

Supplemental Data

Title:

Inter-individual Differences in the Expression of ABC and SLC Family Transporters in Human Skin: DNA Methylation Regulates Transcriptional Activity of the Human *ABCC3* (MRP3) Gene

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Drug Metabolism & Disposition

Supplemental Table 1. Donor information of human skin tissue

ID	Anatomical site	AGE (years)	ID	Anatomical site	AGE (years)
1	abdomen	53	25	abdomen	64
2	abdomen	36	26	abdomen	60
3	abdomen	40	27	abdomen	62
4	abdomen	50	28	abdomen	29
5	abdomen	44	29	abdomen	63
6	abdomen	44	30	abdomen	44
7	abdomen	40	31	abdomen	62
8	abdomen	32	32	abdomen	32
9	abdomen	44	33	abdomen	31
10	abdomen	48	34	abdomen	34
11	abdomen	30	35	abdomen	54
12	abdomen	42	36	abdomen	34
13	abdomen	48	37	abdomen	50
14	breast	49	38	abdomen	61
15	breast	45	39	breast	43
16	breast	54	40	breast	57
17	breast	43	41	breast	49
18	breast	45	42	breast	38
19	abdomen	40	43	breast	51
20	abdomen	49	44	breast	53
21	abdomen	36	45	breast	32
22	abdomen	59	46	breast	62
23	abdomen	41	47	breast	64
24	abdomen	38	48	breast	22

