Supplementary Materials

Influence of Polymorphic OATP1B-Type Carriers on the Disposition of Docetaxel

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METHODS

Determination of Docetaxel Concentrations

Docetaxel was quantified using a validated method involving reversed-phase liquid chromatography coupled to tandem mass-spectrometric detection. Sample extracts were injected onto an Alltima HP C18 HL 3 µm column (50×2.1mm internal diameter, Alltech Applied Science) by a Waters 2795 Separation Module. The mobile phase was composed of acetonitrile and water containing formic acid (0.1% v/v), and was delivered using linear gradient settings at a flow rate of 0.2 mL/min. Detection was performed with a MicroMass Quatro Micro triple-quadrupole mass spectrometer (Waters) in the positive ion mode. The electrospray ionization was set at 3.8 kV and the cone voltage at 18V. The dwell times were set at 150 ms and the inter-channel delay at 50 ms. Multiple reaction monitoring mode was applied for the quantitation with the following parameters: *m/z* 808>527, collision energy at 9 eV for docetaxel and *m/z* 813>532, collision energy at 10 eV for the internal standard docetaxel-d_5. The collision cell pressure was set at ~4×10^{-3} mbar (argon).

Supplementary Figure Legends

Supplementary Fig. S1. (**A**) Influence of Phenol Red on OATP1B1-mediated transport of docetaxel *in vitro*. Transport of docetaxel (concentration, 0.1 µM; 60-min incubations) was evaluated in Flp-In T-Rex293 cells transfected with OATP1B1 in the absence or presence of Phenol Red at a concentration of 10 µg/mL (26.4 µM). Data represent the mean of 3 observations, and are expressed as the average percent of uptake values in cells transfected with an empty vector (VC). Error bars represent the standard error. The *P*-value denotes a statistical comparison of differences in uptake of docetaxel by OATP1B1 in the absence or presence of Phenol Red. (**B**) Visualization of Flp-In T-Rex293 cells transfected with an empty vector (VC) or OATP1B1 cultured in DMEM containing Phenol Red (10 µg/mL) indicating accumulation of Phenol Red in cells expressing OATP1B1.

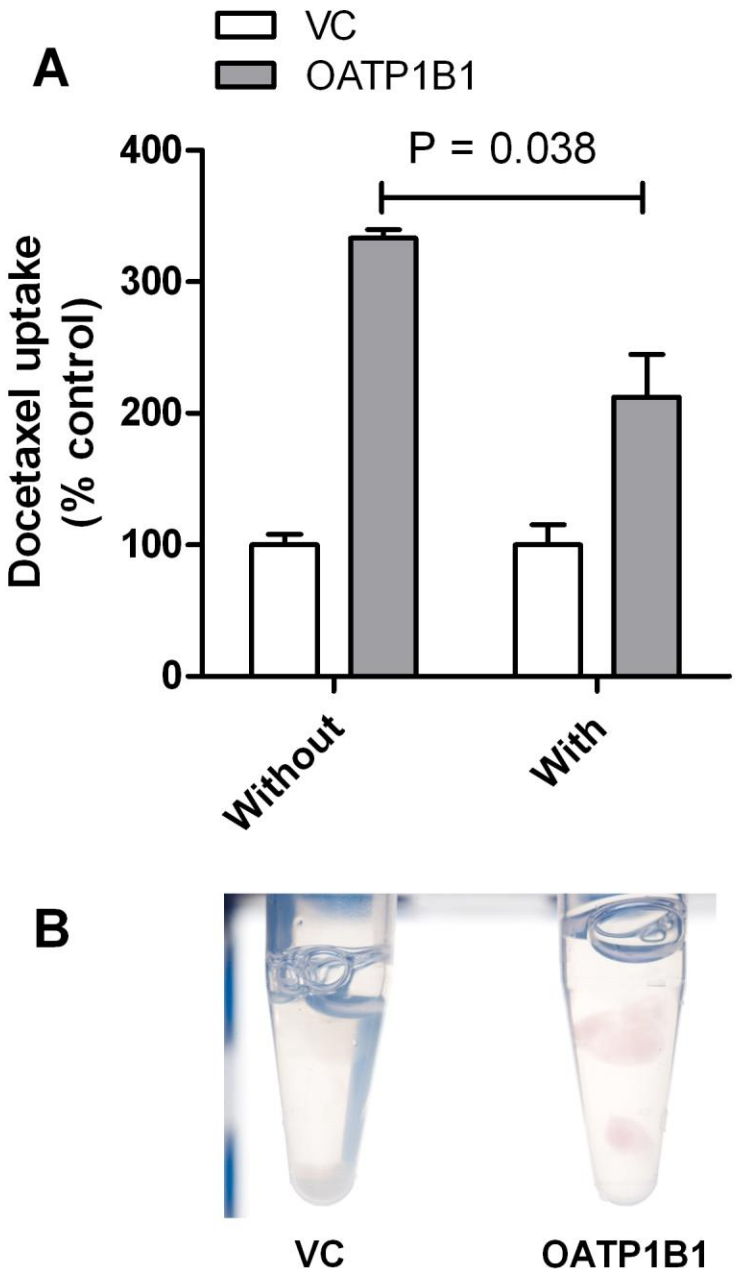
Supplementary Fig. S2. Characterization of paclitaxel concentration-dependent transport by OATP1B1 (**A**) and OATP1B3 (**B**) in CHO cells. Data represent the mean and standard deviation of 2 to 5 independent experiments in cells stably expressing OATP1B1, OATP1B3, or in control cells (VC), and the net difference. Km denotes the Michaelis-Menten constant, and Vmax the maximum velocity.

