APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline

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SUPPLEMENTARY INFORMATION

Supplementary Information Guide

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1. Supplementary Tables (5)

Supplementary Table 1. Hierarchical logistic regression analyses of the blood-brain barrier K_{trans} constant in the hippocampus (HC) and parahippocampal gyrus (PHG) predicting cognitive impairment in *APOE4* and *APOE3* carriers based on clinical dementia rating (CDR) score 0.5 versus 0 after controlling for age, sex, education, HC and PHG volumes, and CSF $A\beta_{1-42}$ and pTau status.

***APOE4* carriers (n=93)**	**HC K_{trans} predicting CDR status**		***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
		Model Parameters for Step 1	*43.477*	*17.131*	*1*	*$3.5x10^{-5}$*
	Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
	0	Age (yrs)	-0.015	0.041	0.133	0.716
	0	Sex (ratio)	-0.077	0.819	0.009	0.925
	0	Education (attainment)	0.269	0.225	1.431	0.232
	0	HC volume (mm^3)	-0.001	<0.001	5.186	0.023
	0	CSF $A\beta_{1-42}$ (status)	-1.252	0.781	2.565	0.109
	0	CSF pTau (status)	0.487	1.031	0.223	0.636
	1	HC BBB K_{trans} (x 10^{-3} min^{-1})	6.700	2.212	9.173	0.002

***APOE4* carriers (n=93)**	**PHG K_{trans} predicting CDR status**		***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
		Model Parameters for Step 1	*44.260*	*13.156*	*1*	*$2.9x10^{-4}$*
	Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
	0	Age (yrs)	-0.032	0.047	0.468	0.494
	0	Sex (ratio)	0.333	0.777	0.183	0.669
	0	Education (attainment)	0.145	0.219	0.439	0.508
	0	PHG volume (mm^3)	-0.001	0.001	0.885	0.347
	0	CSF $A\beta_{1-42}$ (status)	-0.946	0.755	1.570	0.210
	0	CSF pTau (status)	-0.789	0.841	0.880	0.348
	1	PHG BBB K_{trans} (x 10^{-3} min^{-1})	5.969	2.088	8.168	0.004

***APOE3* carriers (n=142)**	**HC K_{trans} predicting CDR status**		***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
		Model Parameters for Step 1	*59.843*	*3.846*	*1*	*0.05*
	Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
	0	Age (yrs)	0.078	0.049	2.471	0.116
	0	Sex (ratio)	0.763	.691	1.221	0.269
	0	Education (attainment)	0.006	.156	0.001	0.970
	0	HC volume (mm^3)	<0.001	<0.001	0.074	0.785
	0	CSF $A\beta_{1-42}$ (status)	0.192	0.731	0.069	0.793
	0	CSF pTau (status)	-0.811	0.891	0.829	0.362
	1	HC BBB K_{trans} (x 10^{-3} min^{-1})	2.493	1.271	3.849	0.050

***APOE3* carriers (n=142)**	**PHG K_{trans} predicting CDR status**		***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
		Model Parameters for Step 1	*57.864*	*5.923*	*1*	*0.02*
	Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
	0	Age (yrs)	0.106	0.053	3.993	0.046
	0	Sex (ratio)	1.148	0.741	2.397	0.122
	0	Education (attainment)	-0.087	0.171	0.260	0.610
	0	PHG volume (mm^3)	<0.001	<0.001	0.164	0.685
	0	CSF $A\beta_{1-42}$ (status)	0.237	0.752	0.099	0.753
	0	CSF pTau (status)	-0.891	0.890	1.003	0.317
	1	PHG BBB K_{trans} (x 10^{-3} min^{-1})	3.908	1.750	4.986	0.030

Supplementary Table 2. Linear mixed model analysis of CSF sPDGFRβ baseline values predicting future cognitive decline on age-, sex-, and education-corrected z-scores on mental status exam and the global cognitive composite of all neuropsychological tests after controlling for CSF Aβ and tau status. *Significance by linear mixed model analysis; no multiple comparison correction applied. All tests are two-tailed (see Methods for further details).*

Total Sample (n=146)

CSF sPDGFRβ Predicting Change in Mental Status Controlling for CSF $Aβ_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.350702	0.137087	128.928	-2.558	0.012
Time	-0.233797	0.121152	96.055	-1.93	0.057
CSF $Aβ_{1-42}$ status	0.085454	0.269908	132.122	0.317	0.752
CSF sPDGFRβ	$-8.95x10^{-5}$	0.000359	128.26	-0.249	0.804
CSF sPDGFRβ x time	-0.000954	0.000307	87.447	-3.103	0.003
Intercept	-0.325414	0.127507	130.073	-2.552	0.012
Time	-0.257617	0.118456	98.676	-2.175	0.032
CSF pTau status	-1.259219	0.275932	130.946	-4.564	$1.1x10^{-5}$
CSF sPDGFRβ	$-2.06x10^{-4}$	0.000336	129.619	-0.613	0.541
CSF sPDGFRβ x time	-0.000955	0.000302	90.817	-3.159	0.002

