Association between imatinib transporters and metabolizing enzymes genotype and response in newly diagnosed chronic myeloid leukemia patients receiving imatinib therapy

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Online Supplementary Table S1. Description of the single nucleotide polymorphisms.

Gene [full name; Protein name and function] SNP ID	METHOD[A]
SLC22A1* [soluble carrier family 22, member 1; OCT1 (organic cation transporter); cellular uptake of imatinib and hepatic transporter of imatinib]	
rs72552763 [MI420I]	RT TaqMan assay C_34211613_10
rs12208357 [R61C]	RT TaqMan assay C_30634096_10
rs683369 [L160P]	RT TaqMan assay C_928536_30
rs4646277 [P283L]	RT TaqMan assay C_30634088_10
rs4646278 [R287G]	RFLP f_GCGATGGCTCCCTTTTG r_TTAGACCCCGACCCAAGACCAC annealing: 56°C; restriction enzyme: ScrF I
rs2282143 [P341L]	RT TaqMan assay C_15877554_40
ABCB1* [ATP-binding cassette sub-family B, member 1; P-gp (P-glycoprotein); cellular efflux of imatinib and intestinal absorption of imatinib]	
rs10245483 [Promoter region]	RT TaqMan assay C_2573447_20
rs3213619 [Promoter region]	RT TaqMan assay C_27487486_10
rs1128501 [G185V]	RT TaqMan assay C_7586664_10
rs1128503 [G412G also C1236T]	RT TaqMan assay C_7586662_10 RFLP [Goreva *et al.*, 2004]
rs60023214 [I1145I also C3435T]	RFLP [Jamroziak *et al.*, 2004]
rs2032582 [A893S/T also G2677T/A]	M [Kurzawski *et al.*, 2006]
ABCG2 [ATP-binding cassette sub-family G, member 2; BCRP (breast cancer resistance protein); cellular efflux of imatinib and intestinal absorption of imatinib]	
rs2231137 [M12V]	RFLP [Hu *et al.*, 2007]
rs2231142 [Q141K]	RT TaqMan assay C_15854163_70
OCTN1 [SLC22A4 (soluble carrier family 22, member 4); organic cation transporter]	
rs1050152 [L503F]	RT TaqMan assay C_3170459_30
OATP1A2 [SLCO1A2; soluble carrier family member 1; OAT (organic anion transporter); cellular uptake of imatinib]	
rs11568563 [E172D]	RT TaqMan assay C_25605897_10]
CYP3A4 [cytochrome P450, family 3, subfamily A, polypeptide 4; CYP3; hepatic metabolism of imatinib]	
rs2740574 [5' Near Gene]	RFLP [Radriguez-Antona *et al.*, 2005]
rs28371759 [L293P]	RT TaqMan assay C_27859823_20]
CYP3A5 [cytochrome P450, family 3, subfamily A, polypeptide 5; CYP3; hepatic metabolism of imatinib]	
rs776746 [Splicing site]	RFLP [Hu *et al.*, 2005]
rs28365083 [T398N]	RFLP [van Schaik *et al.*, 2002]

*[A]RT: real-time PCR with TaqMan allelic discrimination assay [Applera, Foster City, USA]; RFLP: PCR-RFLP and M: Multiplex PCR, analysis carried out according to published methods [reference parenthetically] or as standardized in our laboratory [primer set, T (°C) of annealing and restriction enzymes (Fermentas, Vilnius Lithuania) described]. * Referred to as hOCT1 in the text, figures and following tables. ** Referred to as* MDR1 *in the text, figures and following tables.*

Online Supplementary Table S2A. Genotype frequency of the 20 candidate SNP among Caucasian patients.

