

Supplementary material

LOWER GLUT1 AND UNCHANGED MCT1 IN ALZHEIMER'S DISEASE CEREBROVASCULATURE

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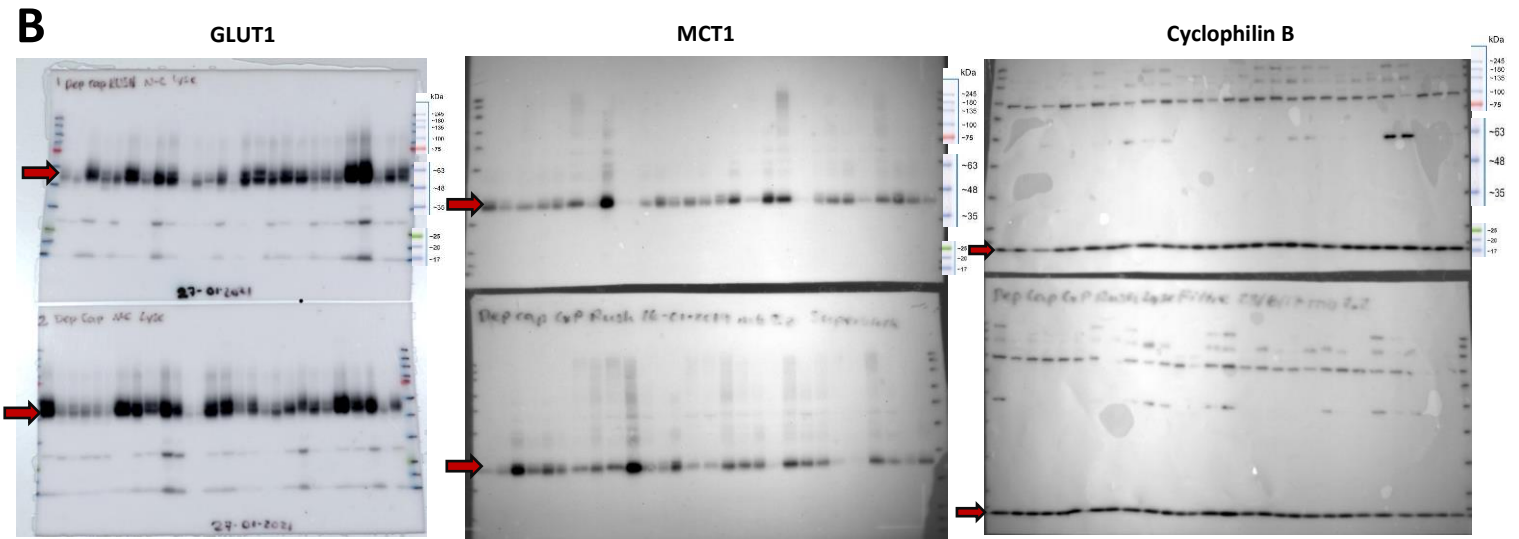
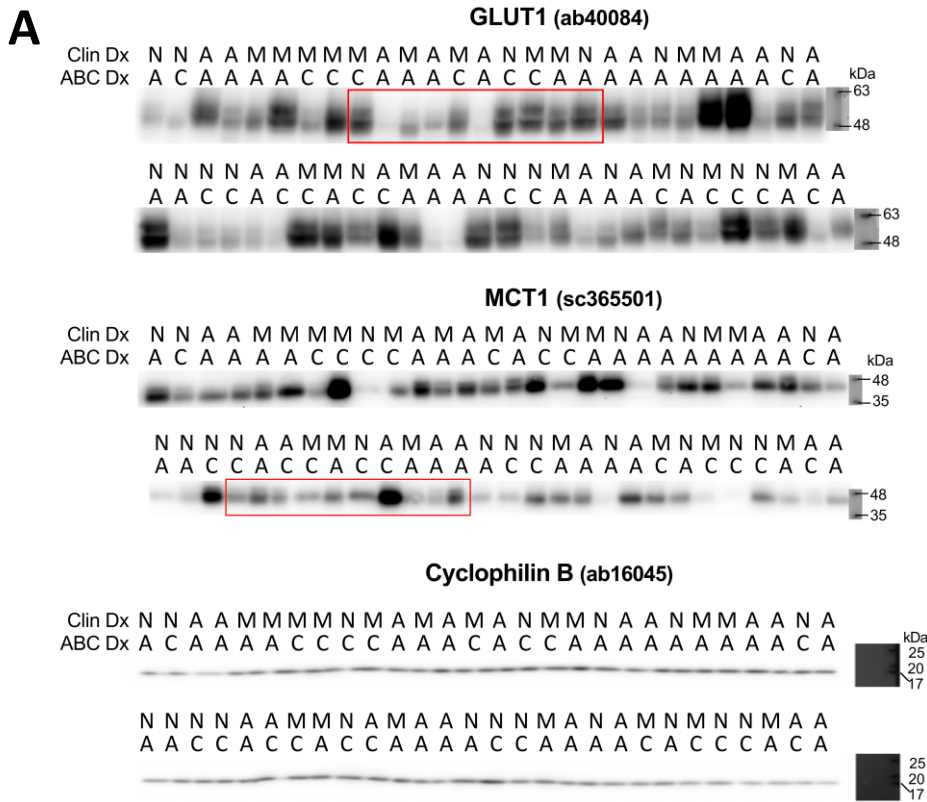
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Protein	Role/localization	Host	Dilution		Company Cat#
			WB	IF	
β-Tubulin III	Neuronal microtubules	Rb	1/8000		Sigma-Aldrich Cat# T2200
Aβ₄₀	Aβ peptide	Ms		1/100	BioLegend Cat# 805401
Aβ₄₂		Ms		1/100	BioLegend Cat# 805501
ApoE	Cholesterol carrier	Rb	1/2000		Novus biologicals Cat# NB110-55466
ApoE4 (clone 4E5)		Ms	1/5000		Millipore Cat# MABN43
Collagen IV	Basal lamina	Gt		1/500	Millipore Cat# AB769
Cyclophilin B	Ubiquitous cellular proteins Loading control	Rb	1/1000		Abcam Cat# 16045
GLUT1	Facilitated diffusion of glucose across a membrane	Ms	1/1000		Abcam Cat# 40084 (discontinued)
		Rb		1/500	Abcam Cat# 115730
MCT1	Facilitates the unidirectional proton-linked transport of monocarboxylates	Ms	1/500	1/500	Santa Cruz Biotech. Cat# sc365501
CD31	Adhesion of endothelial cells	Rb	1/1000		Abcam Cat# ab28364