Supplemental Table 2. Primer sequences used in the real-time PCR analysis

Gene	GenBank Accession No.	Forward (5' to 3')	Reverse (5' to 3')
ABCA1	NM_005502.3	GCACTGAGGAAGATGCTGAAA	AGTTCCTGGAAGGTCTTGTTCAC
ABCA2	NM_001606.4	GTGGGCAACGTGACTCACTA	GCCGCGTTGTCAATGGTATC
ABCA3	NM_001089.2	CCTTCTTCAGCAAAGCCAAAC	CCAGGCCATAGAGCACAGAG
ABCA4	NM_000350.2	AAACATCACCCAGCTGTTCC	ACTGGGAGCTTTCCTCCAAT
ABCA5	NM_018672.3	GGGCCAATGGTAGGAGGTAGAG	TGAGGAATGGGCAAGGGAGGT
ABCA6	NM_080284.2	CCGTCAAGGGGGCTCAGGAA	GATGGCCACACGGTACAC
ABCA7	NM_019112.3	GATGCAGGATGAAGGAAGAAGG	CACGTC AATGTCATGCGGA
ABCA8	NM_007168.2	AGTGC GCGGGCTTCTTTGT	GTTTTCCTTCGCTTTGGTGATA
ABCA9	NM_080283.3	ACGTCTTCCTGCATTTTTGG	CATGATTAGCCCTTCCGTGT
ABCA10	NM_080282	ATGGCTCAGATGATCCCTCCTACA	CTCCGTTTGAATAAGCTCCGTGAA
ABCA12	NM_173076.2	TCTCGCCGAAGTATATGGGATGTT	GGCTTCGGGGAGATGTGATTG
ABCB1	NM_000927.4	GCCAAAGCCAAAATATCAGC	TTCCAATGTGTTCCGGCAT
ABCC1	XM_005255326.1	CGGAAACCATCCACGACCCTAATC	ACCTCCTCAITTCGCATCCACCTTG
ABCC2	NM_000392.3	AATCAGAGTCAAAGCCAAGATGCC	TAGCTTCAGTAGGAATGATTCAGGAGCAC
ABCC3	NM_003786.3	CTTAAGACTTCCCCTCAACATGC	GGTCAAGTTCCTCTTGGCTC
ABCC4	NM_005845.3	TGATGAGCCGTATGTTTTGC	CTTCGGAACGGACTTGACAT
ABCC5	NM_005688.2	AGAGGTGACCTTTGAGAACGCA	CTCCAGATAACTCCACAGACGG
ABCC6	NM_001171.5	TGTCGCTCTTTGGAAAATCC	AGGAACACTGCGAAGCTCAT
ABCC8	NM_000352.3	TGGTCATTGAAGCCTGCTC	CCACCAGACAGGTTGATGC
ABCC10	NM_001198934.1	ATACCTTGGCTGGCCTCTCT	TGGCCTCTGTCTGTGTGAAG
ABCC11	NM_145186.2	TTGCAGTCAAAGTCTCACG	GGACACCACAGGTTGATCT
ABCC12	NM_033226.2	CGCCTCAACACAGTTCTCAA	AGTATGGCAGTGGGGACAAC
ABCG1	XM_005261209.1	CAGTGACAGCCATCCGGTGCT	CGATGAAGTCCAGGTACAGCTTGGC
ABCG2	NM_004827.2	TGGCTGTTCATGGCTTCAGTA	GCCACGTGATTCCTCCACAA
ABCG5	NM_022436.2	CCCAAGGGACTCCGGGGTCA	GACCCATGGACCCTCCGGGG
ABCG8	NM_022437.2	CTTCTACCTCGCCGGGGGCT	CCGCGATGGTGAGGTTCCCG
SLC15A1	NM_005073.3	AATGTTCTGGGCCTTGTTTG	CATCTGATCGGGCTGAATTT
SLC15A2	NM_021082.3	ACCCATGCTGAGAGGAGATG	CCCATTGCAAACACAACAAG
SLC16A1	NM_003051.3	CACCGTACAGCACTATACG	CAATGGTCCGCTTTGTAGA
SLC16A7	NM_001270622.1	TGCACCAAGATTTCCAGTG	CCACCAATTTACCTGCAAGAG
SLC16A8	NM_013356.2	CATGCTAGCCATGCTTACG	GGCAAAGGAAGCTAGGATCA
SLC16A3	NM_001206950.1	ATTGGCCTGGTGCTGCTGATG	CGAGTCTGCAGGAGGCTTGTG
SLC21A3	NM_134431.3	AAGACCAACGCAGGATCCAT	GAGTTTCAACCATTCCACGTACA
SLC21A9	NM_007256.4	CTTCATCTCGGAGCCATAACC	GCTTGAGCAGTTGCCAATTG
SLC21A6	NM_006446.4	TGAACACCGTTGGAATTGC	TCTCTATGAGATGCTACTGGAT
SLC21A11	NM_013272.3	CTTCGTTTGTGGGCTTCAT	AGGATGAAGGCGAAGGATTT
SLC21A12	NM_016354.3	GACCTGCCTCTCCATCTG	ACCACCAGTACCCAAACAA
SLC21A8	NM_019844.3	GTCCAGTCATTGGCTTTGCA	CAACCCAACGAGAGTCTTAGG
SLC22A1	NM_003057.2	TAATGGACCACATCGCTCAA	AGCCCTGATAGAGCACAGA
SLC22A2	NM_003058.3	GGGATCACAATGGCCTATGAGATAGTC	CGAAAACCATCAGCGGGAGC
SLC22A3	NM_021977.3	CCCTGGAATTGCCTACTTCA	GACTCAGGGACCACCAGTA
SLC22A4	NM_003059.2	TGCTGCTGCCACTGTTTGTCT	TTCAGGAATGAACCACCACA
SLC22A5	NM_003060.3	GGATTGTTGTGCCTTCCACT	AGGTTTGAAGCAGATCCAGA
SLC22A6	NM_004790.4	TGCATGACACTGAATGTGGA	AAAAGGCGCAGAGACCAGTA
SLC22A7	NM_006672.3	GAGGATGAACCTGCCACAGT	TCTGCTCACACACCAGATCC
SLC22A8	NM_004254.3	CACCGTCATCTTGAATGTGG	GACGAAGAAGGGAATGGACA
SLC22A9	NM_018484.2	CTTTATCTGGGGCTCCTCT	TCCACTCCACCATCAGTGTCT
SLC22A10	NM_001039752.3	CCCCAGTATGCGTAAAAGGA	CGACGGCCCATATGATTAG
SLC46A1	NM_080669.4	AGTATCTGTGGCACCCTTCA	CCAGTGGGAGGTAAGGGTCTC
SLC47A1	NM_018242.2	ATGCTGTTTTCCCACTTTTG	TCCAACCTTCTGATTTCCACT
SLC47A2	NM_152908.3	GCCAACATCATCTCCAGTT	ATAGGCCCACTCAACAC
GAPDH	NM_002046.4	ATCAAGAAGGTGTTGAAGCAG	TCGCTGTTGAAGTCAGAGGAG

