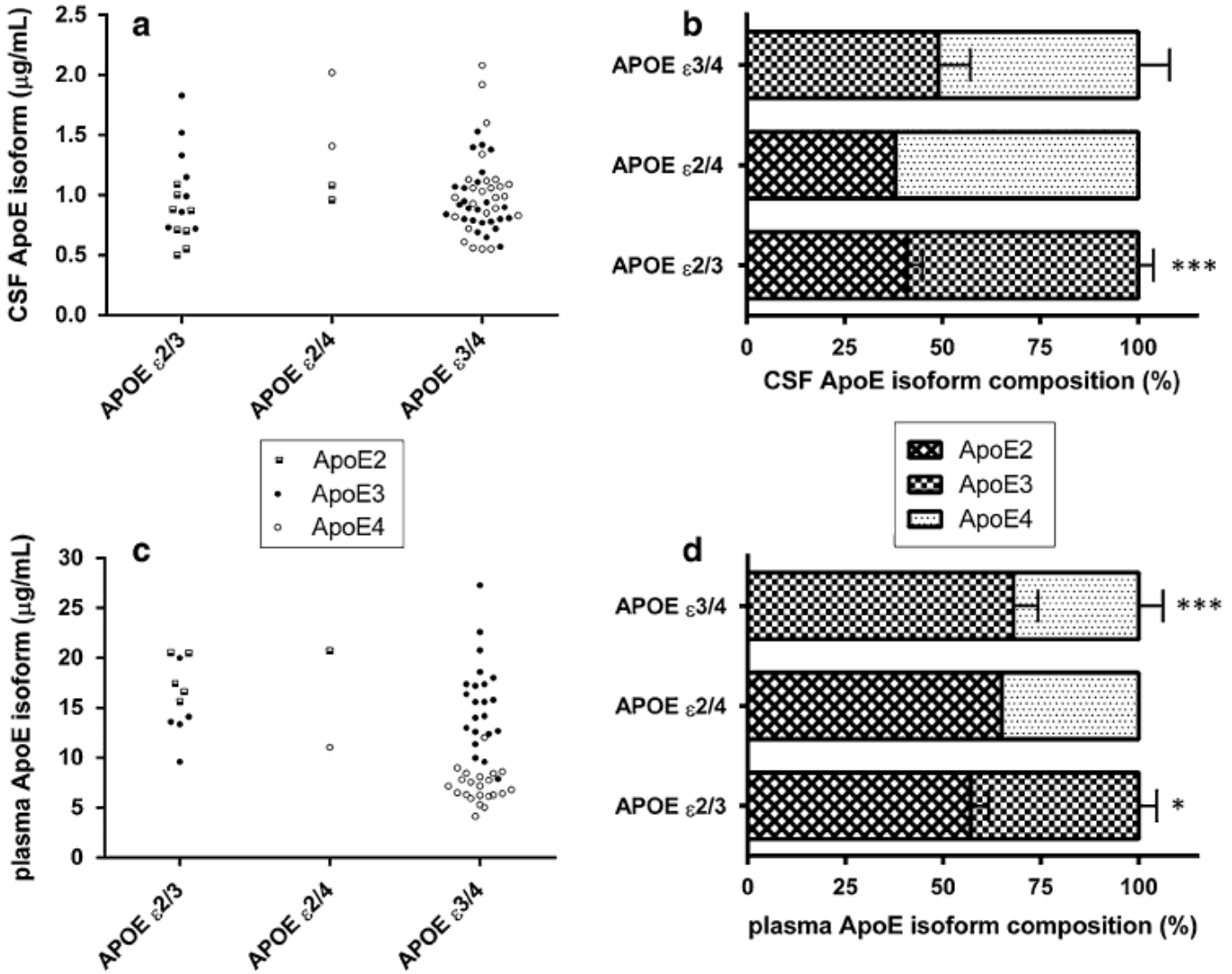




Morris et al 2010

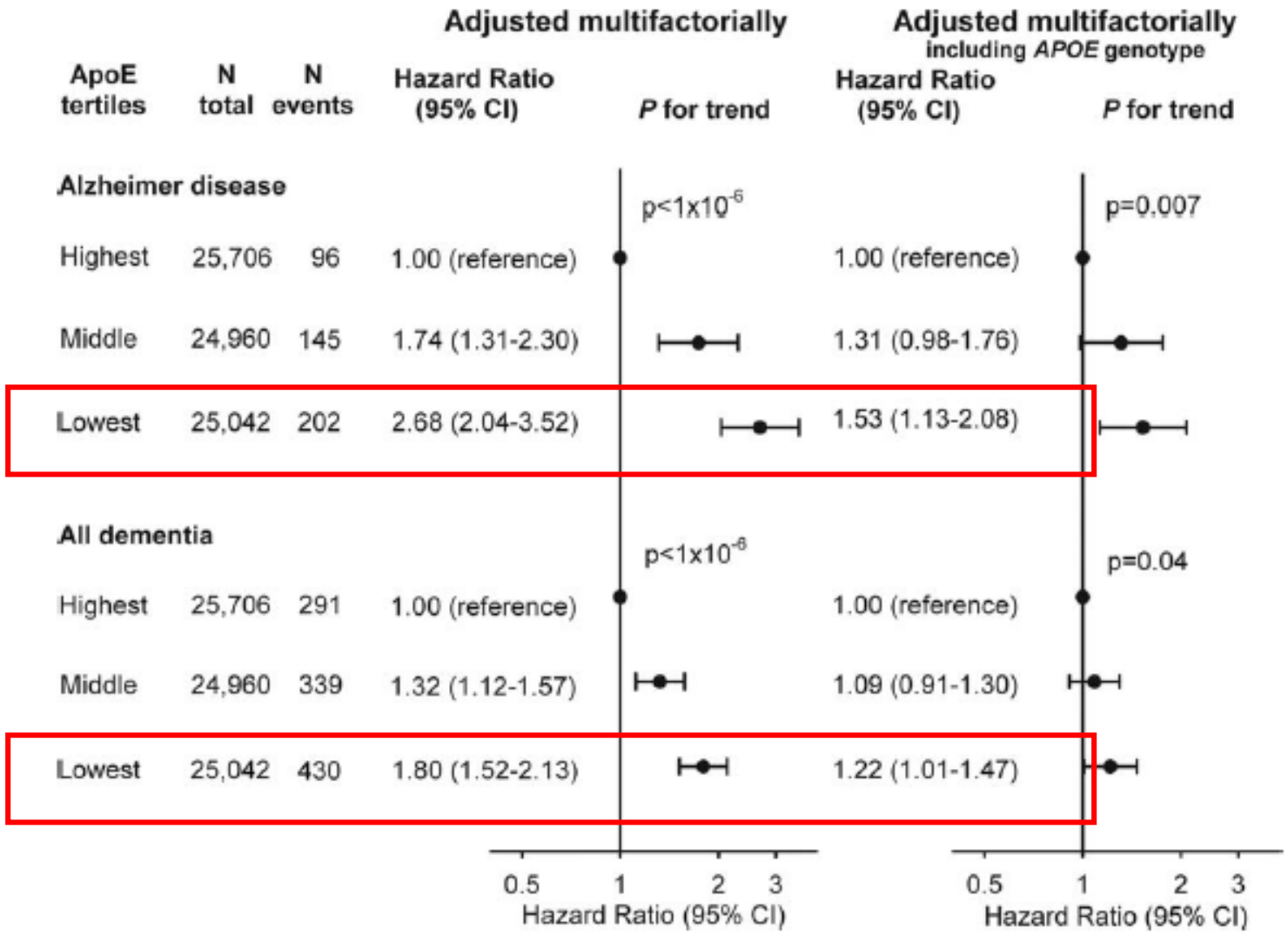
Reduced plasma apoE is an APOEε4-related clinical characteristic





Martinez-Morillo et al 2014

