SUPPLEMENTARY INFORMATION

Efficient isolation of brain capillary from a single frozen mouse brain for protein expression analysis

Seiryo Ogata[1], Shingo Ito[1,2], Takeshi Masuda[1,2], Sumio Ohtsuki[1,2]*

[1]Department of Pharmaceutical Microbiology, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

[2]Department of Pharmaceutical Microbiology, Faculty of Life Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

*Corresponding author: Sumio Ohtsuki, Ph.D.

Department of Pharmaceutical Microbiology, Faculty of Life Sciences, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto 862-0973, Japan

TEL: +81-96-371-4323; FAX: +81-96-371-4329; E-mail: sohtsuki@kumamoto-u.ac.jp

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Table S1. Numbers of brain or brain amount needed for brain capillary isolation of each species using for proteomic analysis.

Author	Species	Numbers of brain or brain amount for isolation	Method	Reference
Gomez-Zepeda et al., 2019	mouse	5 brains	Dextran, mesh filtration	(12)
Chun et al., 2011	mouse	6 brains	Ficoll, mesh filtration, glass beads	(7)
Agarwal et al., 2012	mouse	10 brains	Ficoll, glass beads	(13)
Uchida et al., 2013	mouse	10 brains	Dextran, mesh filtration	(9)
Al Feteisi et al., 2018	rat	3 g brain	Dextran, mesh filtration	(14)
Hoshi et al., 2013	rat, marmoset	3 g brain	Dextran, mesh filtration	(15)
Kubo et al., 2015	pig	-100 g brain	Dextran, mesh filtration	(16)
Uchida et al., 2011	human	1.0-3.2 g brain	Dextran, mesh filtration	(17)
Al-Majdoub et al., 2019	human	3 g brain	Dextran, mesh filtration	(18)

Table S2. Peptide probes and MRM transitions of mouse molecules

Protein	Amino acid sequence	Unlabeled peptide (m/z)					Labeled peptide (m/z)				
		Q1	Q3-1	Q3-2	Q3-3	Q3-4	Q1	Q3-1	Q3-2	Q3-3	Q3-4
Abcb1a (Mdr1a)	ATVSASHIIR	352.2	173.1	348.7	392.2	441.8	354.5	173.1	352.2	395.7	445.3
Abcc4 (Mrp4)	APVLFFDR	482.8	268.2	584.3	697.4	796.4	486.3	268.2	584.3	704.4	803.5
Abcg2 (Bcrp)	ENLQFSAALR	574.8	517.3	664.4	792.4	905.5	578.3	524.3	671.4	799.5	912.5
Slc2a1 (Glut1)	TFDEIASGFR	571.8	466.2	537.3	650.4	894.4	573.8	466.2	541.3	654.4	898.4
Slc16a1 (Mct1)	SDANTDLIGGSPK	637.8	445.2	786.4	887.4	887.5	641.3	445.2	793.5	894.4	894.5
Slc22a8 (Oat3)	YGLSDLFR	485.8	435.3	500.3	637.3	750.4	489.3	442.3	557.3	644.3	757.4
Insr	ESLVISGLR	487.3	432.3	545.3	644.4	757.5	490.8	439.3	552.4	651.4	764.5
Lrp1	GDYSVLVPGLR	588.3	442.3	541.3	654.4	753.5	591.8	449.3	548.4	661.4	760.5
Tfr1	SSVGTGLLLK	487.8	400.8	543.4	701.5	800.5	491.3	404.3	550.4	708.5	807.5
Claudin-5	EFYDPTVPVSQK	705.4	428.3	558.3	855.5	970.5	708.4	431.3	564.3	861.5	976.5

Table S3. Absolute protein expression levels of transporters, receptors and tight junction protein in brain capillary fraction isolated by the standard method and the developed method by MRM.

	Protein name	Protein amount (fmol/μg protein)		Fold change (D/S)	P-value
		Standard	Developed		
Abc transporter	Abcb1a (Mdr1a/P-gp)	9.02 ± 0.92	18.3 ± 1.5	2.03	8.67.E-05
	Abcc4 (Mrp4)	0.547 ± 0.040	0.797 ± 0.044	1.46	2.86.E-02
	Abcg2 (Bcrp)	2.69 ± 0.14	3.62 ± 0.73	1.35	5.71.E-02
Slc transporter	Slc2a1 (Glut1)	105 ± 5	194 ± 9	1.84	3.63.E-04
	Slc16a1 (Mct1)	10.3 ± 0.7	18.2 ± 1.5	1.77	1.42.E-04
	Slc22a8 (Oat3)	1.92 ± 0.18	2.33 ± 0.57	1.21	2.77.E-01
Receptor	Insr	0.952 ± 0.039	1.38 ± 0.14	1.45	7.19.E-03
	Lrp1	0.993 ± 0.082	1.03 ± 0.17	1.04	7.11.E-01
	Tfr1	2.76 ± 0.36	5.49 ± 0.49	1.99	2.29.E-04
Tight junction protein	Claudin-5	6.65 ± 0.71	13.6 ± 1.1	2.04	9.91.E-05

The data represents the mean±SD (n=4). D; Developed, S; Standard

Table S4. Numbers of identified and significantly changed proteins in isolated brain capillary fractions.