Supplemental Table 3. Primer sequences used in the SNP analysis

Position ¹⁾	Forward (5' to 3')	Reverse (5' to 3')	Amplified Length (bp)	Annealing (°C)
-1815 ~ -1535	GCTTAGATATCACCCCTGTCCA	TCCACTCATCCACACATACCC	281	55.7
-1486 ~ -1188	GGGGTAGAAGTCCCTGGTCTG	CTTCCTGCCTTTGCCACAG	299	62.3
-1105 ~ -805	TAAGGAAGACAGGGCAACAGC	AAAGGGTAGGCAAAGCGTATT	301	62.3
-415 ~ -119	TGTTAAATCCCTCTCCCTACG	AGCCCTACCCAGTGCCTCTG	297	62.3

1) Relative to TIS.

Supplemental Table 4. Primer sequences used in the COBRA analysis of *ABCC3*

Position ¹⁾	Forward (5' to 3')	Reverse (5' to 3')	Amplified length (bp)	Annealing (°C)	RE ³⁾	COBRA site ¹⁾
-20117 ~ -19750	TTTTGGGTGGGTTAGTGTAGTTAGT	ATCCCCCTCCAAACATCTATTATAC	368	59.5	<i>Hinf</i> I	-19895
-16272 ~ -15982	GGGATTGTGATTAGGAGTTTATTTGT	ACAAAACCCCAAACCTTAAACTATC	291	59.1	<i>Mbo</i> I	-16169
-15837 ~ -15479 (1 st PCR)	ATTTTAGGTTTTTTTTGGAGGTTTT	TAAAAAATAAAAAATAAACAAAACCTCAC	359	55.7	-	-
-15675 ~ -15479 ²⁾	TTTTTTGATTTATAGGTTAAGGTTAAGTT		197	59.5	<i>Hpy</i> 188III	-15507
-13830 ~ -13429	AAGTTAGAATTTGGGGGTAGGTAA	CTTCCCCAAACAACTAACAATAC	402	51.1	<i>Taq</i> I	-13783
					<i>Hinf</i> I	-13638
-13525 ~ -13140	GTTTGTTTTGGTTTTTTAGTGTTTGT	TACATTACTCTCCAAAACACTAAATAAAAA	386	55.7	<i>Rsa</i> I	-13332
-13536 ~ -13140	GTTTGTAGTGTTTGTTTTGGTTT	TACATTACTCTCCAAAACACTAAATAAAAA	397	55.7	<i>Hinf</i> I	-13222
-10070 ~ -9587	ATTTTGGATTGAGTTATGGGGTATA	TCCCCCAAACATATAAAATTTAAATTT	484	53.0	<i>Hpy</i> 188III	-9718
-560 ~ -120	GTTTGTAGTGGTTTTTGGAAATTTG	ACCCTACCCCAATACCTCTAAATCC	441	62.3	<i>Hinf</i> I	-308
-144 ~ +269	GGATTTAGAGGTATTGGGGTAGGGT	AAAAAAACAAAACAAAACAAATCC	413	55.7	<i>Mbo</i> I	-35
+31 ~ +554	GGTTTTAAGTTTTGGGTAAGG	AAAAAAAATAAAACACTACACAACC	524	53.0	<i>Hpy</i> CH4III	+163
					<i>Tfi</i> I	+457
+9807 ~ +10124	GTGTAGTGGTTTATGTTTGTAATTTT	CCAAACTAATTTTTATTTTCTATTTTTTT	318	55.7	<i>Mbo</i> I	+9877
					<i>Eco</i> R I	+10005
+20175 ~ +20464	AAAAAAAAGAGTGAGGGTTAGG	CAAAATCTCACTCTATTACCAAATAA	290	59.5	<i>Mbo</i> I	+20266
					<i>Eco</i> R I	+20393

1) Relative to TIS.

2) One-tenth of the 1st PCR products were used as templates for semi-nested PCR.

3) Cleavage only occurs if cytosines in the restriction sites are retained during bisulfate modifications as a result of methylation, except for *Bts*C I.