Low plasma apoE associated with increased risk of AD and all dementia



Rasmussen et al 2015

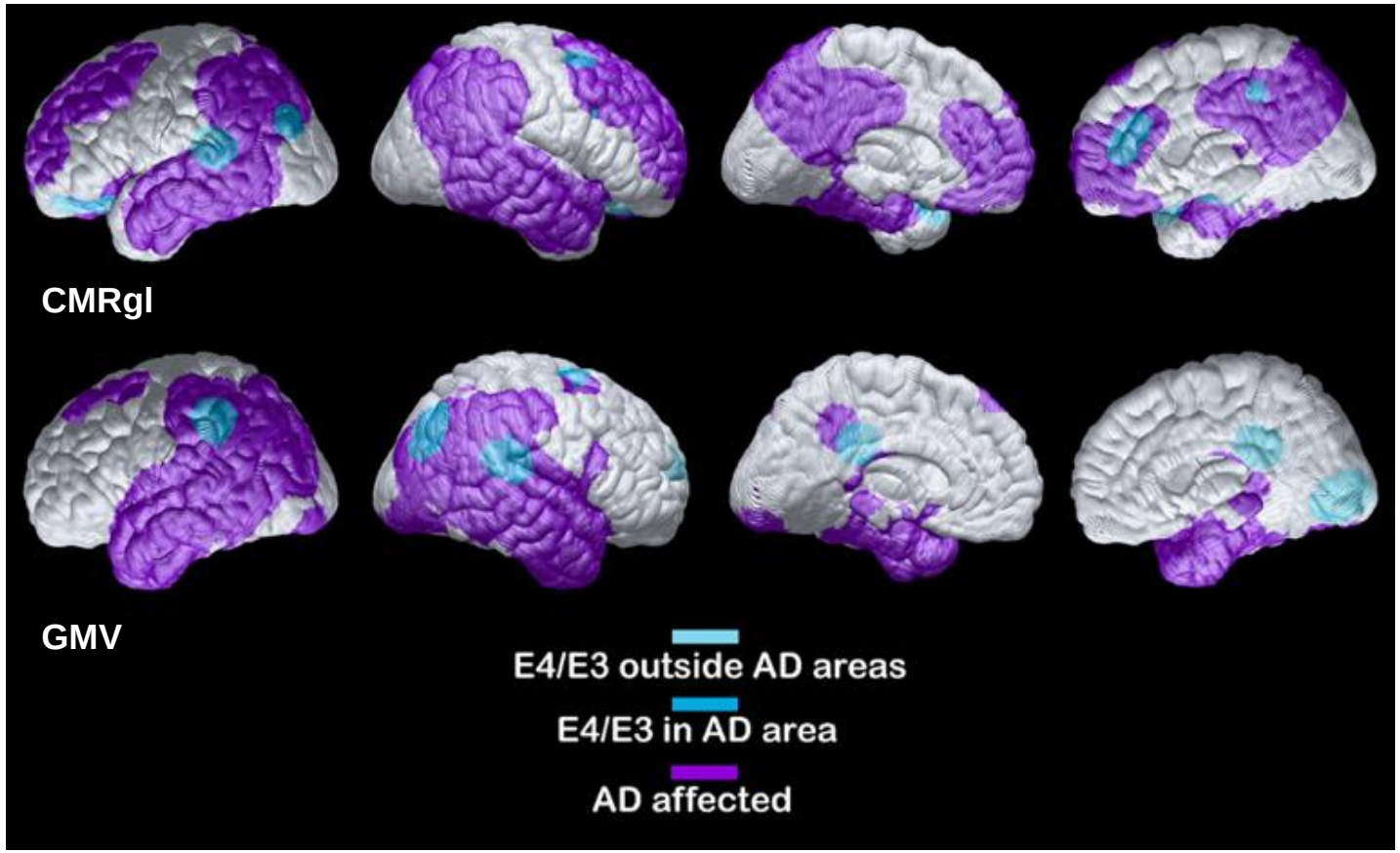
Higher plasma apoE4 to apoE3 ratio is related to structural and metabolic brain alterations

