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Title

Quantitation of plasma membrane drug transporters in kidney tissue and cell lines using a novel proteomic approach enabled a prospective prediction of metformin disposition

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Supplemental Tables

Supplemental Table 1

Tissue Demographics

Donor	Subject ID	Anatomical Site	Tissue Specification	Ethnicity (Race)	Age	Sex	Procurement Type	Cause of death	PMI (hrs)
1	AMS-12-11-S1-31	Kidney, cortex	Normal	Caucasian (White)	38	Male	Autopsy	Traumatic Injury	3
2	AMS-12-11-S2-31	Kidney, cortex	Normal	Caucasian (White)	52	Female	Autopsy	Traumatic Injury	4
3	AMS-12-11-S3-31	Kidney, cortex	Normal	Caucasian (White)	30	Male	Autopsy	Traumatic Injury	4
4	AMS-24-11-S4-31	Kidney, cortex	Normal	Caucasian (White)	30	Male	Autopsy	Traumatic Injury	4
5	AMS-09 11-S6-29	Kidney, cortex	Normal	Caucasian (White)	46	Male	Autopsy	Traumatic Injury	3
6	AMS-09 11-S7-29	Kidney, cortex	Normal	Caucasian (White)	47	Male	Autopsy	Traumatic Injury	3
7	113AFJF185	Kidney, cortex	Normal	Caucasian (White)	60	Male	Autopsy	CVA Stroke; Organ recovery	1
8	113AGET292	Kidney, cortex	Normal	Caucasian (White)	41	Male	Organ recovery	Blunt head trauma	1
9	46-116068	Kidney, cortex	Normal	Caucasian (White)	43	Male	Autopsy	Pulmonary edema	8

PMI, Post-Mortem Interval

Supplemental Table 2

Sequences and multiple reaction monitoring transitions of the surrogate peptides for OCT2, MATE1, MATE2K, Na⁺/K⁺-ATPase and GGT1

Protein	Peptide type	Peptide sequence	MW	Q1	Q3	DP	CE
OCT2	Native	SLPASLQR	870.5	436.1	671.6	30	18
				436.1	574.4	30	27
				436.1	503.3	30	28
				439.6	678.2	41	23
				439.6	581.3	41	29
	SIL	SLPASLQR	877.955	439.6	510.2	41	27
	Native	GGPEATLEVR	1027.529	514.8	688.5	34	30
				514.8	617.3	38	23
				517.2	693.3	50	29
				517.2	622.3	50	27
MATE1	Native	TPEEAHALSAPTSR	1033.09	733.7	802.5	101	45
				733.7	618.4	101	45
				733.7	460.2	101	45
				736.7	808.4	66	45
				736.7	624.4	66	43
	SIL	TPEEAHALSAPTSR	1472.528	736.7	466.2	66	43
	Native	VDNSSLTGESEPQTR	1619.65	540.8	501.3	60	23
				542.7	507.1	60	25
	SIL	VDNSSLTGESEPQTR	1625.618	516.8	717.3	69	28
				516.8	588.3	69	28
MATE2K	Native	AAVPDAVGK	826.94	414.2	586.3	60	26
				417.2	592.3	60	28
	SIL	AAVPDAVGK	832.905	516.8	501.3	69	28
Na ⁺ /K ⁺ -ATPase	Native	ESVESPEQK	1031.48	516.8	588.3	69	28
				516.8	501.3	69	28
GGT1	Native	ESVESPEQK	1031.48	516.8	501.3	69	28

The bold faced letters indicate the location of stable-isotope-label (SIL). Two or three MRM transitions are monitored for each peptide (OCT2, MATE1, MATE2K and GGT1) and the concentrations were calculated using the average. MRM, multiple reaction monitoring; MW, molecular weight; DP, declustering potential; CE, collision energy.

Supplemental Table 3

Liquid chromatography conditions

Samples	Autosampler	Pump	Column	Column Temp	MP A	MP B	Flow rate (µL/min)	Example Gradients		
								Time (min)	MP A %	MP B %
Absolute quantitation (transfected cells and kidney cortex subject #1-6)	Gerstel MultiPurpose Sampler MPS 3C	Eksigent Ekspert microLC 200 UHPLC	3C18-EP120 (50 × 0.5 mm, 3 µm) or HALO C18 (50 × 0.5 mm, 2.7 µm)	40°C	0.1% FA, 0.025% TFA in water	0.1% FA in acetonitrile	35	0	100	0
								0.5	95	5
								4	70	30
								4.5	100	0
Absolute quantitation (kidney cortex subject #7-9)	Gerstel MultiPurpose Sampler MPS 3C	Agilent Technologies 1290 Infinity pump	Acquity UPLC® CSH™ C18 (2.1 × 150 mm, 1.7 µm)	60°C	0.1% FA in water	0.1% FA in acetonitrile	250	0	95	5
								10	50	50
								10.1	5	95
								12.1	5	95
								12.2	95	5
Relative quantitation (kidney cortex subject #7-9)	Gerstel MultiPurpose Sampler MPS 3C	Agilent Technologies 1290 Infinity pump	Kinetex C18 (2.1 × 30 mm, 5 µm)	Room Temp	0.1% FA in water	0.1% FA in acetonitrile	1500	0	97	3
								0.2	97	3
								0.75	50	50
								0.76	5	95
								0.86	5	95
								0.87	97	3

Temp, temperature; MP, mobile phase; FA, formic acid; TFA, trifluoroacetic acid

Supplemental Table 4

Plasma membrane recovery from transfected cells and human kidney cortex

Cells	Replicate #/Subject #	Na ⁺ /K ⁺ -ATPase expression (fmol/10 ⁶ cells)		PM recovery (%)	PM recovery (%)	
		Lysate	PM fraction		Mean	SD
HEK-OCT2	1	1671	114	6.8	20	18
	2	1208	490	41		
	3	1364	175	13		
HEK-MATE1	1	2884	293	10	13	6
	2	2614	222	8.5		
	3	1054	206	20		
HEK-MATE2K	1	2988	195	6.5	7.4	0.8
	2	1902	148	7.8		
	3	2488	196	7.9		
Human kidney cortex	1	123867	16138	13	12	3
	2	150991	24932	17		
	3	147435	14689	10		
	4	130267	10490	8.1		
	5	126211	13418	11		
	6	99343	11074	11		

PM recovery in transfected cells and human kidney cortex was determined using Na⁺/K⁺-ATPase. PM, plasma membrane

Supplemental Table 5

Comparison of basolateral and apical membrane recovery

Tissue	Subject #	Basolateral PM recovery (%)		Apical PM recovery (%)
		Absolute quantification	Relative quantification	Relative quantification
Human kidney cortex	7	12	9.1	20
	8	24	23	38
	9	20	20	23
	Mean	19	18	27
	SD	7	8	9
	CV (%)	35	43	35

Basolateral and apical PM recovery was determined using Na^+/K^+ -ATPase and GGT1, respectively.

Supplemental Table 6

In vitro transport kinetics of metformin by OCT2, MATE1 and MATE2K in individual experiments

Transporter	Experiment	K _m (μM)	V _{max} (nmol/min/mg protein)
OCT2	Exp 1	1874	39.4
	Exp 2	1870	34.6
MATE1	Exp 1	376	19.2
	Exp 2	532	12.1
MATE2K	Exp 1	1418	16.8
	Exp 2	2261	17.1

Exp, experiment