SNP ID	Major/Minor allele	Homozygous Major	Heterozygous	Homozygous Minor	MAF	HWE *P* value
hOCT1						
rs72552763	GAT/-	106	37	8	0.18	0.08
rs12208357	C/T	135	18	1	0.06	0.48
rs683369	C/G	105	39	8	0.18	0.10
rs4646277	C/T	135	0	0	0.00	---
rs4646278	C/G	135	0	0	0.00	---
rs2282143	C/T	152	2	0	0.01	1.00
MDR1						
rs60023214	C/T	38	79	34	0.49	0.62
rs1128503	C/T	53	73	28	0.42	0.74
rs10245483	G/T	46	62	46	0.50	0.01
rs3213619	A/G	149	6	0	0.02	1.00
rs1128501	C/A	135	0	0	0.00	---
rs2032582	G/T or A **	47	77	22	0.41	0.39
ABCG2						
rs2231137	G/A	138	11	1	0.04	0.24
rs2231142	G/T	129	23	2	0.09	0.32
OCTN1						
rs1050152	C/T	50	74	31	0.44	0.74
OATP1A2						
rs11568563	T/G	138	15	1	0.06	0.38
CYP3A4						
rs2740574	A/G	140	14	0	0.05	1.00
rs28371759	A/G	134	0	0	0.00	---
CYP3A5						
rs776746	G/A	129	17	2	0.07	0.15
rs28365083	C/A	134	0	0	0.00	---

** MAF: minor allele frequency. ** Includes three alleles, forming six genotypes. In all analyses the two rare alleles, A and T, are combined and treated as a single minor allele group.*

Online Supplementary Table S2B. Genotype frequency of the 20 candidate SNP among Asian patients.

SNP ID	Major/Minor allele	Homozygous Major	Heterozygous	Homozygous Minor	MAF	HWE *P* value
hOCT1						
rs72552763	GAT/-	23	0	0	0.00	---
rs12208357	C/T	23	0	0	0.00	---
rs683369	C/G	18	5	0	0.11	1.00
rs4646277	C/T	23	0	0	0.00	---
rs4646278	C/G	23	0	0	0.00	---
rs2282143	C/T	17	5	1	0.15	0.41
MDR1						
rs60023214	C/T	13	6	4	0.30	0.13
rs1128503	C/T	3	13	7	0.59	0.67
rs10245483	G/T	13	8	2	0.26	0.61
rs3213619	A/G	20	3	0	0.07	1.00
rs1128501	C/A	23	0	0	0.00	---
rs2032582	G/T or A **	7	11	5	0.46	1.00
ABCG2						
rs2231137	G/A	8	14	1	0.35	0.18
rs2231142	G/T	12	11	0	0.24	0.28
OCTN1						
rs1050152	C/T	23	0	0	0.00	---
OATP1A2						
rs11568563	T/G	23	0	0	0.00	---
CYP3A4						
rs2740574	A/G	23	0	0	0.00	---
rs28371759	A/G	23	0	0	0.00	---
CYP3A5						
rs776746	G/A	14	8	1	0.22	1.00
rs28365083	C/A	23	0	0	0.00	---

** MAF: minor allele frequency. **Includes three alleles, forming six genotypes. In all analyses the two rare alleles, A and T, are combined and treated as a single minor allele group.*

Online Supplementary Table S2C. Genotype frequency of the 20 candidate SNP among patients of other races.

SNP ID	Major/Minor allele	Homozygous Major	Heterozygous	Homozygous Minor	MAF	HWE *P* value
hOCT1						
rs72552763	GAT/-	8	1	1	0.15	0.15
rs12208357	C/T	9	1	0	0.05	1.00
rs683369	C/G	7	3	0	0.15	1.00
rs4646277	C/T	10	0	0	0.00	---
rs4646278	C/G	10	0	0	0.00	---
rs2282143	C/T	8	2	0	0.10	1.00
MDR1						
rs60023214	C/T	6	4	0	0.20	1.00
rs1128503	C/T	5	5	0	0.25	1.00
rs10245483	G/T	2	6	2	0.50	1.00
rs3213619	A/G	7	3	0	0.15	1.00
rs1128501	C/A	10	0	0	0.00	---
rs2032582	G/T or A **	6	4	0	0.20	1.00
ABCG2						
rs2231137	G/A	8	2	0	0.10	1.00
rs2231142	G/T	8	1	1	0.15	0.15
OCTN1						
rs1050152	C/T	5	5	0	0.25	1.00
OATP1A2						
rs11568563	T/G	9	1	0	0.05	1.00
CYP3A4						
rs2740574	A/G	7	2	1	0.20	0.31
rs28371759	A/G	10	0	0	0.00	---
CYP3A5						
rs776746	G/A	4	6	0	0.30	0.48
rs28365083	C/A	10	0	0	0.00	---

** MAF: minor allele frequency. **Includes three alleles, forming six genotypes. In all analyses the two rare alleles, A and T, are combined and treated as a single minor allele group.*

Online Supplementary Table S3. CgR and MR according to the candidate genotypes in the overall population.