Cognitively healthy APOEε3/E ε4 carriers from the Arizona APOE cohort

Table 3 Correlations between regional glucose metabolism, gray matter volume and plasma apoE concentrations

apoE4/E3 ratio negative association	Brain region	Coordinates (*X*, *Y*, *Z*)	*p* value
CMRgl	Hippocampus_R	26, −12, −12	2.49×10^{-4} [a]
GMV	Cingulate_Post	4, −40, 21	5.60×10^{-4} [b]
	Hippocampus_L	−16, −34, 9	4.91×10^{-3}
	Lateral Temporal_L	−48, −64, −3	2.81×10^{-3}
	Lateral Temporal_R	60, −31, 13	1.83×10^{-4}
	Medial Temporal_L	−16, −34, 9	4.91×10^{-3}
	Precuneus_R	8, −43, 18	1.87×10^{-3}

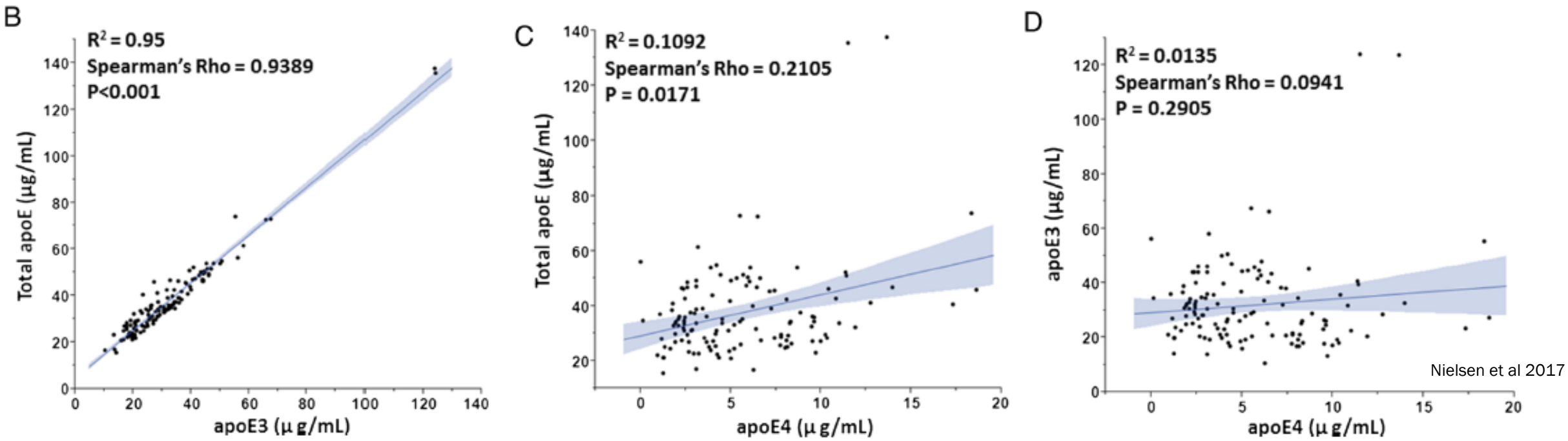
[a] Survived correction at a 0.05 level
[b] Survived correction at a 0.059 level
CMRgl cerebral metabolic rate of glucose, *GMV* gray matter volume