A

	Identified	Significant (p or FDR<0.05)	Significant and fold>2.0	Significant and fold<0.5
Student t-test	1352	1065	100	258
Benjamini–Hochberg method		1051	99	258

B

	Identified	Significant (p or FDR<0.05)	Significant and fold>1.25	Significant and fold<0.8
Student t-test	1100	79	28	1
Benjamini–Hochberg method		0	0	0

(A) Number of identified and significantly changed proteins in isolated brain capillary fractions of standard method and the developed method. The volcano plot and original data were shown in Figure 3A and Table S7, respectively. (B) Number of identified and significantly changed proteins in isolated brain capillary fractions of Wt and Glut1$^{+/-}$ mice. The volcano plot and original data were shown in Figure 5A and Table S8, respectively. The p-value was evaluated using Student's t-test, and FDR (q-value) was evaluated using Benjamini–Hochberg method.

Table S5. Significantly different proteins in brain capillary fraction isolated from Wt and Glut1$^{+/-}$ mouse using proteomic analysis.

Uniprot ID	Protein name	Fold change (Glut1$^{+/-}$/Wt)	P-value	
E9QA15	Caldesmon 1	1.85	8.86.E-03	(2)
Q9QWI6	SRC kinase signaling inhibitor 1	1.61	2.79.E-04	
Q7TPR4	Alpha-actinin-1	1.46	1.48.E-02	(2)
Q62261	Spectrin beta chain, non-erythrocytic 1	1.46	1.69.E-02	
E9Q0S6	Tensin 1	1.44	1.79.E-02	(2)
Q9D2N4	Dystrobrevin alpha	1.44	9.02.E-03	
P16546	Spectrin alpha chain, non-erythrocytic 1	1.43	9.70.E-03	
P97823	Acyl-protein thioesterase 1	1.43	9.56.E-03	(2)
P26041	Moesin	1.41	6.41.E-03	(2)
Q9WV02	RNA-binding motif protein, X chromosome	1.40	1.03.E-02	
Q61234	Alpha-1-syntrophin	1.38	2.44.E-02	
Q9Z204	Heterogeneous nuclear ribonucleoproteins C1/C2	1.37	1.79.E-02	(2)
P26039	Talin-1	1.37	1.63.E-02	
P06837	Neuromodulin	1.36	1.17.E-02	
Q9CY58	Plasminogen activator inhibitor 1 RNA-binding protein	1.36	2.65.E-03	(2)
P11531	Dystrophin	1.36	8.90.E-03	
Q9QXS1	Plectin	1.32	8.43.E-04	
P55284	Cadherin-5	1.32	4.41.E-04	(1)
Q61033	Lamina-associated polypeptide 2, isoforms alpha/zeta	1.32	1.27.E-02	
Q8BTM8	Filamin-A	1.31	1.26.E-02	(2)
P57780	Alpha-actinin-4	1.31	1.08.E-02	(2)
P26040	Ezrin	1.30	2.80.E-02	
Q62417	Sorbin and SH3 domain-containing protein 1	1.29	1.72.E-02	
Q8VHM5	Heterogeneous nuclear ribonucleoprotein R	1.28	4.36.E-02	
Q07417	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	1.27	5.57.E-03	

P46638	Ras-related protein Rab-11B	1.26	1.76.E-02	
P53994	Ras-related protein Rab-2A	1.26	1.69.E-02	
O54724	Polymerase I and transcript release factor	1.25	8.44.E-03	(1)
P04370	Myelin basic protein	0.738	3.54.E-02	

The data was extracted from the proteomic analysis results. Proteins were extracted by using the following criteria: $p < 0.05$ and >1.25-fold. (1) and (2) in the right-most column indicate the brain capillary endothelial cell (BCEC) specific and selective protein [(expression levels at most highly expressed cell types/expression levels at secondary highly expressed cell types) extracted from the mouse brain RNA-sequencing database is over 10 and 1.0-fold].

Table S6. Expression levels of brain cells marker protein of both isolated brain capillary fraction and whole brain fraction using proteomic analysis.

Cell types	Protein name	Fold change (Brain capillary/Whole brain)	P-value
Brain capillary endothelial cell	Mdr1a	46.6	3.53.E-05
Astrocyte	Gfap	4.10	7.32.E-04
Pericyte	Mcam	4.78	4.09.E-04
Neuron	Syp	0.183	3.00.E-08
Oligodendrocyte	Mog	0.0818	1.04.E-07
Microglia	Coro1a	0.364	1.82.E-04

Table S7. Identified proteins in the brain capillary fraction of mice isolated by the standard method and the developed method using proteomic analysis.

Table S8. Identified proteins in brain capillary fraction isolated from Wt and Glut1$^{+/-}$ mice using proteomic analysis.