	MCgR		CCgR		MMR		CMR	
SNP ID	*P*	Hazard ratio [95% CI] *	*P*	Hazard ratio [95% CI] *	*P*	Hazard ratio [95% CI] *	*P*	Hazard ratio [95% CI] *
hOCT1								
rs72552763	0.41	0.89 (0.68, 1.18)	0.96	0.99 (0.75, 1.31)	0.11	0.79 (0.59, 1.06)	0.66	0.90 (0.57, 1.44)
rs12208357	0.85	1.04 (0.66, 1.66)	0.87	1.04 (0.65, 1.66)	0.75	0.93 (0.57, 1.50)	0.12	0.50 (0.18, 1.34)
rs683369	0.80	0.96 (0.73, 1.28)	0.59	0.93 (0.70, 1.23)	0.18	0.81 (0.60, 1.11)	0.29	0.77 (0.47, 1.26)
rs2282143	**0.05**	1.87 (1.06, 3.29)	0.11	1.70 (0.93, 3.11)	0.99	1.00 (0.52, 1.96)	0.30	0.53 (0.13, 2.07)
MDR1								
rs60023214	0.77	1.03 (0.83, 1.29)	0.39	1.11 (0.88, 1.39)	0.23	0.86 (0.68, 1.10)	**0.06**	0.70 (0.49, 1.02)
rs1128503	0.38	1.11 (0.88, 1.39)	0.12	1.20 (0.95, 1.52)	0.93	1.01 (0.81, 1.26)	0.46	0.88 (0.62, 1.24)
rs10245483	0.21	0.89 (0.73, 1.07)	0.13	0.85 (0.70, 1.05)	0.17	0.87 (0.70, 1.07)	0.44	1.14 (0.82, 1.57)
rs3213619	**0.06**	1.92 (1.03, 3.56)	**0.07**	1.85 (0.99, 3.44)	0.26	1.48 (0.78, 2.82)	**0.07**	2.27 (1.03, 5.00)
rs2038502	0.61	1.06 (0.84, 1.34)	0.50	1.09 (0.85, 1.39)	0.79	0.97 (0.76, 1.23)	0.31	0.83 (0.57, 1.20)
ABCG2								
rs2231137	0.21	1.28 (0.88, 1.88)	0.26	1.27 (0.85, 1.87)	0.75	0.94 (0.64, 1.38)	0.79	0.92 (0.50, 1.69)
rs2231142	0.38	0.86 (0.62, 1.20)	0.33	0.84 (0.59, 1.20)	0.48	0.88 (0.60, 1.28)	0.42	1.25 (0.74, 2.11)
OCTN1								
rs1050152	**0.06**	0.81 (0.65, 1.01)	0.17	0.85 (0.68, 1.07)	**0.03**	0.79 (0.63, 0.98)	0.09	0.74 (0.52, 1.06)
OATP1A2								
rs11568563	0.53	0.84 (0.50, 1.44)	0.43	0.82 (0.49, 1.37)	0.40	0.80 (0.47, 1.37)	0.11	0.46 (0.15, 1.40)
CYP3A4								
rs2740574	0.48	1.23 (0.70, 2.19)	0.61	1.17 (0.64, 2.12)	0.25	1.35 (0.83, 2.33)	0.39	1.40 (0.68, 2.86)
CYP3A5								
rs776746	0.38	1.18 (0.83, 1.67)	0.50	1.14 (0.79, 1.64)	0.50	1.13 (0.79, 1.62)	0.25	1.39 (0.81, 2.38)
SNP combinations								
hOCT1	0.76	1.03 (0.86, 1.22)	0.92	1.01 (0.84, 1.21)	**0.03**	1.23 (1.01, 1.50)	**0.08**	1.31 (0.96, 1.79)
MDR1	0.54	0.98 (0.90, 1.06)	0.36	0.96 (0.88, 1.05)	0.54	1.03 (0.93, 1.11)	0.43	1.05 (0.93, 1.19)
ABCG2	0.96	1.01 (0.78, 1.30)	0.89	0.98 (0.76, 1.27)	0.44	0.90 (0.69, 1.18)	0.73	1.07 (0.72, 1.61)
CYP3A	0.37	0.89 (0.70, 1.14)	0.50	0.91 (0.71, 1.18)	0.35	0.89 (0.71, 1.13)	0.26	0.81 (0.57, 1.15)
IM Uptake	0.10	1.11 (0.98, 1.27)	0.19	1.09 (0.96, 1.24)	**0.004**	1.21 (1.06, 1.37)	**0.01**	1.30 (1.05, 1.61)
IM Efflux	0.66	0.98 (0.91, 1.06)	0.49	0.97 (0.89, 1.05)	0.71	1.02 (0.94, 1.10)	0.34	1.06 (0.94, 1.20)