Nielsen et al 2017

Plasma apoE3 and apoE4 levels are not correlated

Cognitively healthy APOEε3/E ε4 carriers from the Arizona APOE cohort

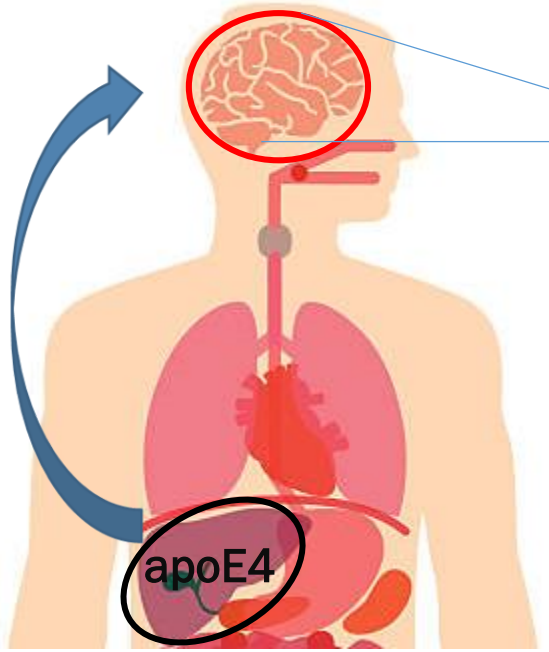


Nielsen et al 2017

No correlation between plasma apoE3 and apoE4 (n=128) plasma levels suggesting differential regulation mechanisms

Hypothesis

Perpheral liver-derived apolipoprotein E either directly or indirectly promotes neuropathological processes in the brain leading to the development of neurodegenerative disease unless counteracted by yet to be identified protective mechanisms



- **Glucose hypometbolism and insulin resistance**
- **Gray matter atrophy**
- **Inflammation**
- **Synaptic degeneration**

Amyloid-beta, tau

AD

Aims

To in detail characterize a potential peripheral phenotype that can be predict the development of Alzheimer's disease in APOEε4-carriers

- Investigate effect of peripheral apoE isoforms on pathological processes in the brains of FRGN mice with humanized livers

- Identify the cause of plasma apoE deficiency in APOEε4 carriers
  - RNA sequencing analysis of liver biopsies from APOEε4-carriers versus non-carriers
  - Perform a large scale 44K antigen screen to investigate potential presence of auto-antibodies in the plasma of APOEε4

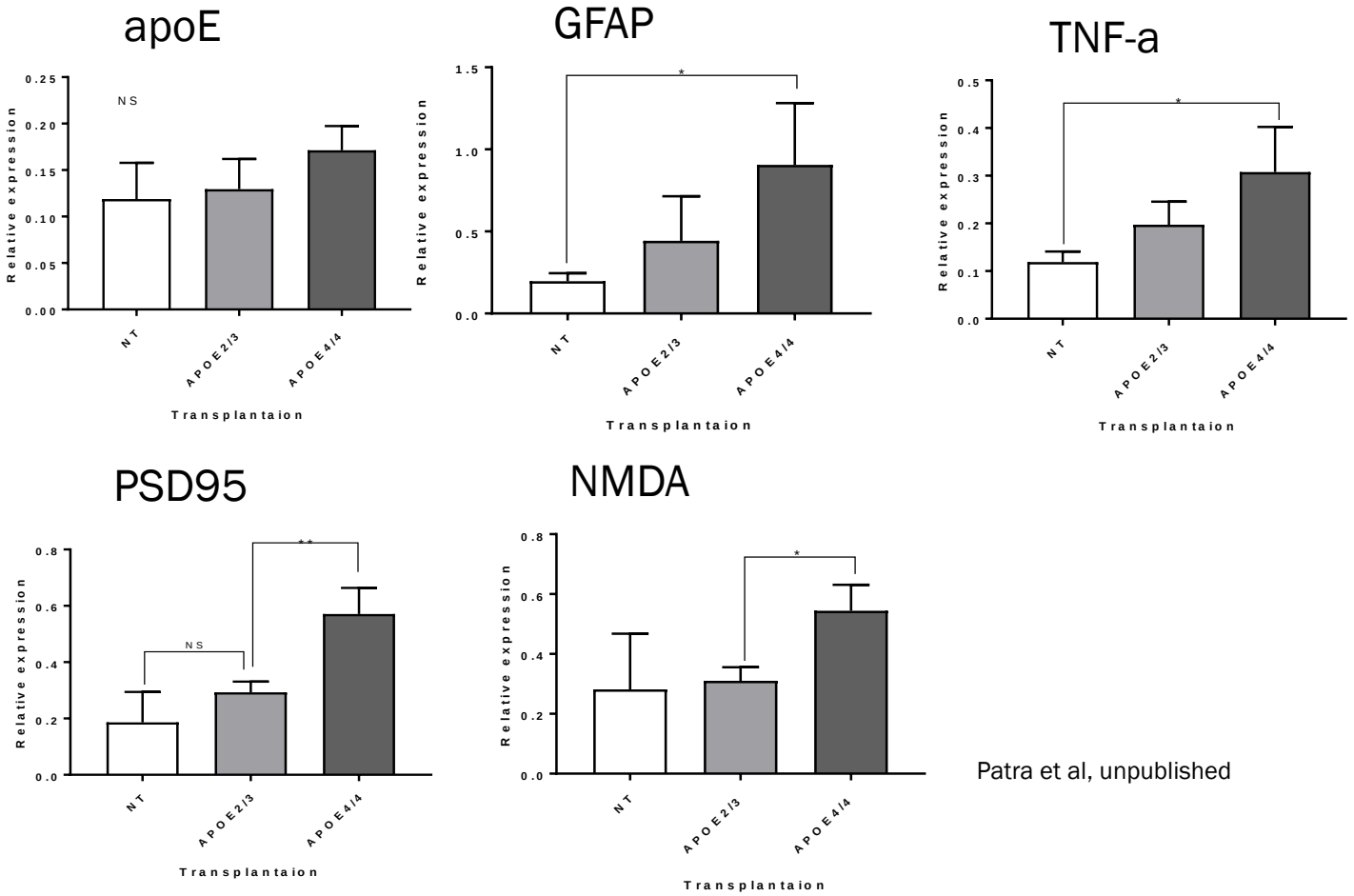
FRGN mice with human livers relevant model to study effect of peripheral apoE on brain

