Supplemental Information

Several transporters previously identified in the human BBB, such as *SLC7A5*, *SLCO2B1*, *ABCB1*, and *ABCG2*, were also enriched in our BMV samples (Figure 1A). Enrichment of these and several other genes (*SLC22A3*, *SLC47A1*, *SLC47A2* and *SLC5A6*) was confirmed by TaqMan gene expression analysis of three paired cerebral cortex-brain microvessel (BMV) samples (Figure S2). OpenArray results indicated that *SLC19A2, SLCO1A2,* and *SLCO3A1* were not enriched in BMV samples, and were included as negative controls. Furthermore, linear regression analysis on the OpenArray and TaqMan enrichment values yielded an r^2 = 0.60 (Figure S2B), indicating good agreement between the two RT-PCR technologies, and confirming the OpenArray-based expression profiling results.

Materials and Methods

Tissue acquisition. Four partial human cerebral cortex samples were procured from the National Disease Research Interchange (Philadelphia, PA). Donor demographics and medical history are provided in Table S1. Normal human liver and kidney tissue were commercially obtained (Asterand, Detroit, MI) for comparative expression profiling. All tissues were acquired in accordance with UCSF Institutional Review Board and ethics committee guidelines (IRB # 11-06153).

BMV isolation. BMVs were isolated using a previously established protocol (1). Approximately one gram of tissue was homogenized in ice cold Hanks' balanced salt solution (HBSS; Invitrogen, Carlsbad, CA) with a Potter–Elvehjem homogenizer

(Thomas Scientific, Swedesboro, NJ). The homogenate was centrifuged for 5 min at 1000 *g* at 4°C, and the supernatant was aspirated. The pellet was resuspended in an autoclaved 17% dextran solution (Sigma-Aldrich, St Louis, MO) and centrifuged for 15 min at 4250 *g* at 4°C. The myelin-enriched supernatant was aspirated and the resulting BMV-enriched pellet was resuspended in ice cold HBSS. This solution was applied to a 40 µm nylon mesh filter, and BMVs retained on the filter were washed with 40 ml of ice cold HBSS. BMVs were recovered from the filter, and centrifuged for 5 min at 4250 *g* at 4°C. The resulting pellet was then used immediately for RNA extraction or IHC.

RNA extraction and reverse transcriptase PCR. Total RNA was extracted from the BMV-enriched samples (n=3), cerebral cortex (n=3), liver (n=60), and kidney (n=59) tissue homogenates using TRIzol Reagent and RNeasy Plus Micro Kit (Qiagen, Valencia, CA), according to the kit instructions. After separation of the organic and aqueous phases by centrifugation, total RNA was isolated from the aqueous phase using an RNeasy Plus Micro Kit. After isolation, RNA samples were stored at -80°C. Total RNA (up to 1 µg) was reverse transcribed using the SuperScript VILO cDNA Synthesis Kit (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. The resulting cDNA samples were stored at -80°C.

Real Time-PCR. High-throughput, real-time PCR (RT-PCR) was performed using a customized OpenArray® system (Life Technologies) as previously described (2). cDNA at a concentration of 108 ng/µL and SYBR Green qPCR reagent (Fast Start DNA SYBR Green kit, Roche, CA) were dispensed into custom plates containing 448 pre-validated

real-time SYBR Green PCR assays. RT-PCR occurred in a computer-controlled imaging thermal cycler where 9216 PCR amplifications and dissociation curves were implemented in less than four hours. The transporter genes analyzed and the PCR primers used are described in a previous study (2). Post-acquisition data processing generated fluorescence amplification and melt curves for each through-hole in the array, from which cycle threshold (C_T) and melt temperature (T_m) were computed and used for further data analysis.