Table S9. Identified proteins in isolated brain capillary fraction and whole brain fraction using proteomic analysis.

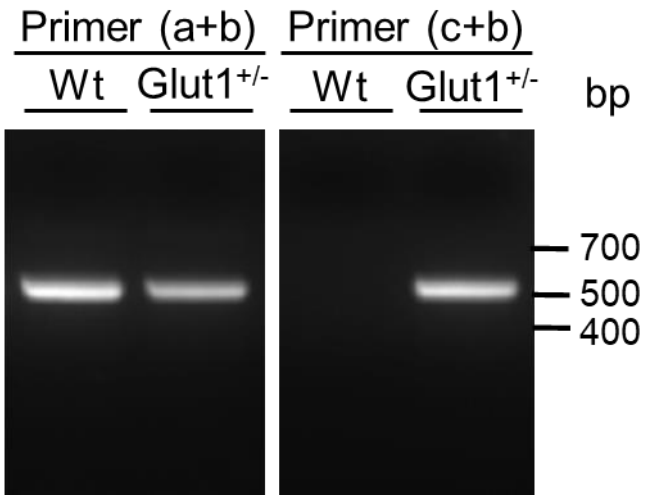

Figure S1. Genotyping of Glut1 by genomic PCR analysis. The genotyping of all offsprings was analyzed using PCR of genomic DNA from the tail, as previously reported[19]. Primer (a+b) detected the wild type allele, and primer (c+b) detected the mutated allele.

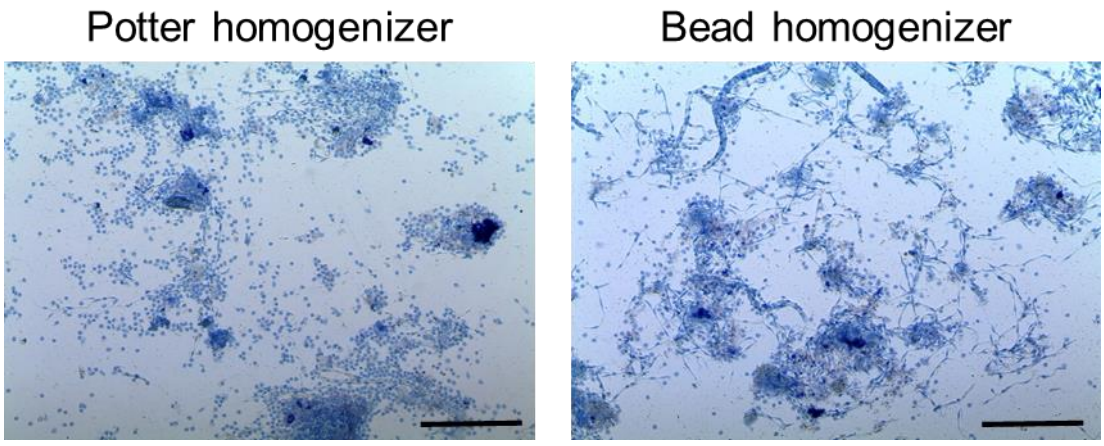

Figure S2. Images of suspended sample of the pellet which is concentrated after dextran centrifuge of brain homogenized by Potter homogenizer (left) and bead homogenizer (right). Each sample was stained by trypan blue. Scale bar = 250 μm.

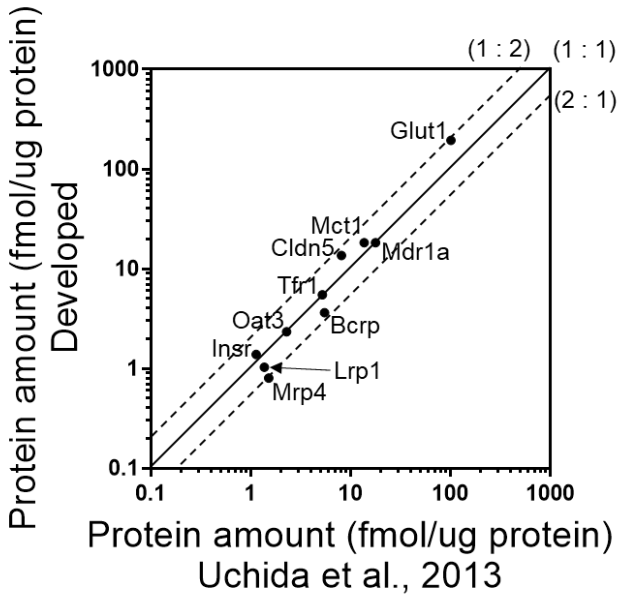

Figure S3. Absolute protein expression levels of transporters, receptors, and tight junction protein between brain capillary fraction isolated by the developed method and previous report[9]. The solid line passing through the origin represents the line of identity, and the broken lines represent 2-fold differences.

Figure S4. Full unedited blot for Figure 2D.

Figure S5. Full unedited blot for Figure 4C.

Figure S6. Full unedited blot for Figure 6A.