CSF sPDGFRβ Predicting Change in Global Composite Controlling for CSF $Aβ_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.238899	0.070962	140.235	-3.367	0.001
Time	-0.077554	0.044723	135.214	-1.734	0.085
CSF $Aβ_{1-42}$ status	0.071522	0.145093	140.405	0.493	0.623
CSF sPDGFRβ	-0.000278	0.000192	139.208	-1.446	0.15
CSF sPDGFRβ x time	-0.000304	0.000119	127.458	-2.544	0.012
Intercept	-0.234876	0.068014	139.987	-3.453	0.001
Time	-0.088201	0.043783	136.92	-2.015	0.046
CSF pTau status	-0.498812	0.154003	140.05	-3.239	0.001
CSF sPDGFRβ	-0.000297	0.000185	138.916	-1.602	0.111
CSF sPDGFRβ x time	-0.000313	0.000117	129.855	-2.665	0.009

Supplementary Table 3. Linear mixed model analysis of CSF sPDGFRβ baseline values predicting future cognitive decline on age-, sex-, and education-corrected z-scores on mental status exam and the global cognitive composite of all neuropsychological tests in *APOE4* carriers after controlling for CSF Aβ and tau status. *Significance by linear mixed model analysis; no multiple comparison correction applied. All tests are two-tailed (see Methods for further details).*

***APOE4* carriers (n=58)**

CSF sPDGFRβ Predicting Change in Mental Status Controlling for CSF $A\beta_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.493185	0.21685	53.123	-2.274	0.027
Time	-0.066464	0.229312	54.021	-0.29	0.773
CSF $A\beta_{1-42}$ status	0.209097	0.400371	54.583	0.522	0.604
CSF sPDGFRβ	0.000334	0.000546	52.841	0.612	0.543
CSF sPDGFRβ x time	-0.001621	0.000542	45.708	-2.993	0.004
Intercept	-0.349128	0.199119	54.509	-1.753	0.085
Time	-0.127275	0.222438	55.358	-0.572	0.57
CSF pTau status	-1.313143	0.399477	54.433	-3.287	0.002
CSF sPDGFRβ	3.39×10^{-5}	0.000503	53.885	0.067	0.946
CSF sPDGFRβ x time	-0.001616	0.000525	47.055	-3.077	0.003

CSF sPDGFRβ Predicting Change in Global Composite Controlling for CSF $A\beta_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.334356	0.103951	53.211	-3.216	0.002
Time	-0.104365	0.071676	47.613	-1.456	0.152
CSF $A\beta_{1-42}$ status	0.126515	0.194343	45.506	0.651	0.518
CSF sPDGFRβ	-0.000118	0.000263	53.224	-0.449	0.655
CSF sPDGFRβ x time	-0.00042	0.000168	39.136	-2.502	0.017
Intercept	-0.297598	0.099654	53.767	-2.986	0.004
Time	-0.113505	0.06901	50.395	-1.645	0.106
CSF pTau status	-0.323346	0.198942	43.959	-1.625	0.111
CSF sPDGFRβ	-0.000147	0.000253	53.64	-0.58	0.564
CSF sPDGFRβ x time	-0.000434	0.000162	42.223	-2.679	0.01

Supplementary Table 4. Linear mixed model analysis of the overall incremental predictive value of CSF sPDGFRβ baseline values in relation to cognitive decline on age-, sex-, and education-corrected z-scores on mental status exam and the global cognitive composite of all neuropsychological tests in *APOE3* carriers after controlling for CSF Aβ and tau status. *Significance by linear mixed model analysis; no multiple comparison correction applied. All tests are two-tailed (see Methods for further details).*

***APOE3* carriers (n=88)**

CSF sPDGFRβ Not Predicting Change in Mental Status Controlling for CSF $A\beta_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.351175	0.183267	366.785	-1.916	0.056
Time	-0.119878	0.145479	112.947	-0.824	0.412
CSF $A\beta_{1-42}$ status	-0.037947	0.36085	272.065	-0.105	0.916
CSF sPDGFRβ	-0.000446	0.000497	369.322	-0.897	0.37
CSF sPDGFRβ x time	-0.000264	0.000402	111.691	-0.658	0.512
Intercept	-0.380945	0.171377	306.273	-2.223	0.027
Time	-0.125378	0.142834	119.044	-0.878	0.382
CSF pTau status	-1.236054	0.375561	223.335	-3.291	0.001
CSF sPDGFRβ	-0.000478	0.000467	307.686	-1.024	0.307
CSF sPDGFRβ x time	-0.00023	0.000399	117.444	-0.577	0.565