Microplate-based SYBR green and TaqMan RT-PCR was used to measure cell type marker gene expression and confirm array-based gene expression results. Two ng of BMV or cerebral cortex cDNA was used as a template, and mixed with PCR primers at for either platelet endothelial cell adhesion molecule (*PECAM1*), vascular endothelial cadherin (*VEC*), glucose transporter 1 (*GLUT1*), synaptophysin (*SYP*), or glial fibrillary acidic protein (*GFAP*) for SYBR green RT-PCR (see Table S3 for primer sequences). For TaqMan RT-PCR, the following TaqMan Gene Expression Assays (Life Technologies) were mixed with two ng of template cDNA: GAPDH (Hs99999905_m1), ACTB (Hs01060665_m1), PGK1 (Hs99999906_m1), ABCB1 (Hs00184491_m1), ABCG2 (Hs01053790_m1), SLC2A1 (Hs00892681_m1), SLC5A6 (Hs00221573_m1), SLC7A5 (Assay ID: Hs01001190_m1), SLC19A2 (Hs00949696_m1), SLC22A3 (Hs01009568), SLC47A1 (Hs00217320), SLC47A2 (Hs00398719_m1), SLCO1A2 (Hs00366488_m1), SLCO2B1 (Hs_00200670), and SLCO3A1 (Hs00203184_m1). RT-PCR reactions were carried out in 96-well reaction plates in a volume of 10 µL using either Fast SYBR green Universal Master Mix or TaqMan Fast Universal Master Mix (Life Technologies). Reaction plates were run on the Applied Biosystems 7900HT Fast

Real-Time PCR System with the following profile: 95°C for 20 seconds followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds.

The relative expression of each gene in the different tissues was calculated by the $\Delta\Delta C_T$ comparative expression method (3). The ΔC_T values for all the genes in each sample were calculated by subtracting the mean C_T values for three housekeeping genes (GAPDH, β-Actin, and β2 microglobulin) from the C_T for each target gene. The relative quantity of each gene was then determined by calculating the $2^{-\Delta C_T}$ value and multiplying by 100 to obtain the percent of housekeeping genes. To determine the fold change in gene expression relative to a reference tissue, the log2-transformed relative quantity values from each tissue were compared to each other.

Immunohistochemistry. IHC analysis of isolated BMVs was performed as previously described (4). BMV-enriched pellets were resuspended in ice cold HBSS and seeded onto positively charged glass slides, and fixed with 4% paraformaldehyde for 15 min at 4°C. Slides were then washed and stored in HBSS at 4°C until use (within one week of fixing). Fixed BMVs were permeabilized and nonspecific antibody binding blocked by treatment with 30% normal goat serum in phosphate-buffered saline (PBS) containing 0.1% tween 20 (PBST) at pH 7.4 for 1 hr at room temperature, and incubated overnight at 4°C in PBS containing 3% normal goat serum and primary antibodies against the following target proteins: LAT1 (Epitomics, Burlingame, CA), OCT3 (Genway, San Diego, CA), RFC (Sigma-Aldrich), MATE1 (Sigma-Aldrich), OATP1B3 (Sigma-Aldrich) and Rfc (LifeSpan Biosciences, Seattle, WA). Negative control sections were stained

without primary antibody at this step. Slides were then washed with PBS 6x 5 min and incubated with an Alexa 488 conjugated secondary antibody (Life Technologies) for 1 hr at room temperature. Slides were washed with PBS 6x 5 min again, and mounted in VECTASHIELD Mounting Medium with DAPI (Vector Labs, Burlingame, CA). Fluorescent imaging was done on a Zeiss AxioImager M1 microscope with AxioCam Mrm camera (Carl Zeiss Microscopy, Thornwood, NY), and bright field imaging was done on a Leica DM IL LED microscope with DFC400 camera (Leica Microsystems, Buffalo Grove, IL). Image files were processed with ImageJ (5).

