ApoE4 disrupts the cerebral microcirculation and undermines white matter integrity and cognitive function

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Introduction

• Microvascular alterations leading to white matter injury are a major cause of cognitive impairment (Dichgans & Leys, 2017);

• The ApoE4 allele is the leading genetic risk factor for sporadic Alzheimer’s disease and may also be involved in vascular cognitive impairment (Iadecola, 2013);

• Individuals homozygous for ApoE4 have reduced cerebral blood flow (Tai et al., 2016) and a higher risk of white matter lesions (Brickman et al., 2014);

• However, it remains to be elucidated whether ApoE4 alters the cerebral microcirculation and exacerbates white matter injury and cognitive impairment.
Objective

To investigate whether ApoE4 alters the cerebral microcirculation and contributes to the white matter injury and cognitive impairment induced by chronic cerebral hypoperfusion
Methods

• Male wild-type, and homozygous human ApoE3 target replacement (TR) and ApoE4-TR mice on a C57BL/6J genetic background at the age 3–4 months were used;

• Cerebral blood flow, white matter damage and cognitive function were tested in chronic cerebral hypoperfusion induced by bilateral common carotid artery stenosis (BCAS).
Study 1: Is cerebral blood flow (CBF) altered in ApoE4-TR mice?
Resting CBF is reduced in ApoE4-TR compared to WT and ApoE3-TR mice

**A**

ASL-MRI

![Brain images of WT, ApoE3, and ApoE4 mice](image)

**B**

Somatosensory Cortex

![Bar chart showing CBF comparison](image)

- WT
- ApoE3
- ApoE4

*, p<0.05 vs. WT or ApoE3; N=5-6/group
Vascular diameter and RBC velocity assessed by 2-photon microscopy do not differ between ApoE3-TR and ApoE4-TR mice.

*, p<0.05 vs. WT or ApoE3; N=5-6/group
Microvascular density is reduced in neocortex and corpus callosum of ApoE4-TR compared to WT and ApoE3-TR mice.

* , p<0.05 vs. WT or ApoE3; N=5-6/group
Study 2:
Is neurovascular regulation altered in ApoE4-TR mice?
Methods for cerebrovascular assessment

- Mice were anesthetized (urethane-chloralose) and artificially ventilated with controlled arterial pressure and blood gases;

- CBF was monitored by laser Doppler flowmetry in a cranial window placed over the somatosensory cortex;

- The CBF increase evoked by neural activity (functional hyperemia) was tested by mechanical stimulation of the facial whiskers;

- CBF response to neocortical superfusion with the endothelial agonist acetylcholine (10 µM) was also tested.
The increase in CBF evoked by whisker stimulation or ACh is reduced in ApoE4-TR mice

* p<0.05 from WT or ApoE3 (n = 5/group)
The free radical scavenger MnTBAP reverses the neurovascular dysfunction in ApoE4-TR mice

* $p<0.05$ from WT; n = 5/group
Study 3:
Are ApoE4-TR mice more susceptible to chronic hypoxic-ischemic injury?

- BCAS: Chronic cerebral hypoperfusion with WM injury
- CBF measurements:
  - Laser speckle flowmetry
  - 3-photon microscopy

(Shibata et al., Stroke 2004)
BCAS induces more marked CBF reductions in ApoE4-TR mice

A

WT-BCAS

ApoE3-BCAS

ApoE4-BCAS

B

WT-sham

ApoE3-sham

ApoE4-sham

WT-BCAS

ApoE3-BCAS

ApoE4-BCAS

* , p<0.05 vs. Sham; #, p<0.05 vs. WT-BCAS or ApoE3-BCAS; N=8/group
Corpus callosum CBF is significantly lower in ApoE4-TR than ApoE3-TR mice before and after BCAS.

*, p<0.05 vs ApoE3 sham; #, p<0.05 vs ApoE3/E4-sham ApoE3-BCAS
Study 4:
Is the more severe hypoperfusion in ApoE4-TR mice associated with more severe WM injury and cognitive deficit?
BCAS damages WM more severely in ApoE4-TR mice

**A**

Klüver-Barerra (KB)

<table>
<thead>
<tr>
<th>Sham</th>
<th>WT-BCAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE3-BCAS</td>
<td>ApoE4-BCAS</td>
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</tbody>
</table>

Bar graph showing the percentage positive area of KB:

- WT: 95%
- WT: 85%
- ApoE3: 90%
- ApoE4: 80%

* p<0.05 vs. Sham; # p<0.05 vs. WT- or ApoE3-BCAS; N=6/group

**B**

Nav1.6/Caspr

<table>
<thead>
<tr>
<th>WT-Sham</th>
<th>WT-BCAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE3-BCAS</td>
<td>ApoE4-BCAS</td>
</tr>
</tbody>
</table>

Bar graph showing the length of Nav1.6 (µm):

- WT: 2.0 µm
- WT: 1.5 µm
- ApoE3: 1.0 µm
- ApoE4: 2.5 µm

* p<0.05 vs. Sham; # p<0.05 vs. WT- or ApoE3-BCAS; N=6/group
BCAS damages cognition more severely in ApoE4-TR mice

**A**  
*Y-maze*

Trial phase  
- Delay 30 min

Testing phase

- Arm alternation (%)
  - WT: 60 ± 5
  - WT: 55 ± 5
  - ApoE3: 65 ± 5
  - ApoE4: 70 ± 5

* p<0.05 vs. sham; # p<0.05 vs. WT ApoE3; N = 10/group

**B**  
*Novel object recognition*

Training phase  
- Delay 30 min

Testing phase

- Exploration time (% vs familiar)
  - WT: 80 ± 10
  - WT: 75 ± 10
  - ApoE3: 85 ± 10
  - ApoE4: 90 ± 10

* p<0.05 vs. sham; # p<0.05 vs. WT ApoE3; N = 10/group
Summary and Conclusions

- ApoE4-TR mice exhibit reduced resting CBF and neurovascular dysfunction;
- These alteration are related to vascular oxidative stress;
- BCAS reduces neocortical and corpus callosum CBF more severely in ApoE4-TR than in ApoE3-TR or WT mice;
- BCAS induces more severe white matter injury and cognitive impairment in ApoE4-TR than in ApoE3-TR or WT mice;
- ApoE4-induced microvascular dysfunction may be responsible for the heightened susceptibility to white matter injury and cognitive impairment in ApoE4 carriers.