FRG *[**F**ah(−/−)**R**ag2(−/−)Il2r**g**(−/−)]*) mice repopulated with primary human hepatocytes (Ellis et al 2013)

Total Cholesterol mmol/L		% VLDL	% LDL	% HDL	Ratio LDL/HDL
WT	1.6	6.9	13.3	79.8	0.2
Human	4.7	7.7	57.3	35.1	1.6
45% repop.	1.9	8.6	43.5	47.9	0.9
88% repop.	5.8	1.3	49.9	48.8	1.0
90% repop.	1.0	6.2	56.5	37.4	1.5

- Mouse model exhibits human-like plasma lipid profile alongside production of human liver-derived proteins including apoE, alpha-1-antitrypsin, albumin etc
- Proof-of-concept model as human apoE variants are produced in the periphery but not in the brain

FRGN mice with APOEε4 livers exhibit cortical alterations



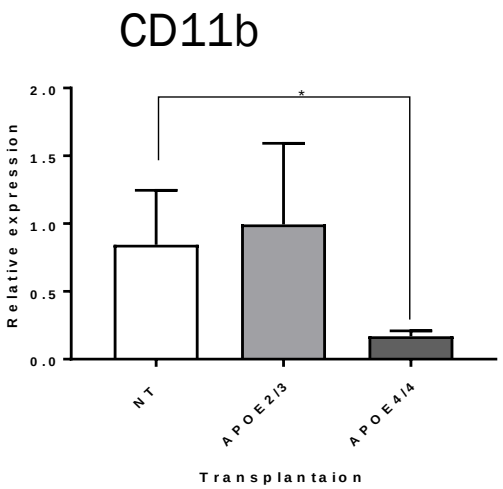
Patra et al, unpublished

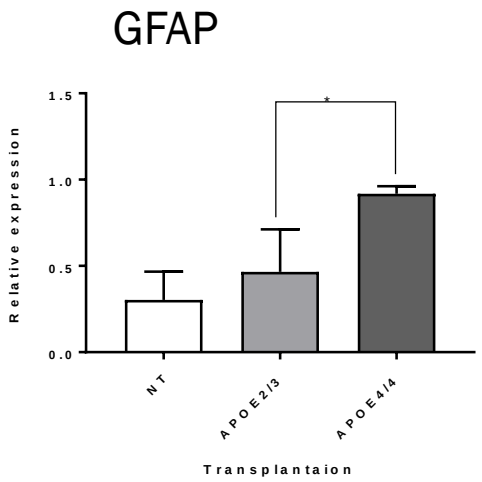
N=3 per group

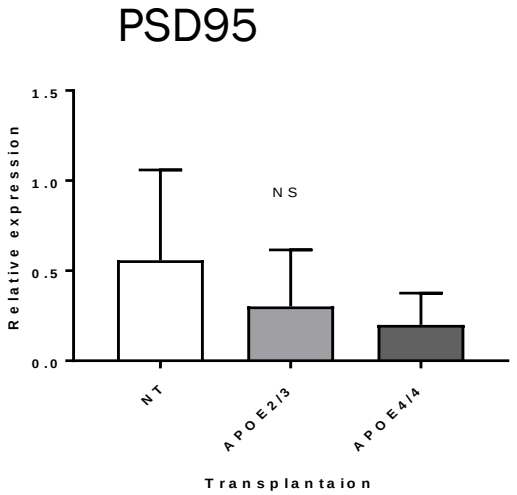
In the **cortex** mice with APOEε4/ε4 livers exhibited:

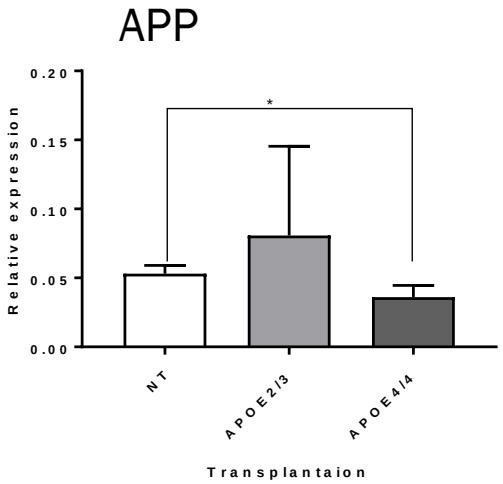
- unaltered levels of apoE
- increased astrocytosis (GFAP)
- increased levels of proinflammatory cytokine TNF-a
- altered levels of synaptic markers PSD95 and NMDA receptors