Western blot analysis. Protein was extracted from BMVs, and transporter- and empty vector transfected human embryonic kidney cells by incubating with prechilled CellLytic Mcell lysis buffer (Sigma-Aldrich, St Louis, MO) containing a protease inhibitor cocktail for 20 min at 4°C. Homogenates were centrifuged for 10 min at 15,000 rpm at 4°C, and the protein concentration of the supernatant determined by BCA protein assay (Thermo Fisher Scientific, Rockford, IL) using the manufacturer's protocol. Up to 50 µg of total protein was subjected to SDS-PAGE using a tris-glycine 4-15% polyacrylamide gel (Bio-Rad, Hercules, CA), and then transferred onto a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA). Membranes were blocked in Protein-free T20 Blocking Buffer (Thermo Fisher Scientific, Rockford, IL) for 1 hr at room temperature, and then incubated with primary antibodies diluted in blocking buffer overnight at 4°C (see Materials and Methods section for antibody information). Membranes were then washed with tris-buffered saline containing 0.1% tween 20 (TBST) at pH 7.4 6x 5 min prior to incubating with HRP-conjugated goat anti-rabbit IgG diluted in TBST for 1 hr at room

temperature. Membranes were then washed 6x 5 min in TBST again, and developed with SuperSignal West Femto Kit (Thermo Fisher Scientific, Rockford, IL) using the manufacturer's protocol. All scanned membrane images were processed using ImageJ.

Supplemental Figures

Figure S1. Validation of BMV enrichment from cerebral cortex samples. (A) Representative image of an aliquot from a BMV enriched sample. The scale bar is set to 50 µm. (B) Expression of cell-type marker genes in BMV and paired cerebral cortex samples from donor #1, #2, and #3 (see Table S1). Messenger RNA expression levels in BMVs are normalized to paired cerebral cortex samples. Platelet endothelial cell adhesion molecule (*PECAM1*) and vascular endothelial cadherin (*VEC*) are pan-endothelial cell markers, while glucose transporter 1 (*GLUT1*) is a brain endothelial cell marker. Synaptophysin (*SYP*) is a neuronal cell marker and glial fibrillary acidic protein (*GFAP*) is an astrocytic cell marker. Values represent the mean ± SEM (n=3).

Figure S2. Validation of OpenArray gene expression results. (A) The mRNA expression levels of 12 genes in BMV and paired cerebral cortex samples from donor #1, #2, and #3 (see Table S1) were determined by TaqMan RT-PCR and compared to OpenArray results. (B) Linear regression of the relative mRNA expression in BMV samples determined by TaqMan RT-PCR versus those determined by OpenArray RT-PCR (r^2=0.60). All values represent the mean ± SD. Linear regression was done using Prism 5 GraphPad software.

Figure S3. Confirmation of SLC transporter protein expression in BMVs and antibody specificity. Western blot analysis of BMVs isolated from donor one detected the expression of OCT3 (A), MATE1 (B), and RFC (C). (D) OATP1B3 was included as a negative control and was not detected in BMVs. In all panels, antibody specificity was determined using HEK cells transfected with OCT3, MATE1, RFC, OATP1B3, or EV. All molecular weights indicated are in kDa. TT, transporter transfected cell lysate; EV, empty vector transfected cell lysate.

References

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(4) Roberts, L.M. *et al.* Subcellular localization of transporters along the rat blood-brain barrier and blood-cerebral-spinal fluid barrier by in vivo biotinylation. *Neuroscience* 155, 423-38 (2008).

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Table S1. Tissue donor information.

Donor	Brain Region	Analysis	Age	Sex	Race	Cause of Death	Comorbidities
1	Cortex	OpenArray, TaqMan, IHC, WB	62	M	C	Respiratory arrest	COPD, cardiomyopathy, high blood pressure
2	Cortex	OpenArray, TaqMan	63	M	C	Cardio-pulmonary arrest	COPD, hypertension, heart disease, hypothyroidism
3	Cortex	TaqMan	68	F	C	Intracranial hemorrhage	Hypertension
4	Cortex	IHC	65	M	C	Cardiac arrest	Heart disease, hypertension, bladder cancer, anemia, gout, type II diabetes

IHC, immunohistochemistry; WB, western blot; M, Male; F, Female; C, Caucasian;

COPD, chronic obstructive pulmonary disease.

Table S2. Genes expressed in human BMVs in comparison to housekeeping genes and paired cerebral cortex samples.