CSF sPDGFRβ Not Predicting Change in Global Composite Controlling for CSF $A\beta_{1-42}$ and pTau status

	β	***SE***	***df***	***t***	***p-value***
Intercept	-0.191169	0.09844	85.805	-1.942	0.055
Time	-0.048517	0.060892	90.359	-0.797	0.428
CSF $A\beta_{1-42}$ status	0.028411	0.197739	86.711	0.144	0.886
CSF sPDGFRβ	-0.000344	0.000281	85.181	-1.223	0.225
CSF sPDGFRβ x time	-0.000176	0.000178	93.73	-0.989	0.325
Intercept	-0.209294	0.094928	85.528	-2.205	0.03
Time	-0.054147	0.060094	90.311	-0.901	0.37
CSF pTau status	-0.50794	0.215262	86.808	-2.36	0.021
CSF sPDGFRβ	-0.000356	0.000272	84.783	-1.311	0.193
CSF sPDGFRβ x time	-0.000165	0.000177	94.172	-0.933	0.353

Supplementary Table 5. Hierarchical logistic regression analyses of CSF sPDGFRβ baseline values predicting cognitive impairment in *APOE4* but not in *APOE3* carriers based on clinical dementia rating (CDR) score 0.5 versus 0 after controlling for age, sex, education, HC and PHG volumes, and CSF $A\beta_{1-42}$ and pTau status.

***APOE4* carriers (n=58)**	**CSF sPDGFRβ predicting CDR status**	***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
	Model Parameters for Step 1	*122.370*	*6.582*	*1*	*0.01*
Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
0	Age (yrs)	0.037	0.026	2.062	0.151
0	Sex (ratio)	0.57	0.459	1.543	0.214
0	Education (attainment)	-0.006	0.18	0.001	0.974
0	CSF $A\beta_{1-42}$ (status)	-0.902	0.451	4.002	0.045
0	CSF pTau (status)	-0.975	0.492	3.928	0.047
1	CSF sPDGFRβ (ng/mL)	0.001	0.001	6.127	0.013

***APOE3* carriers (n=88)**	**CSF sPDGFRβ predicting CDR status**	***-2 Log Likelihood***	***Chi-square***	***df***	***p-value***
	Model Parameters for Step 1	*166.319*	*0.076*	*1*	*0.78*
Step	***Predictor***	***β***	***SE***	***Wald***	***p-value***
0	Age (yrs)	0.069	0.024	8.472	0.004
0	Sex (ratio)	1.105	0.411	7.215	0.007
0	Education (attainment)	-0.273	0.158	3	0.083
0	CSF $A\beta_{1-42}$ (status)	0.106	0.418	0.065	0.799
0	CSF pTau (status)	-0.675	0.433	2.433	0.119
1	CSF sPDGFRβ (ng/mL)	$1.0x10^{-4}$	0.001	0.077	0.782

2. Supplementary Methods

Quantification of the Blood-Brain Barrier Permeability

Post-processing analysis was performed using *Rocketship* software[1] running with Matlab. To account for a possible confounding effect of blood flow on DCE-MRI measurements, we determined in each studied individual the arterial input function (AIF) curve from the internal carotid artery (ICA), which provides a dynamic profile of a gadolinium tracer concentration in the arterial blood after the i.v. injection, instead of using an average value from the superior sagittal venous sinus to determine tracer concentration in blood[2–5]. Although not as ideal as simultaneous measurements of the blood flow on the same subjects, using the individual AIF dynamic profile measurements of the tracer concentration in the arterial blood self-corrects for possible differences in the blood flow that may affect delivery of the tracer to the brain via flow across the ICA, which tends to minimize possible confounding effects of changes in blood volume and blood flow that could potentially affect the K_{trans} measurements, we reported[6,7]. The AIF, which was extracted from a region-of-interest (ROI) positioned at the ICA, was fitted with a bi-exponential function prior to fitting with the Patlak model[7,8]. In a few cases when the ICA was not clearly visible a nearby large arterial vessel was used.

The Patlak linearized regression mathematical analysis was used to generate the BBB permeability K_{trans} maps, as we previously reported[1,6–8]. The high spatiotemporal resolution allowed not only simultaneous measurements of the regional BBB permeability in different white and gray matter regions, but also accurate calculations of the K_{trans} values in small anatomical regions as thin as cortical gray matter areas.