Supplementary Fig. S3. Docetaxel clearance as a function of observed OATP1B1 (*SLCO1B1*) or OATP1B3 (*SLCO1B3*) diplotypes. Data were obtained in 141 predominantly white cancer patients receiving docetaxel-based chemotherapy. Each symbol represents an individual patient, and horizontal lines indicate median values. The *P*-value denotes a statistical comparison of the clearance of docetaxel in the different diplotype group. The composition and frequencies (Freq) of the observed haplotypes in OATP1B1 and OATP1B3 are shown below the figures and compared with reported frequency values in the literature. **Ramsey et al**. refers to:

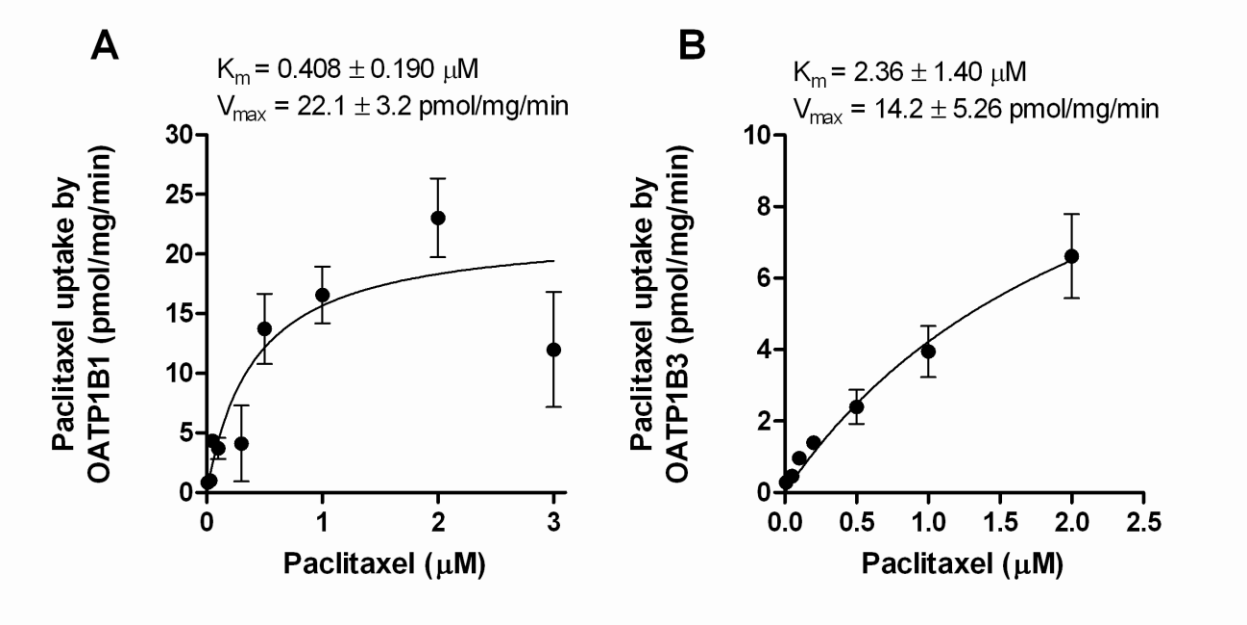
Ramsey LB, Bruun GH, Yang W, et al: Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. Genome Res 22:1-8, 2012; **Smith et al.** refers to: Smith NF, Marsh S, Scott-Horton TJ, et al: Variants in the SLCO1B3 gene: interethnic distribution and association with paclitaxel pharmacokinetics. Clin Pharmacol Ther. 81:76-82, 2007.