Gene	Relative Expression (% of Housekeeping genes)	Relative Expression (Fold over cerebral cortex)
ABCA10	0.377	ND
ABCA12	0.127	0.518
ABCA13	0.281	0.927
ABCA2	6.59	2.07
ABCA3	0.772	1.00
ABCA5	1.11	1.46
ABCA6	0.482	2.65
ABCA7	0.191	1.67
ABCA8	0.224	0.729
ABCA9	1.077	3.12
ABCB1	13.1	10.4
ABCB10	0.821	1.99
ABCB11	0.132	0.447
ABCB4	0.195	1.19
ABCB6	3.87	2.96
ABCB7	0.509	2.57
ABCB8	0.201	1.88
ABCB9	0.209	ND
ABCC1	0.806	0.987
ABCC10	0.895	3.77
ABCC12	0.499	1.53
ABCC2	0.278	2.27
ABCC3	0.110	1.17
ABCC4	0.337	0.678
ABCC5	5.81	2.11
ABCC6	0.187	1.87
ABCC7	0.205	0.647
ABCC8	2.30	4.58
ABCC9	1.10	1.86
ABCD1	0.134	ND
ABCD2	0.481	0.856
ABCD3	5.14	1.36
ABCD4	0.257	0.939
ABCE1	0.549	1.22
ABCF1	2.00	1.26
ABCF2	0.984	1.56

ABCF3	0.867	3.99
ABCG1	1.62	0.773
ABCG2	4.15	8.96
ABCG4	0.693	1.18
ABCG5	0.186	1.05
ABCG8	0.515	3.17
SLC10A1	0.191	ND
SLC10A3	0.165	1.55
SLC10A4	0.510	1.34
SLC10A5	0.327	0.581
SLC10A6	0.192	1.46
SLC10A7	0.194	1.84
SLC11A2	0.322	0.600
SLC12A1	0.206	2.23
SLC12A2	0.519	1.63
SLC12A5	12.9	0.504
SLC12A6	0.909	1.09
SLC12A7	6.28	1.71
SLC13A3	0.179	1.65
SLC14A1	1.07	0.693
SLC15A1	0.115	1.27
SLC15A3	0.440	2.89
SLC15A4	0.837	0.985
SLC16A1	0.591	1.20
SLC16A10	0.243	0.662
SLC16A11	0.320	1.90
SLC16A13	0.0957	ND
SLC16A2	0.880	0.967
SLC16A4	0.298	ND
SLC16A6	0.357	1.93
SLC16A7	0.835	0.933
SLC16A8	0.323	2.86
SLC16A9	0.218	1.32
SLC17A2	0.256	1.54
SLC17A3	0.223	3.07
SLC17A4	0.301	ND
SLC17A5	0.478	0.906
SLC17A6	0.382	0.466
SLC17A7	28.3	1.06
SLC18A2	0.279	1.40
SLC19A1	0.642	ND

SLC19A2	0.293	0.696
SLC19A3	2.73	5.01
SLC1A1	2.63	0.834
SLC1A2	33.8	0.788
SLC1A3	0.663	1.21
SLC1A4	5.75	1.48
SLC1A6	0.172	2.15
SLC20A1	1.56	1.02
SLC20A2	5.86	1.06
SLC22A1	0.206	1.76
SLC22A10	0.357	1.46
SLC22A13	0.178	1.03
SLC22A15	0.788	1.30
SLC22A17	1.34	2.20
SLC22A18	0.280	1.04
SLC22A2	0.192	0.573
SLC22A23	0.495	2.23
SLC22A2 splice	0.113	0.724
SLC22A3	0.684	2.46
SLC22A5	1.20	1.51
SLC22A6	0.395	1.95
SLC22A7	0.146	1.22
SLC22A8	0.219	1.85
SLC22A9	1.94	1.38
SLC23A2	3.12	0.776
SLC24A1	0.183	1.16
SLC24A2	2.15	0.454
SLC24A3	0.334	0.489
SLC24A4	0.383	1.16
SLC24A6	0.191	1.55
SLC25A1	0.396	1.66
SLC25A10	0.164	1.40
SLC25A11	0.671	1.40
SLC25A12	2.22	0.816
SLC25A13	0.941	1.04
SLC25A14	0.271	1.10
SLC25A15	0.169	1.15
SLC25A16	0.491	1.12
SLC25A17	0.403	0.839
SLC25A18	1.57	1.41