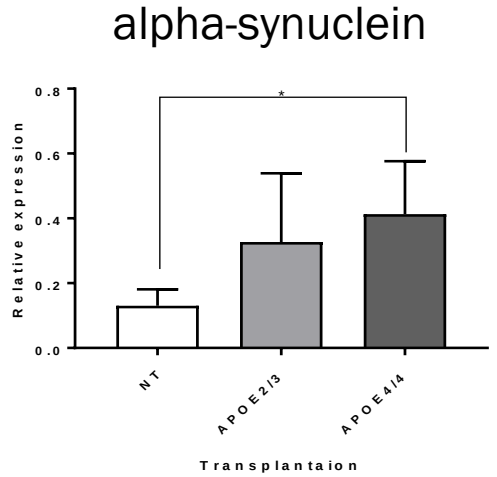
FRGN mice with APOEε4 livers exhibit hippocampal alterations











Patra et al, unpublished

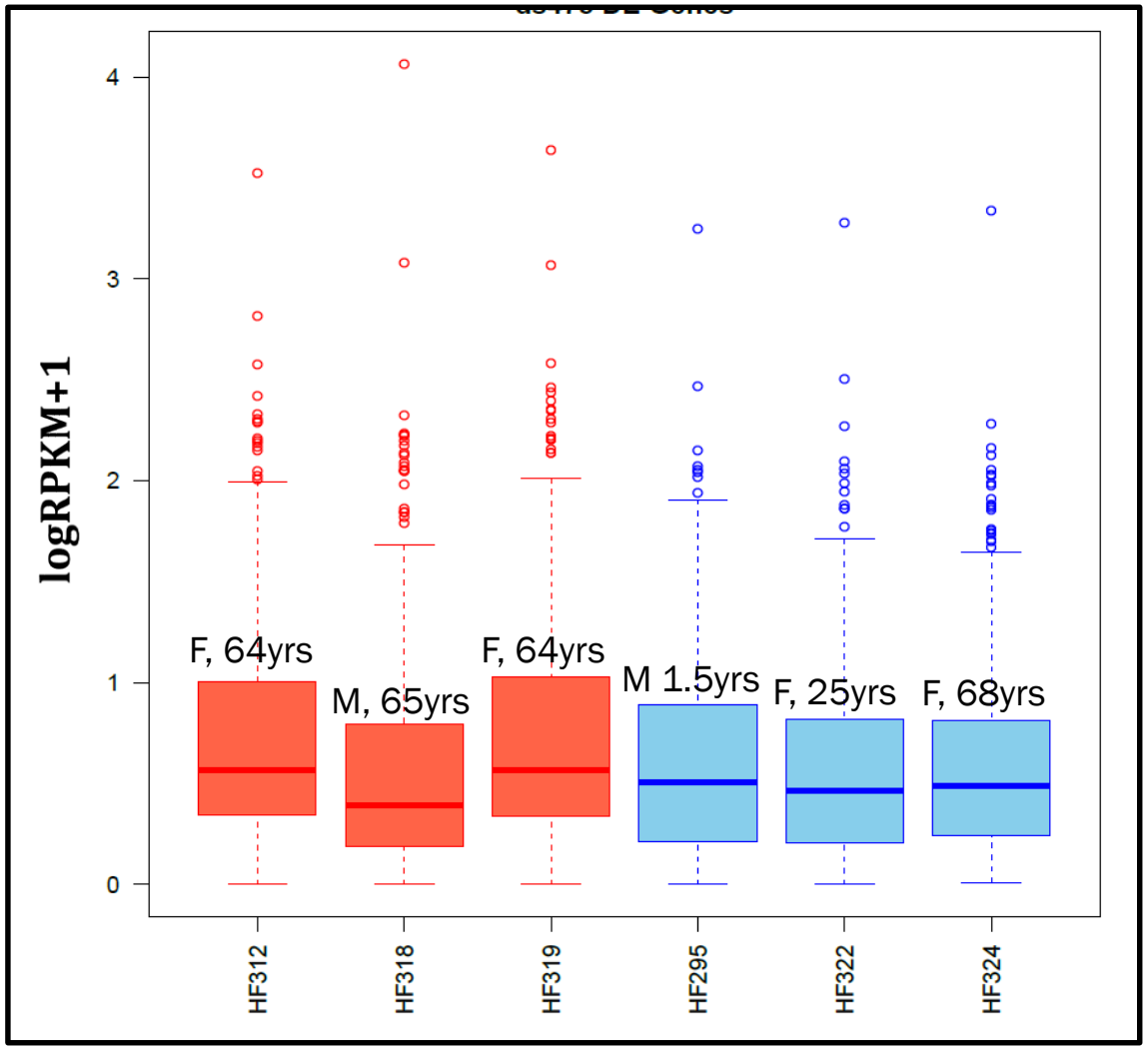
N=3 per group

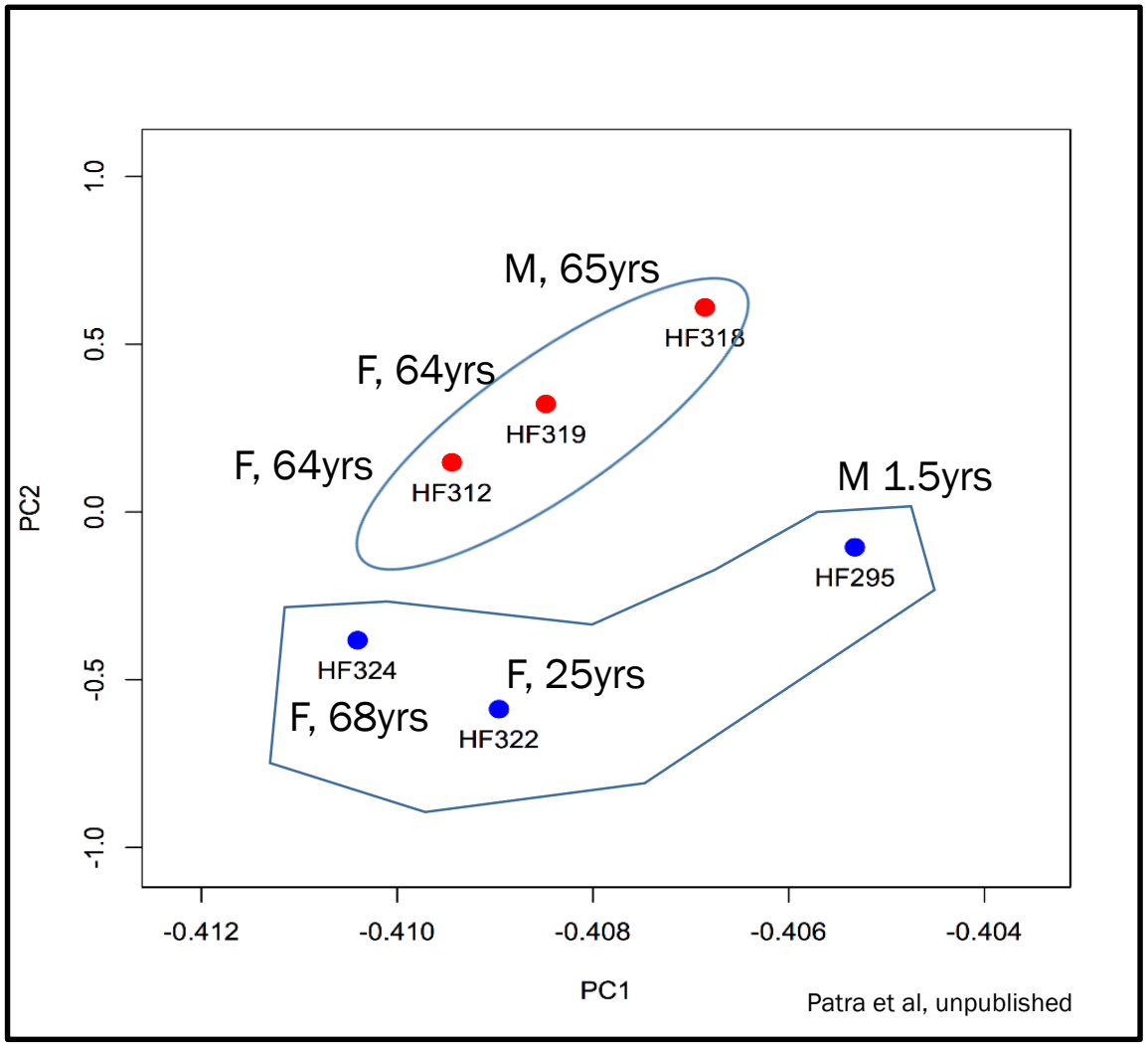
In the **hippocampus** mice with APOEε4/ε4 livers exhibited:

- increased astrocytosis (GFAP)
- decreased amount of microglia marker CD11b
- altered levels of PSD95, APP and alpha-synuclein