The present analysis requires that the tracer's diffusion across the BBB remains unidirectional during the acquisition time. The total tracer concentration in the tissue, $C_{tissue}\ (t)$, can be described as a function of the blood concentration, $C_{AIF}\ (t)$, the intravascular blood volume, v_p, and a blood-to-brain transfer constant, K_{trans}, that represents the flow from the intravascular to the extravascular extracellular space using equation below:

$$C_{tissue}(t) = K_{trans} \int_0^t C_{AIF}(u)\, du \; + \; v_p AIF(t)$$

We did not observe statistically significant intersubject variability in the measurement of v_p value. For instance, v_p (mean ± SEM) in HC was 0.0166 ± 0.0003 (n=128; CDR 0 *APOE3*), 0.0167 ± 0.0005 (n=68; CDR 0 *APOE4*), 0.0183 ± 0.0009 (n=14; CDR 0.5 *APOE3*), and 0.0164 ± 0.0009 (n=25; CDR 0.5 *APOE4*). In PHG, v_p was 0.0172 ± 0.0003 (n=128; CDR 0 *APOE3*), 0.0171 ± 0.0004 (n=68; CDR 0 *APOE4*), 0.0180 ± 0.0009 (n=14; CDR 0.5 *APOE3*), and 0.0180 ± 0.0008 (n=25; CDR 0.5 *APOE4*). ROI-averaged analysis of DCE-MRI output maps was performed by an experienced neuroradiologist who manually drew ROIs on T1-weighted (FA 12°) pre-contrast MR images for each participant based on their own anatomy to minimize variability between individuals as seen at a macroscopic level (*e.g.*, enlarged ventricles, cortical atrophy, hippocampal shrinkage). Thus, the regional BBB K_{trans} permeability were measured in 10 different gray matter ROIs including the hippocampus (HC), parahippocampal gyrus (PHG), caudate nucleus, thalamus, striatum, orbital frontal cortex (OFC), and inferior temporal gyrus (ITG), and white matter ROIs including subcortical watershed white matter fibers, corpus callosum, and internal capsule.

3. Supplementary Discussion

Although our data demonstrate self-autonomous activation of the CypA-MMP9 pathway in human iPSC-derived *APOE4* pericytes, earlier work in transgenic mice and pericyte cultures has shown that astrocyte-derived apoE4, but not apoE3, can also lead to activation of CypA-MMP9 pathway in pericytes in a non-cell-autonomous manner[9]. Therefore, whether the pericyte is a double culprit, *i.e.*, both an activator of the BBB breakdown process (being the producer of apoE4 protein and of the basement membrane-degrading enzyme MMP9) and subsequently a victim in the process (since they die and release sPDGFRβ), leading to further BBB breakdown, remains to be seen, as well as the cell-specific sources of apoE4 contributing to this process.

BBB breakdown in HC and PHG regions in *APOE4* carriers provides clear anatomical substrate for episodic memory impairment likely caused by neuronal stress related to leaked blood-borne neurotoxic proteins that enter these regions after BBB disruption[10]. Since other cognitive functions such as attention, executive function, working memory, semantic fluency, etc., require connecting pathways linked to HC and medial temporal lobe regions[6], disruption of these connections by BBB breakdown in the medial temporal lobe could also contribute to the observed cognitive deficits beyond memory, as seen in *APOE4* carriers (**Fig. 3**). Additionally, BBB breakdown in the caudate nucleus, that we show progresses with cognitive impairment in *APOE4* carriers (**Extended Data Fig. 1**), may contribute to the overall cognitive decline.

APOE3 homozygotes also develop BBB breakdown during the early stages of cognitive impairment that is much less pronounced than in *APOE4* carriers, and is independent of Aβ and tau (**Fig. 1b-d,l,m**), but in contrast to *APOE4* carriers does not implicate the CypA-MMP9 BBB-degrading pathway (**Fig. 4h,i,k)** and/or pericyte injury (**Fig. 4a,b),** as a major driver of BBB dysfunction during this early stage. Since loss of low density lipoprotein receptor-related protein 1 (LRP1) has been shown to limit the ability of apoE3 to suppress the CypA-MMP9 pathway in transgenic *APOE3* knock-in mice[9], and LRP1 is reduced in blood vessels by aging and Alzheimer's disease[11,12] (see a recent review[10]), it is possible that reduced LRP1 levels with disease progression could potentially lead to activation of the CypA-MMP9 pathway in *APOE3* homozygotes. This possibility needs to be addressed by future longitudinal studies.

4. Supplementary References

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