

Supplemental Material

Multiple transporters contributed to FTC accumulation in JEG-3 cells. FTC accumulation in JEG-3 cells was studied to investigate further if transporters were involved in the placental transport of FTC. As shown in Fig. S1, accumulation of FTC (10 μ M, 3 min) in JEG-3 cells was inhibited remarkably by 1 μ M NBTI ($P < 0.01$) and 100 μ M NBTI (an inhibitor of ENTs; $P < 0.001$), 200 μ M phlorizin (an inhibitor of CNTs; $P < 0.01$) and 200 μ M adenosine (a substrate/inhibitor of ENTs and CNTs; $P < 0.01$), which indicated that ENTs and CNTs contributed to FTC transport.

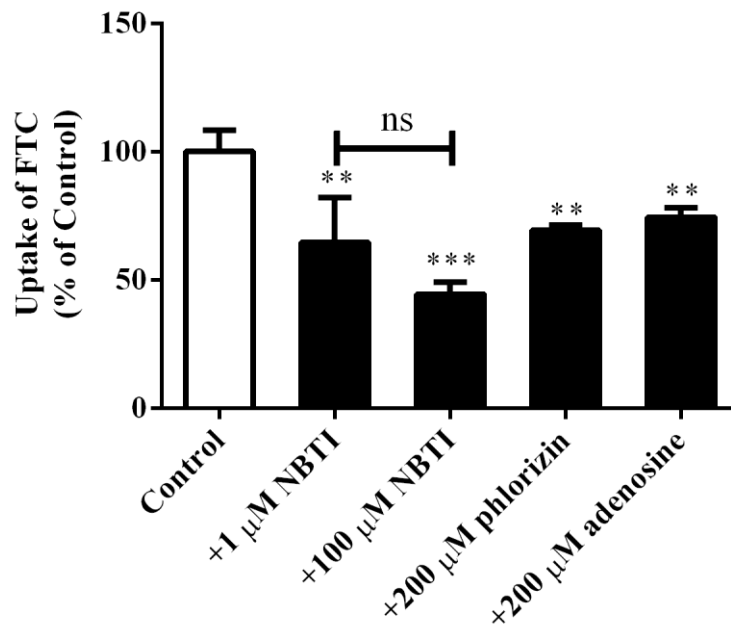


FIG. S1 Effect of specific inhibitors of ENTs (NBTI and dipyridamole) or CNTs (phlorizin) and a general inhibitor of nucleoside transporters (adenosine) on accumulation of FTC (10 μ M, 3 min) in JEG-3 cells, compared with the control, ** $P < 0.01$, *** $P < 0.001$; comparison between with an inhibitor of NBTI, ns, $P > 0.05$.

Expression of CNTs mRNA in first-trimester and full-term human placentas. We measured mRNA expression of CNTs in first-trimester and full-term human placentas by RT-PCR. As shown in Fig. S2, CNT1, CNT2 and CNT3 were detected in full-term human placentas. mRNA expression of CNT1 increased significantly ($P < 0.05$). mRNA expression of CNT2/3 increased, but not significantly.

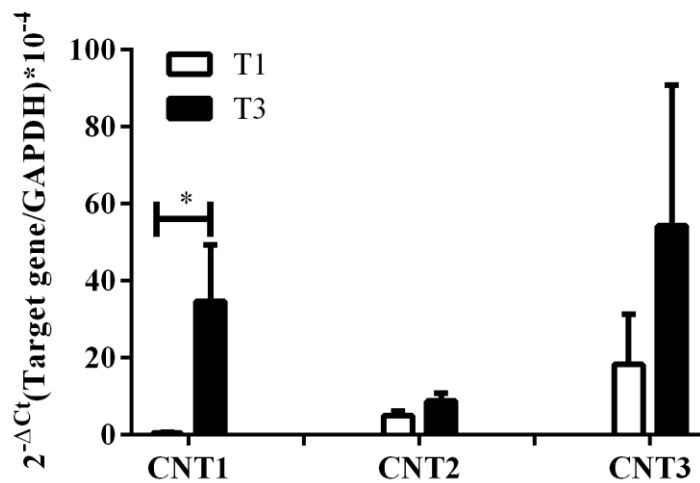


FIG. S2 Expression of CNTs mRNA in the first-trimester (T1, n = 8) and full-term placenta (T3, n = 8), compared with T1, * $P < 0.05$.

FTC is a substrate of MRP1. FTC was reported to be a substrate of MRP1. For further confirmation, we compared FTC accumulation in mock and MDCK-hMRP1 cells. As shown in Fig. S3, accumulation of FTC (10 μ M, 60 min) in MDCK-hMRP1 cells was 72% of that observed in mock cells ($P < 0.05$), and 20 μ M MK571 (an inhibitor of MRPs) increased FTC accumulation in MDCK-hMRP1 cells markedly ($P < 0.01$).

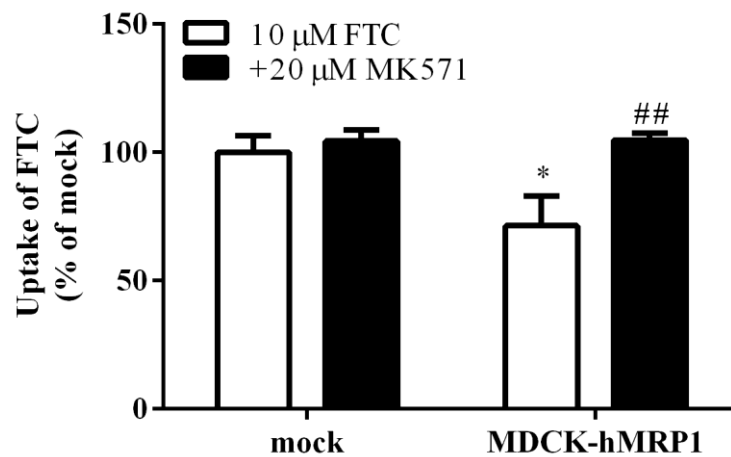


FIG. S3 FTC accumulation in mock and MDCK-hMRP1 cells. Cells were exposed to FTC with or without MK571 (20 μ M) for 90 min, compared with accumulation in mock cells, * $P < 0.05$; compared with accumulation without an inhibitor, ** $P < 0.01$.

TABLE S1 siRNA sequences

Name	Sense (5' to 3')
siENT1-A1	ACCAAGUUGGACCUCAUUA
siENT1-A2	ACCAAUGAAAGCCACUCUA
NC	UUCUCCGAACGUGUCACGU

TABLE S2 Primers used in RT-PCR

Gene	Direction	Sequence (5' to 3')	Product Length
CNT1	Forward	TGAAGTGACAAGGCAAGCCA	276 bp
	Reverse	TCCAGCTGCTCCTGATCTCT	
CNT2	Forward	ACTGAGGAGCCAGAGGGAAT	235 bp
	Reverse	CACCGACTCCTCCTCTGGTA	
CNT3	Forward	GCAGGTGGCTTTCTGACAT	416 bp
	Reverse	AAAATGCCCCAGATGATGTGC	