Supplementary Fig. S4. Docetaxel clearance as a function of observed OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*) genotypes. Data were pooled from the prospective cohort of 141 patients and a retrospective analysis on a cohort of 72 patients receiving docetaxel-based chemotherapy (Baker SD, Verweij J, Cusatis GA, et al. Pharmacogenetic pathway analysis of docetaxel elimination. Clin Pharmacol Ther 85:155-63, 2009). Each symbol represents an individual patient, and horizontal lines indicate median values. The P-value denotes a statistical comparison of the clearance of docetaxel in the different genotype group.

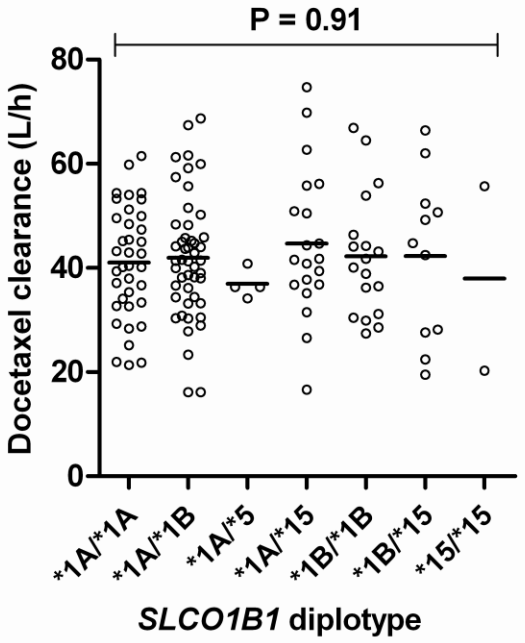
Supplementary Figure S1

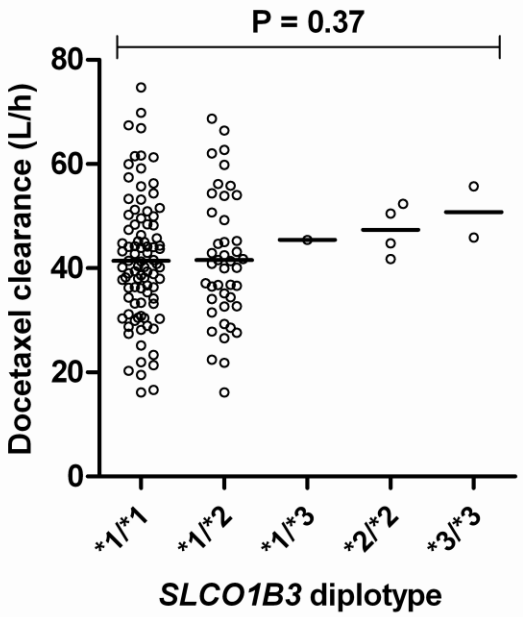


Supplementary Figure S2



Supplementary Figure S3

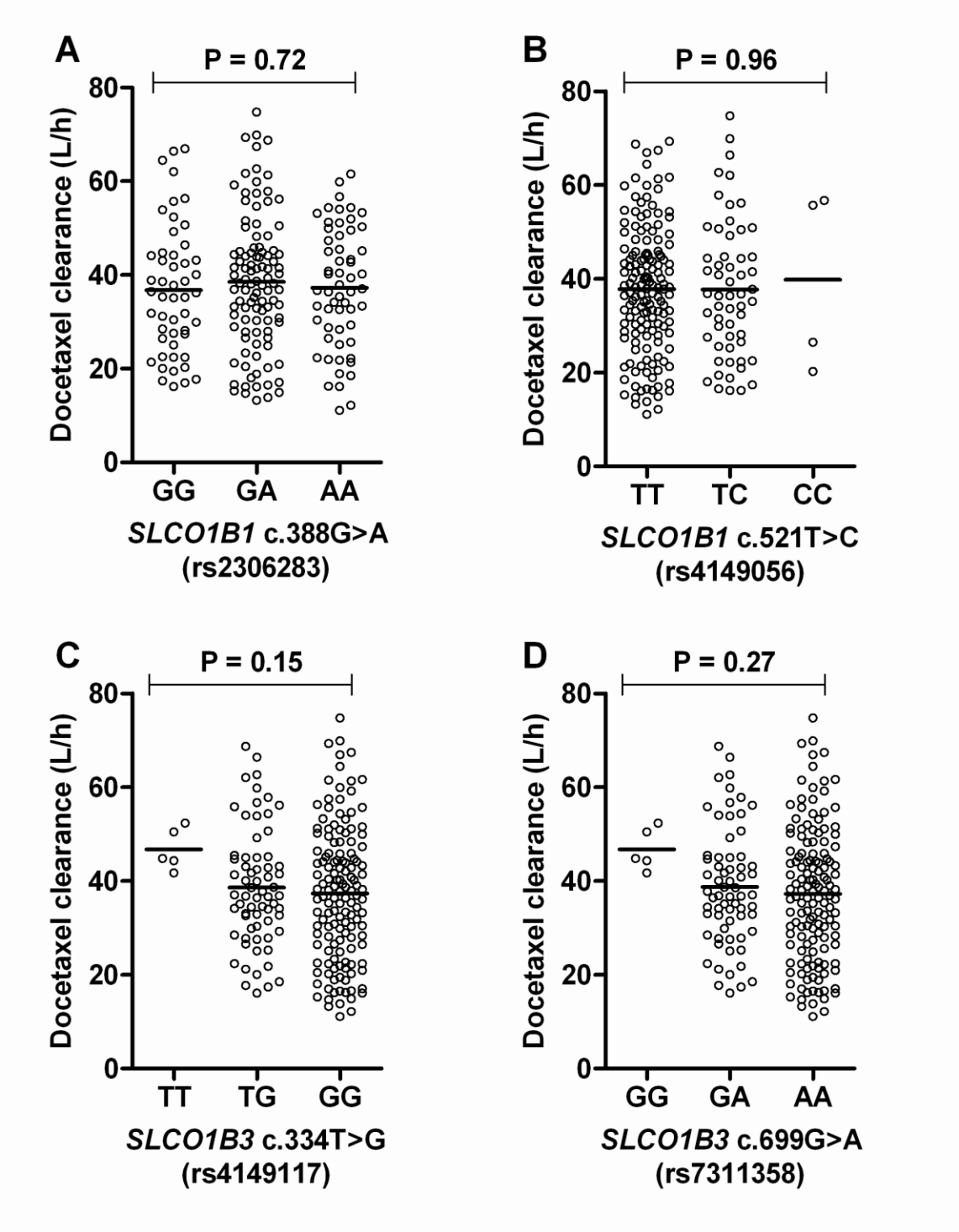




	Ramsey et al Freq	Current Freq	c.388	c.521
*1A	0.540	0.521	A	T
*1B	0.140	0.339	G	T
*5	0.005	0.014	A	C
*15	0.120	0.125	G	C

	Smith et al Freq	Current Freq	c.344	c.699
*1	0.804	0.799	G	A
*2	0.168	0.183	T	G
*3	0.022	0.018	G	G
*4	0.006	0.000	T	A

Supplementary Figure S4



Supplementary Table S1. Genotyped variants in OATP1B1 (*SLCO1B1*) and OATP1B3 (*SLCO1B3*)