SLC25A20	0.302	0.928
SLC25A22	4.22	0.667
SLC25A23	2.94	2.20
SLC25A24	0.298	2.68
SLC25A25	0.553	1.71
SLC25A27	1.10	0.476
SLC25A28	3.59	0.827
SLC25A29	0.149	1.48
SLC25A3	5.82	0.559
SLC25A30	0.754	1.45
SLC25A32	0.234	1.63
SLC25A36	3.01	1.55
SLC25A38	0.500	1.25
SLC25A39	0.759	2.93
SLC25A4	1.85	0.807
SLC25A41	0.249	ND
SLC25A42	0.207	1.50
SLC25A44	0.508	0.722
SLC25A5	3.88	0.627
SLC25A6	5.45	1.27
SLC25A9	0.180	1.43
SLC26A1	0.230	ND
SLC26A10	0.193	ND
SLC26A11	0.149	1.72
SLC26A2	0.968	5.11
SLC26A3	0.148	ND
SLC26A4	0.240	1.44
SLC26A6	0.382	3.69
SLC26A7	0.218	1.15
SLC26A8	0.210	0.902
SLC27A1	2.83	ND
SLC27A4	0.533	1.06
SLC27A6	0.168	1.43
SLC28A2	0.477	0.879
SLC28A3	0.117	ND
SLC29A1	0.419	2.34
SLC29A2	0.611	2.62
SLC29A3	0.272	1.47
SLC2A1	1.09	4.80
SLC2A2	0.162	1.06
SLC2A3	22.6	1.88

SLC2A4	0.149	ND
SLC2A5	0.356	1.04
SLC2A6	0.621	4.12
SLC2A8	0.455	0.901
SLC2A9	0.184	ND
SLC30A4	0.529	1.28
SLC30A7	0.279	1.30
SLC30A9	1.88	0.682
SLC31A1	1.86	1.39
SLC31A2	0.274	0.325
SLC32A1	0.339	0.589
SLC33A1	0.616	0.955
SLC35A1	2.42	1.51
SLC35A2	0.715	0.728
SLC35A3	0.252	2.36
SLC35A4	0.961	1.21
SLC35A5	0.330	1.04
SLC35B1	0.627	0.553
SLC35B2	0.268	0.622
SLC35B3	0.169	0.887
SLC35B4	0.615	0.540
SLC35C1	0.235	1.82
SLC35C2	0.277	0.990
SLC35D2	0.594	3.24
SLC35E1	1.24	0.538
SLC35E2	5.43	1.57
SLC35F1	0.902	0.346
SLC35F2	0.391	1.04
SLC35F3	0.105	ND
SLC35F5	4.03	1.51
SLC36A1	0.793	0.843
SLC36A4	0.613	0.745
SLC37A1	0.527	1.30
SLC37A3	0.177	1.31
SLC37A4	0.665	1.04
SLC38A1	1.20	0.453
SLC38A10	0.157	1.85
SLC38A11	1.90	10.5
SLC38A2	19.9	1.15
SLC38A3	6.39	2.30
SLC38A5	11.2	11.2

SLC38A6	0.173	ND
SLC38A7	0.223	2.45
SLC38A9	0.739	0.828
SLC39A1	0.751	1.86
SLC39A10	2.86	1.05
SLC39A12	0.285	0.444
SLC39A13	0.226	1.05
SLC39A14	0.333	0.738
SLC39A2	5.45	0.779
SLC39A3	1.28	1.00
SLC39A6	0.775	0.958
SLC39A7	0.933	2.97
SLC39A8	0.853	3.36
SLC39A9	0.248	0.942
SLC3A1	6.01	0.530
SLC3A2	3.66	1.31
SLC40A1	0.705	2.25
SLC41A1	0.714	1.21
SLC41A2	0.539	0.454
SLC41A3	0.342	0.383
SLC42A3	0.173	ND
SLC43A2	1.10	1.13
SLC43A3	0.414	1.78
SLC44A1	3.80	0.688
SLC44A2	7.07	1.17
SLC44A5	0.446	1.22
SLC45A1	0.186	1.19
SLC45A3	0.104	ND
SLC45A4	2.15	1.41
SLC46A2	0.192	ND
SLC46A3	0.517	0.996
SLC47A1	0.735	8.42
SLC47A2	0.205	2.06
SLC48A1	1.51	0.872
SLC4A10	13.3	0.767
SLC4A4	2.06	0.711
SLC4A5	0.178	2.21
SLC4A7	0.132	1.35
SLC4A8	0.580	0.391
SLC5A1	0.110	0.792
SLC5A10	0.194	2.48