P-gp	Efflux pump regulating the distribution and bioavailability of drug	Rb	1/2000		Abcam Cat# ab170904
GAP43	Schwann cells component	Rb	1/4000		Abcam Cat# ab75810
INSRα	Human INSR extracellular α chain between His28-Lys944	Gt	1/500- 1/1000		R and D Systems Cat# AF1544
INSRβ (E9L5V)	INSR cytosolic β chain and pro-INSR	Rb	1/500		Cell Signaling Technology Cat# 23413
Total Tau (OST)	Tau cytoskeletal protein	Rb	1/3000		Osenses Cat# OST00329W
p-Tau T231/S235 (AT180)		Ms	1/1000		Thermo Fisher Scientific Cat# MN1040
p-Tau S396/404 (AD2)		Ms	1/50		Biorad Cat# 56484
anti-Rabbit-HRP	HRP-conjugated	Gt	1/20 000- 1/60 000		Jackson ImmunoResearch Labs Cat# 111-035-144
anti-Mouse-HRP		Gt	1/20 000- 1/60 000		Jackson ImmunoResearch Labs Cat# 115-035-166
anti-Goat-HRP		Dk	1/30 000- 1/40 000		Jackson ImmunoResearch Labs Cat# 705-035-147
anti-Goat-AF790		Dk		1/500	Jackson ImmunoResearch Labs Cat# 705-655-147
anti-Mouse-AF555		Dk		1/500	Thermo Fisher Scientific Cat# A-31570
anti-Rabbit-AF555		Dk		1/500	Thermo Fisher Scientific Cat# A-31572