** Hazard ratio estimates adjusted for randomized drug dose.*

Online Supplementary Table S4. Summary measures for the *hOCT1* gene.

SNP ID	Major / Minor allele	Favorable allele	Number of favorable alleles
Combination of SNP in the *hOCT1* gene			
rs72552763	GAT/-	GAT	GAT/GAT = 2; GAT/- 1; -/- = 0
rs12208357	C/T	C	CC = 2; CT = 1; TT = 0
rs683369	C/G	C	CG = 2; CG = 1; GG = 0
rs2282143	C/T	C	CC = 2; CT = 1; TT = 0

Study population	N. of patients	Total number of favorable alleles among SNP in the *hOCT1* gene*				
		0-4	5	6	7	8
All patients	189	0	11	26	59	84
Caucasian	156	0	10	22	49	66
Asian	23	0	0	2	8	13
Other races	10	0	1	2	2	5

**A total of 0 favorable alleles represents homozygosity for the unfavorable allele in all SNP in the combination; the maximum total of eight represents homozygosity for the favorable allele in all SNP in the* hOCT1 *gene.*

Online Supplementary Table S5. Summary measures for genes involved in imatinib uptake.

SNP ID	Major / Minor allele	Favorable allele	Number of favorable alleles
Combination of SNP in genes involved in imatinib uptake			
hOCT1 rs72552763	GAT/-	GAT	GAT/GAT = 2; GAT/- 1; -/- = 0
rs12208357	C/T	C	CC = 2; CT = 1; TT = 0
rs683369	C/G	C	CC = 2; CG = 1; GG = 0
rs2282143	C/T	C	CC = 2; CT = 1; TT = 0
OCTN1 rs1050152	C/T	C	CC = 2; CT = 1; TT = 0
OATP1A2 rs11568563	T/G	T	TT = 2; TG = 1; GG = 0

Study population	N. of patients	Total number of favorable alleles among SNP in genes involved in imatinib uptake*						
		0-6	7	8	9	10	11	12
All patients	189	0	3	16	22	43	60	36
Caucasian	156	0	3	14	21	39	52	18
Asian	23	0	0	0	0	2	8	13
Other races	10	0	0	2	1	2	0	5

**A total of 0 favorable alleles represents homozygosity for the unfavorable allele in all SNP in the combination; the maximum total of 12 favourable alleles represents homozygosity for the favorable allele in all SNP in imatinib uptake genes.*

Online Supplementary Table S6. CgR and MR according to the candidate genotypes among Caucasians.