SLC5A11	0.235	1.34
SLC5A4	0.779	1.68
SLC5A6	2.04	4.42
SLC6A1	1.93	2.48
SLC6A11	0.239	0.474
SLC6A12	2.61	12.0
SLC6A13	0.808	7.93
SLC6A15	0.817	0.394
SLC6A16	0.141	1.75
SLC6A17	2.48	0.665
SLC6A2	0.282	2.41
SLC6A20	0.251	2.05
SLC6A4	0.117	0.621
SLC6A7	1.44	0.756
SLC6A8	4.70	0.805
SLC7A1	4.80	2.72
SLC7A10	0.220	1.97
SLC7A11	1.54	1.16
SLC7A14	0.381	0.300
SLC7A2	0.518	3.10
SLC7A4	0.180	1.59
SLC7A5	6.91	13.4
SLC7A6	4.64	1.39
SLC7A7	0.656	2.32
SLC7A8	3.33	0.910
SLC7A9	0.272	1.27
SLC8A1	1.35	0.429
SLC8A2	1.66	0.599
SLC8A3	0.889	1.16
SLC9A1	0.893	0.773
SLC9A11	0.197	0.775
SLC9A2	0.676	1.11
SLC9A3R2	2.68	4.44
SLC9A5	0.322	ND
SLC9A6	1.76	0.412
SLC9A7	0.250	0.676
SLC9A8	0.287	1.98
SLC9A9	0.824	1.95
SLCO1A2	5.62	1.39
SLCO1B3	0.317	2.52
SLCO1C1	0.434	0.679

SLCO2A1	0.400	1.07
SLCO2B1	19.1	8.34
SLCO3A1	0.744	0.870
SLCO4A1	1.00	3.10
SLCO5A1	0.843	3.96

ND, not determined.

All values represent the mean of n=2 BMV samples.

Table S3. PCR primers for cell-type marker genes analyzed by microplate SYBR green-based RT-PCR.

Gene Symbol	Forward Primer	Reverse Primer
GFAP	AGATGGCCCGCCACG	GCACGGGAATGGTGATCCGGT
PECAM1	GCGAGTCATGGCCCC	GCCACATCGTGGCCCCTTGG
SYP	CACCTCGGTGGTGTT	CGGGTGCCGGTTGTTTCTCG
VEC	TGGCCTGTGTTCACGG	CTGAGGTCCCCACAGCCGAC

A

B

Relative Expression
(Fold over cerebral cortex)

70
60
50
40
30
10
8
6
4
2
0

BMV
CC

PECAM1 VEC GLUT1 SYP GFAP

A

Relative Expression
(Fold over cerebral cortex)

30
20
10
0

TaqMan
OpenArray

ABCB1
ABCG2
SLC19A2
SLC22A3
SLC2A1
SLC47A1
SLC47A2
SLC5A6
SLC7A5
SLCO1A2
SLCO2B1
SLCO3A1

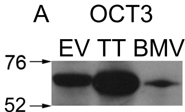
B

TaqMan
(BMV fold over cerebral cortex)

25
20
15
10
5
0

0
5
10
15

OpenArray
(BMV fold over cerebral cortex)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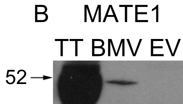
OCT3
EV TT BMV
76
52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TT BMV EV
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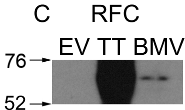
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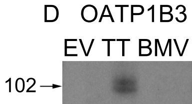
RFC
EV TT BMV
76
52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