Gene	Position	Location	Effect[a]	Activity[b]	NCBI ID	MAF[c]	MAF[d]	Assay[e]	Ref[f]
SLCO1B1	c.388A>G	Exon 4	p.Asn130Asp	Increased	Rs2306283	0.46 A	0.41 A	C__1901697_20	(1-6)
	c.521T>C	Exon 5	p.Val174Ala	Decreased	Rs4149056	0.14 C	0.12 C	C__30633906_10	(1,6-14)
	g.-11187 G>A	Promotor	-	Decreased	Rs4149015	0.08 A	0.07 A	C__32325356_10	(6,14,15)
SLCO1B3	c.334 T>G[g]	Exon 3	p.Ser112Ala	Unchanged	Rs4149117	0.19 T	0.29 T	C__25639181_40	(16-21) [g]
	c.699 G>A[g]	Exon 6	p.Met233Ile	Unchanged	Rs7311358	0.19 G	0.29 G	C__25765587_40	(16-20) [g]
	IVS12-567A>G	Intron 12	-	Decreased	Rs11045585	0.13 G	0.17 G	C__31106434_10	(22,23)

[a] Number represents amino-acid codon
[b] Functional activity in vivo of the variant allele relative to the reference allele
[c] MAF in studied population
[d] MAF in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP)
[e] TaqMan® (Applied Biosystems, CA, USA) genotyping assays used
[f] References for activity in vivo of the variant allele relative to the reference allele
[g] c.699 G>A and c.334 T>G are in complete linkage disequilibrium

Abbreviations: MAF; minor allele frequency, NCBI ID; National Center for Biotechnology Information identification number

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Supplementary Table S2. Patient characteristics[a]

Characteristic	Value
Number of patients	141
Age, years	55 (18 – 85)
Sex	
Male	54 (38)
Female	87 (62)
BSA, m^2	1.86 (1.37 – 2.60)
Racial ancestry	
Caucasian	131 (93)
Sub-Saharan	2 (1.4)
Northern African	1 (0.7)
Eastern Asian	3 (2.1)
Hindustan	3 (2.1)
Unknown	1 (0.7)
Docetaxel dose, mg/m^2	75 (30 – 100)
Docetaxel dose, mg	150 (50 – 230)
Primary tumor	
Breast	74 (53)
Prostate	21 (15)
Melanoma	11 (7.8)
Head and neck	10 (7.1)
Sarcoma	7 (5.0)
Lung	5 (3.5)
Miscellaneous	13 (9.2)

[a]Continuous data are given as median with range in parentheses, and categorical data are given as number of patients with percentage of the total population in parentheses.

Supplementary Table S3. Inhibitors of OATP1B1 and OATP1B3

	OATP1B1		OATP1B3	
Compound	**IC_{50} (μM)[a]**	**Ref**	**IC_{50} (μM)[a]**	**Ref**
Beclomethasone	6.7	(1)	1.4	(1)
Bromocryptine	0.7	(1)	1.8	(1)
Clarithromycin	8.26[b]-96	(2,3)	32	(2)
Cyclosporine	0.2[b]-2.2	(3-9)	0.06	(9)
Ergocryptine	0.8	(1)	2.2	(1)
Erythromycin	11.4[a]-217	(2,3)	34	(2)
Estropipate	0.06	(1)	19.3	(1)
Everolimus	4.1	(10)	3.7	(10)
Moricizine	8.1	(1)	2.7	(1)
Niflumic acid	3.7	(1)	22.0	(1)
Ramipril	4.0	(1)	3.3	(1)
Repaglinide	1.1-2.2	(1,11)	4.8	(1)
Resveratrol	11.2	(1)	23.7	(1)
Rifampicin	0.477[b]-17[b]	(1,3,4,6,12-14)	1.5-5[b]	(1,13)
Roxithromycin	153	(2)	37	(2)
Sirolimus	9.8	(10)	1.3	(10)
Telithromycin	121	(2)	11	(2)
Ursolic acid	12.5	(1)	2.3	(1)

[a] Inhibitor concentration producing 50% inhibition of transporter activity.
[b] Value represent inhibition constant (Ki) provided instead of IC_{50}.

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