SNP ID	MCgR P	MCgR Hazard ratio [95% CI]	CCgR P	CCgR Hazard ratio [95% CI]	MR P	MR Hazard ratio [95% CI]	MCR P	MCR Hazard ratio [95% CI]
hOCT1								
rs72552763	0.75	0.95 (0.72, 1.27)	0.72	1.05 (0.79, 1.41)	0.28	0.85 (0.63, 1.15)	0.60	0.88 (0.55, 1.42)
rs12208357	0.87	1.04 (0.65, 1.68)	0.92	1.02 (0.63, 1.66)	0.88	0.96 (0.58, 1.58)	0.13	0.50 (0.18, 1.35)
rs683369	0.91	1.02 (0.75, 1.38)	0.72	0.95 (0.70, 1.28)	0.25	0.83 (0.59, 1.15)	0.33	0.78 (0.47, 1.31)
rs2282143	0.68	1.36 (0.34, 5.56)	0.90	0.92 (0.23, 3.73)	0.08	0.25 (0.03, 1.79)	0.12	0.00 (---)
MDR1								
rs60023214	0.52	1.08 (0.85, 1.38)	0.24	1.17 (0.90, 1.50)	0.09	0.79 (0.60, 1.04)	0.005	0.56 (0.37, 0.84)
rs1128503	0.67	1.06 (0.82, 1.36)	0.25	1.16 (0.90, 1.50)	0.95	0.99 (0.77, 1.27)	0.70	0.93 (0.63, 1.37)
rs10245483	0.45	0.92 (0.75, 1.14)	0.35	0.90 (0.72, 1.12)	0.54	0.93 (0.74, 1.17)	0.17	1.28 (0.90, 1.80)
rs3213619	0.10	2.19 (0.95, 5.02)	0.24	1.71 (0.75, 3.93)	0.14	2.13 (0.86, 5.30)	0.08	2.78 (1.01, 8.02)
rs2038502	0.47	1.10 (0.85, 1.43)	0.39	1.13 (0.86, 1.48)	0.92	1.01 (0.77, 1.33)	0.24	0.78 (0.51, 1.18)
ABCG2								
rs2231137	0.97	0.99 (0.57, 1.71)	0.80	1.08 (0.61, 1.90)	0.76	1.09 (0.63, 1.90)	0.62	1.25 (0.54, 2.87)
rs2231142	0.39	0.84 (0.55, 1.28)	0.46	0.85 (0.55, 1.32)	0.20	0.75 (0.47, 1.19)	0.39	1.31 (0.73, 2.36)
OCTN1								
rs1050152	0.24	0.87 (0.68, 1.10)	0.42	0.90 (0.70, 1.16)	0.03	0.77 (0.61, 0.97)	0.07	0.70 (0.48, 1.03)
OATP1A2								
rs11568563	0.89	0.96 (0.55, 1.68)	0.66	0.89 (0.52, 1.51)	0.91	0.97 (0.57, 1.66)	0.13	0.47 (0.16, 1.43)
CYP3A4								
rs2740574	0.25	1.44 (0.79, 2.63)	0.26	1.44 (0.79, 2.62)	0.07	1.78 (1.00, 3.17)	0.17	1.83 (0.81, 4.09)
CYP3A5								
rs776746	0.72	1.08 (0.70, 1.67)	0.90	1.03 (0.66, 1.62)	0.30	1.25 (0.83, 1.87)	0.29	1.42 (0.77, 2.63)
SNP combination								
hOCT1	0.83	1.02 (0.85, 1.23)	0.88	1.01 (0.84, 1.23)	0.05	1.24 (1.00, 1.53)	0.09	1.31 (0.94, 1.83)
MDR1	0.44	0.97 (0.89, 1.05)	0.30	0.95 (0.87, 1.04)	0.67	1.02 (0.93, 1.12)	0.43	1.06 (0.92, 1.21)
ABCG2	0.43	0.87 (0.61, 1.24)	0.57	0.90 (0.62, 1.30)	0.36	0.85 (0.59, 1.22)	0.36	1.27 (0.77, 2.10)
CYP3A	0.47	0.90 (0.68, 1.19)	0.56	0.92 (0.69, 1.22)	0.16	0.82 (0.63, 1.06)	0.21	0.77 (0.53, 1.13)
imatinib Uptake	0.29	1.08 (0.93, 1.25)	0.33	1.08 (0.93, 1.25)	0.01	1.23 (1.06, 1.44)	0.01	1.37 (1.08, 1.75)
imatinib Efflux	0.43	0.97 (0.88, 1.05)	0.37	0.96 (0.87, 1.05)	0.82	1.01 (0.92, 1.11)	0.26	1.08 (0.94, 1.24)

** Hazard ratio estimates adjusted for randomized drug dose.*

Online Supplementary Table S7. *MDR1* haplotype frequencies.

Number haplotype [C3435T-C1236T-G2677T/A]	Haplotype frequecies ± standard error
1. C-C-A	0.0118±0.00915
2. C-C-G	0.416±0.0283
3. C-C-T	0.0208±0.0116
4. C-T-A	0.0107±0.00681
5. C-T-G	0.0641±0.0150
6. C-T-T	0.0363±0.121
7. T-C-A	0.00290±0.00291
8. T-C-G	0.100±0.0172
9. T-C-T	0.0108±0.00783
10. T-T-G	0.0133±0.00712
11. T-T-T	0.313±0.0258