Supplemental Table 5. Primer sequences used in reporter gene construction

Position ¹⁾	Forward (5' to 3')	Reverse (5' to 3')	Amplified Length (bp)	Annealing (°C)	Cloning sites
-10157 ~ -9265 (1 st PCR)	GGTCATGCCTATCTGGTCGG	CCTCTGCCTAGACTTCCCCT	893	59.5	–
-9966 ~ -9624 ²⁾	TTTTTGAGTTT A G A T CTTGTGCCCAG	ACGCTCA A G C T TCAGGACACACAC	343	59.5	<i>Bgl</i> II <i>Hind</i> III
-2023 ~ +13	GCCCAGAGCAA G C T T GTGCCTATG	ACAG A G A GTCCATGGGGCCGAG	2036	64.0	<i>Hind</i> III <i>Nco</i> I

1) Relative to TIS.

2) One-six hundred and fortieth of the 1st PCR product was used as a template for nested PCR.

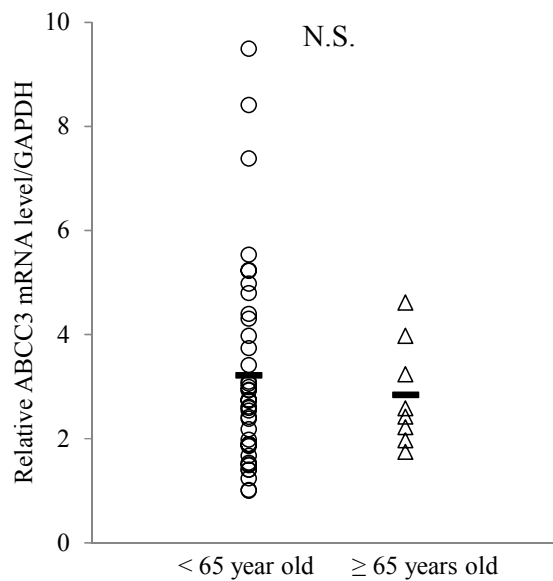
The mutated base is in bold.

Supplemental Table 6. gRNA oligo and primer sequences used for CRISPR/Cas9

Name	Strand	Sequence
gRNA-1	Top	5'– CCGGGGTCATGCCTATCTGGTCGG –3'
	Bottom	5'– AAACCCGACCAGATAGGCATGACC –3'
gRNA-2	Top	5'– CCGGTGTGTCCACGCTCATGCCTC –3'
	Bottom	5'– AAACGAGGCATGAGCGTGGACACA –3'
Primer pair-1 (deletion check)	Forward	5'– TGCACCCAGTTCCTTGTTCAC –3'
	Reverse	5'– TGAGCTGAGGCTGCAATGATC –3'
Primer pair-2 (deletion check)	Forward	5'– GAGCTCCCAACCTCAGATGATC –3'
	Reverse	5'– ATGGCATGGGCTGTGGCCTTATC –3'
<i>ACTB</i> locus qPCR	Forward	5'– AACACTGGCTCGTGTGACAA –3'
	Reverse	5'– GCTAAGTGTGCTGGGGTCTT –3'