Global gene expression in livers from APOEε3/ε4 versus APOEε2/ε3-carriers

Liver biopsies from: N=3 APOEε3/ε4, N=3 APOEε2/ε3





Differential gene expression in APOEε3/ε4-carriers

In total n=624 differentially expressed genes in APOEε3/ε4 versus APOEε2/εE3-carriers (p<0.05)

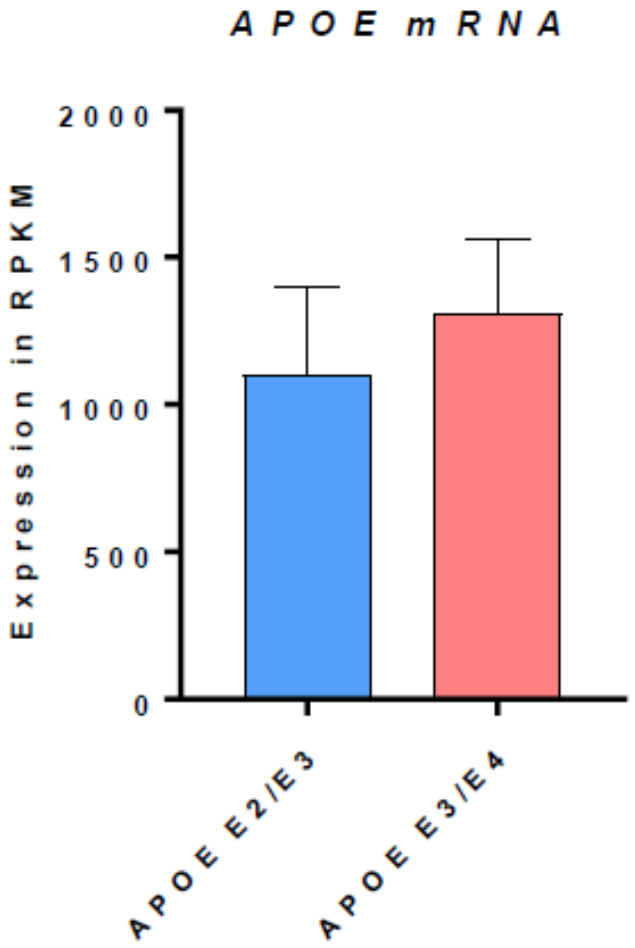
Top 10 most significant genes of which the expression was altered in APOEε3/ε4 vs APOEε2/εE3-carriers