Supplementary Table 1 – List of antibodies used in this study for Western blotting and immunofluorescence

Abbreviations: Aβ, β-amyloid peptides; AF, Alexa Fluor; ApoE, Apolipoprotein E; CD31, Platelet endothelial cell adhesion molecule; Dk, Donkey; GAP43, Growth Associated Protein 43; GLUT1, Glucose transporter 1; Gt, Goat; HRP, Horseradish peroxidase; IF, Immunofluorescence; INSR, Insulin receptor; MCT1, Monocarboxylate transporter 1; Ms, Mouse; p, Phospho; P-gp, P-glycoprotein; Rb, Rabbit; WB, Western blot.



Supplemental Figure 1 – Western blots of GLUT1 and MCT1 in human brain microvascular extracts

Unedited Western immunoblotting image from experiments in human brain microvessels with MCT1 and GLUT1 with their respective antibody numbers (A) and full unedited blot for Figure 2 (B). Cyclophilin B was used as a loading control. Clinical diagnoses are given above each sample. An equal amount (8 μg) of proteins per sample was loaded. Red arrows indicate the level of protein migration and red rectangles

indicate the bands that were taken as representative photo examples in Fig.2. Samples were loaded in a random order to avoid quantification biases.

Abbreviations: A, Alzheimer's Disease; C, Control; CypB, Cyclophilin B; Clin Dx, Clinical diagnosis; GLUT1, Glucose transporter 1; M, Mild cognitive impairment; MCT1, Monocarboxylate transporter; N, Healthy control with no cognitive impairment.

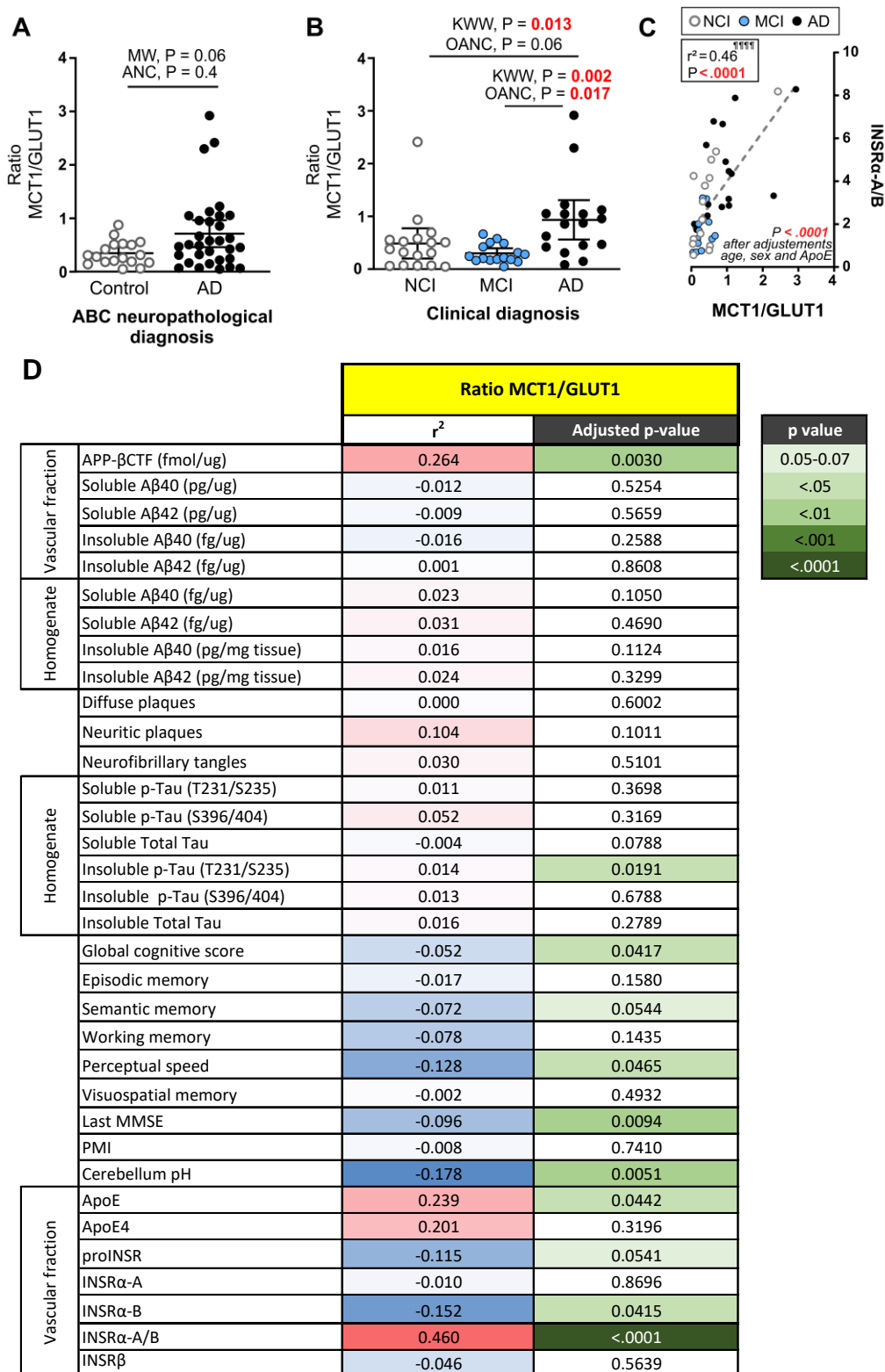
Proteins	Analyzed factor	F ratio test effect	p-values	Statistical analysis in Figure
MCT1	ABC Dx	$F_{1,52} = 0.76$	0.39	ANC
	Clinical Dx	n/a^*	n/a^*	KW
		$F_{2,53} = 1.25$	0.30	ANC
	Thal	$F_{1,53} = 0.03$	0.87	ANC
	Braak	$F_{1,53} = 0.35$	0.56	ANC
	CERAD	$F_{1,53} = 1.22$	0.28	ANC
	CAA	n/a^*	n/a^*	KW
GLUT1	ABC Dx	$F_{1,52} = 9.08$	0.004	ANC
	Clinical Dx	n/a^*	n/a^*	KW
		$F_{2,53} = 5.78$	0.006	ANC
	Thal	$F_{1,53} = 8.31$	0.006	ANC
	Braak	$F_{1,53} = 4.92$	0.032	ANC
	CERAD	$F_{1,53} = 17.02$	0.0002	ANC
	CAA	n/a^*	n/a^*	KW
MCT1/GLUT1	ABC Dx	n/a^*	n/a^*	MW
		$F_{1,50} = 0.87$	0.36	ANC
	Clinical Dx	n/a^*	n/a^*	KW
		$F_{2,50} = 4.25$	0.022	ANC