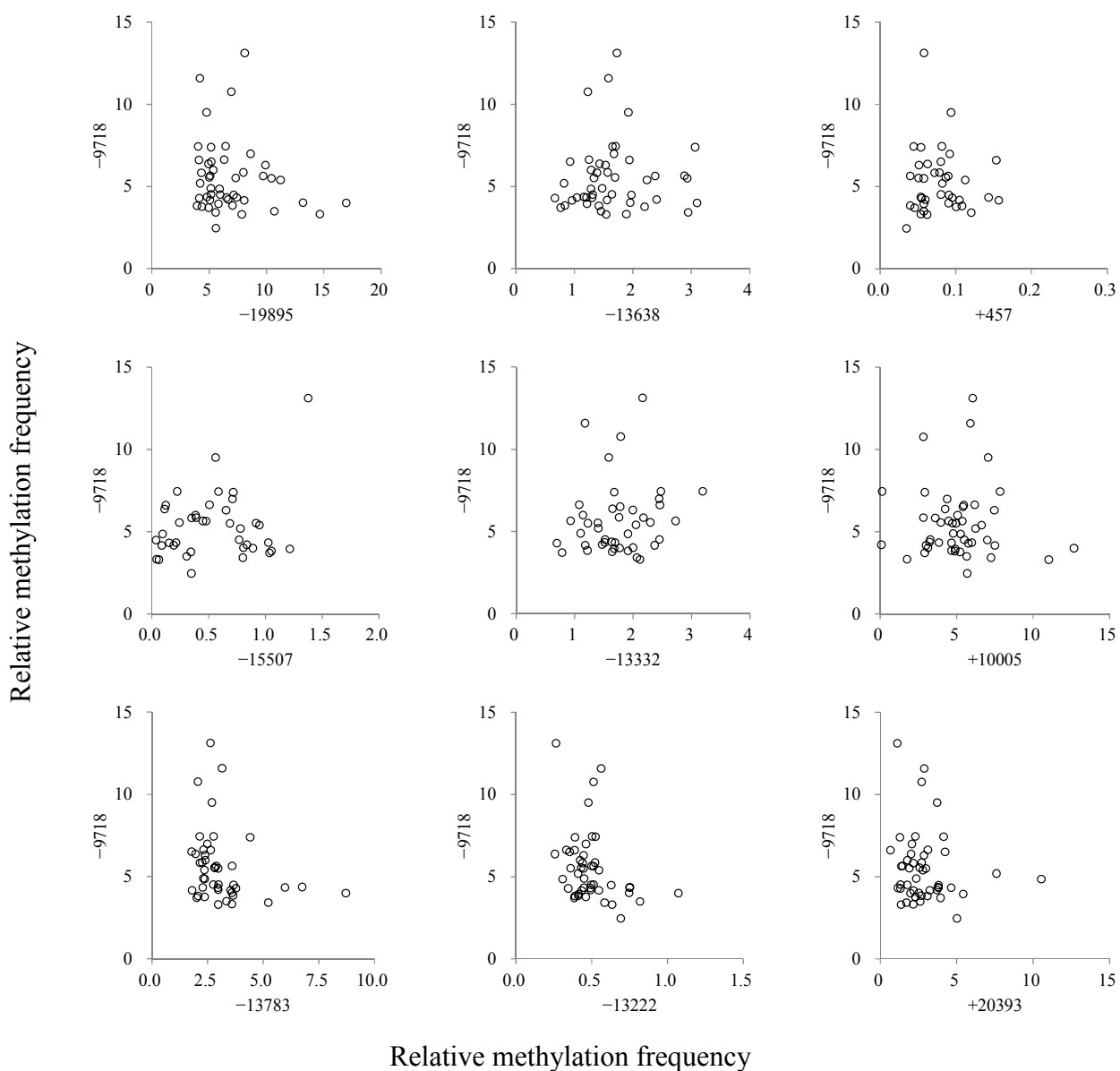
Supplemental Table 7. Typical substrates of ABCC3, SLCO3A1, and SLC22A3

Isoforms	References	Endogenous substrates	Substrate drugs
ABCC3	Kool et al., 1999		Methotrexate Etoposide
	Patel et al., 2016	Estradiol-17 β -glucuronide	Fexofenadine Acetaminophen glucuronide
	Zhou et al., 2008	Bilirubin glucuronide Leukotriene C4	
	Schinkel and Jonker, 2003	Bile salts	
SLCO3A1	Tamai et al., 2000	Estrone-3-sulfate Prostaglandin E2	
	Huber et al., 2007	Vasopressin Prostaglandin E1 Thyroxine	
SLC22A3	Wu et al., 1998	Dopamine Testosterone Progesterone Norepinephrine Histamine	Cimetidine
	Chen et al., 2015		Metformin
	Koepsell, 2015		Atropine Nicotine Prazosin



Supplemental Figure 1. Effects of age on ABCC3 mRNA expression in human skin.

No significant differences were observed in expression levels between samples under and over 65 years old. Statistical comparison was performed with the Mann-Whitney U test ($P < 0.05$). N.S. means not significant.

**Supplemental Figure 2. Correlation of methylation levels between CG sites.**

There were no positive correlations between the CG site (-9718) and the other CG sites.