GeneID	GeneName	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
ENSG00000064205	WISP2	188.06	5.39	0.49	10.92	9.54E-28	2.37E-23
ENSG00000189292	FAM150B	202.03	3.37	0.40	8.37	5.56E-17	6.90E-13
ENSG00000197956	S100A6	1236.27	2.28	0.32	7.22	5.11E-13	4.22E-09
ENSG00000119782	FKBP1B	126.89	3.34	0.47	7.08	1.45E-12	9.00E-09
ENSG00000182795	C1orf116	100.93	3.37	0.50	6.77	1.27E-11	6.29E-08
ENSG00000084453	SLCO1A2	186.26	-3.74	0.56	-6.68	2.38E-11	8.86E-08
ENSG00000135052	GOLM1	2898.73	2.93	0.44	6.67	2.50E-11	8.86E-08
ENSG00000165023	DIRAS2	82.32	3.18	0.48	6.65	2.96E-11	9.19E-08
ENSG00000214264	KCTD9P4	596.88	-2.95	0.47	-6.25	4.14E-10	1.14E-06
ENSG00000132470	ITGB4	335.79	1.95	0.32	6.14	8.24E-10	2.04E-06

SLCO1A2 variant implicated in PSP in an eGWAS study (Zou et al 2012) and in an ADNI sample of AD as a modifier of the effect of cortical amyloid-beta burden on cognitive impairment and temporal lobe atrophy in AD (Roostaei et al 2017).

Patra et al, unpublished

No difference in APOE expression between APOEε3/ε4 vs APOEε2/ε3-carriers



Expression of APOE was similar in APOEε2/ε3 versus APOEε3/ε4-carriers with a trend to increased levels in the APOEε3/ε4-carriers

Patra et al, unpublished

Plasma autoantibody screening strategy

Discovery phase:

Assessment of autoimmune plasma IgG reactivities using a 44K antigen array based on the Human Proteome Atlas

Total of n=4 plasma pools including

n=4 (F/M) MCI-AD patients APOEε3/ε3

n=4 (F/M) MCI-AD patients APOEε4/ε4

n=4 (F/M) MCI-MCI patients APOEε3/ε3

n=4 (F/M) MCI-MCI patients APOEε4/ε4

Plasma autoantibody screening results

Pending mid-December 2017

Ongoing and future efforts

- Expand on the investigation of FRGN mice with humanized livers

- Confirm and assess physiological relevance of top 10 genes with altered expression in APOEε4-carriers using primary human hepatocyte cultures with different APOE genotypes (n=40) and plasma from patients with MCI and AD

- Assess levels of specific IgG reactivities identified in the discovery phase of our autoantibody screening in plasma samples from controls, MCI and AD patients with different APOE genotypes

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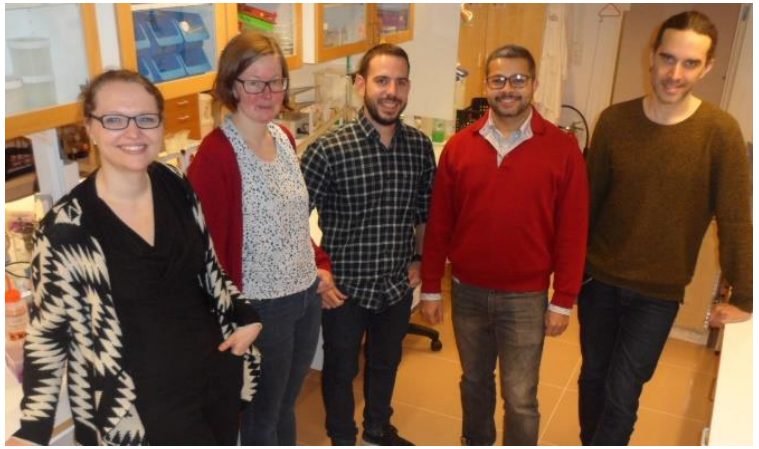
Anna Edlund, PhD student
Andreas Giannisis, master student
Maliheh Keshavarzi, research trainee
(*Simon Moussaud, researcher*)



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CONCLUSIONS

- Peripheral apoE4 promotes neuroinflammatory events and synaptic alterations in proof-of-concept FRGN mice with humanized livers

- Plasma apoE deficiency in APOEε4-carriers cannot be explained by reduced APOE expression levels – allele-specific expression still to be performed

- Differential gene expression in livers with an APOEε4-phenotype may reveal disease-promoting mechanisms with implications for neurodegeneration and liver transplantation routines