Supplementary Table 2 – Supplemental statistical analyses

Estimate effect (F ratio and p-value) of statistical analyses in Figures 2 and 3, and Supplemental Figure 2.

*A non-parametric test was used and is depicted in the Figure.

Abbreviations: ABC, Dx Neuropathological Diagnosis; ANC, Analysis of covariance; CAA, Cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; Clinical Dx, Clinical Diagnosis; GLUT1, Glucose transporter 1; KW, Kruskal-Wallis one-way analysis on ranks; MCT1, Monocarboxylate transporter 1; MW, Mann-Whitney two groups comparison.



Supplemental Figure 2 – Cerebrovascular MCT1/GLUT1 ratios are higher in clinically diagnosed AD participants, and significantly associated with INSR α -A/B ratios

(A) Subjects were compared according to the neuropathological diagnosis following ABC criteria. (B) Subjects were grouped according to the clinical diagnosis. Protein content in human microvessel extracts

were determined by Western immunoblotting. Data are represented as scatterplots, with horizontal lines depicting means of relative optical density values with 95% confidence intervals. Additional statistical analyses are available in **Suppl. Table 2**. **(C)** MCT1/GLUT1 ratio was positively associated with global INSR α -A/B. **(D)** Linear regressions between the ratio of MCT1/GLUT1 in microvessel extracts and levels of other vascular/neuropathological proteins and cognitive scores (same as in Figure 4). Linear regressions include several AD-relevant variables in human parietal cortex, soluble and insoluble proteins, APP- β CTF, A β concentrations, levels of phosphorylated-Tau T231/S235 (AT180), S396/404 (AD2), total Tau, antemortem cognitive evaluation, post-mortem interval (PMI), cerebellum pH; as well as insulin receptor (INSR) microvessel-enriched fractions of human parietal cortex.

Statistical analysis: **(A)** Mann-Whitney test to identify significant differences between two groups (MW). Analysis of covariance F-test with sex, age at death and ApoE genotype as covariates (ANC). **(B)** Kruskal-Wallis one-way analysis of variance followed by a Wilcoxon's post hoc test (KWW), One-Way Analysis of covariance F-test with sex, age at death and ApoE genotype as covariates (OANC). **(C)** Linear regressions adjusted for the following covariates: gender, age at death and APOE genotype. Coefficients of determination (r^2) are shown. **(D)** Cells highlighted in pink or darker blue, respectively, indicate significant positive and negative correlations. Deeper color shades indicate stronger r^2 values. The intensity of green color indicates the significance.

Abbreviations: A β , β -amyloid protein; ABC, Dx Neuropathological Diagnosis; AD, Alzheimer's disease; ANC, Analysis of covariance; APP- β CTF, Amyloid Precursor Protein C-terminal fragment; ApoE4, apolipoprotein E epsilon 4; GLUT1, Glucose transporter 1; INSR, Insulin receptor; KWW, Kruskal-Wallis followed by Wilcoxon's test; MCI, Mild cognitive impairment; MCT1, Monocarboxylate transporter 1; MMSE, Mini-Mental State Examination; MW, Mann-Whitney two groups comparison; NCI, Healthy controls with no cognitive impairment; OANC, One-way analysis of covariance; PMI, Postmortem Interval; proINSR, INSR precursor.