Association of *SLC22A1,SLCO1B3* Drug Transporter Polymorphisms and Smoking with Disease Risk and Cytogenetic Response to Imatinib in Patients with Chronic Myeloid Leukemia

Fatemeh Mohammadi, PhD,[1] *Golale Rostami, PhD,*[2] *Dlnya Assad, PhD,*[3] *Mohammad Shafiei, PhD,*[1,4] *Mohammad Hamid, PhD,*[2,*] *Hasan Jalaeikhoo, MD*[5]

Laboratory Medicine 2021;52:584-596

DOI: 10.1093/labmed/lmab023

ABSTRACT

Objective: To determine whether polymorphisms of *SLC22A1* and *SLC01B3* genes could predict imatinib (IM) response and chronic myeloid leukemia (CML) risk.

Methods: We genotyped *SLC22A1 (c.480G > C, c.1222A > G)* and *SLC01B3 (c.334T > G, c.699G > A)* polymorphisms in 132 patients with CML and 109 sex- and age-matched healthy subjects. The patients were evaluated for cytogenetic response by standard chromosome banding analysis (CBA).

Results: Polymorphism analysis showed significant increased risk of IM resistance for *SLC22A1c.1222AG* ($P = .03$; OR = 2.2), *SLC01B3c.334TT/TG* genotypes ($P = .007$; OR = 4.37) and 334T allele ($P = .03$; OR = 2.86). The double combinations of *SLC22A1c.480CC* and *c.1222AG* polymorphisms with *SLC01B3c.334TT/TG* were significantly associated with complete cytogenetic response (CCyR) ($P < .05$; OR> 7). The interaction between all polymorphisms and smoking were associated with CML development and IM resistance ($P \leq .04$; OR> 3).

Conclusions: Our study results suggest the influence of *SLC22A1* and *SLC01B3* polymorphisms and the interaction of smoking on CML development and IM response.

Keywords: chronic myeloid leukemia, complete cytogenetic response, imatinib mesylate, *SLC22A1*, *SLC01B3*, *smoke*

Abbreviations:

CML, chronic myeloid leukemia; IM, imatinib mesylate; CP, chronic phase; AP, accelerated phase; BP, blastic phase; MCyR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response; ELN, european leukemia net; OR, odds ratio; CI, confidence interval; EFS, event- free survival; OS, overall survival; SLC22A1, solute carrier 22A1; SLCO1B3, solute carrier organic anion transporter family member 1B3; PCR–RFLP, Polymerase Chain Reaction–Restriction Fragment Length Polymorphism; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; LOR, loss of response.

[1]Department of Biology, School of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran, [2]Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, [3]Department of Biology, College of Science, Sulaimani University, Sulaymanyah, Iraq, [4]Biotechnology and Biological Science Research Center, Shahid Chamran University of Ahvaz, Ahvaz, Iran, [5]AJA Cancer Epidemiology Research and Treatment Center (AJA-CERTC), AJA University of Medical Sciences, Tehran, Iran

*To whom correspondence should be addressed.
hamid143@yahoo.com

Fatemeh Mohammadi and Golale Rostami contributed equally to this manuscript.

Chronic myeloid leukemia (CML) is a myeloproliferative disorder marked by the attendance of the Philadelphia (Ph) chromosome resulting from a balanced translocation between chromosomes 9 and 22, t (9; 22) (q34; q11), which creates a fusion oncogene, *BCR-ABL1*. This gene encodes a *Bcr-Abl1* chimeric protein with constitutively tyrosine kinase activity that leads to uncontrolled cell division.[1] Imatinib mesylate (IM; trade name, Glivec or Gleevec), a tyrosine kinase inhibitor (TKI), is a powerful Bcr-Abl1–targeting drug that is currently used as the first-line treatment for patients with newly diagnosed CML in the chronic phase (CML-CP).[2]

Despite significant improvements in clinical response rates and survival outcomes in patients with CML who undergo imatinib therapy, approximately 30% to 40% of patients develop failure in the cytogenetic and molecular response.[3,4] Several resistance mechanisms have been proposed; the most important include gene amplification, clonal

chromosome abnormalities in ph + cells, and mutations in the tyrosine kinase domain of *BCR-ABL1*. Further, the decrease bioavailability of IM in leukemic cells has been proposed as a major pharmacokinetic factor that participates in the development of resistance to IM.[5]

Seven of 55 *SLC* gene families encode drug-carrier transporters that participate in the influx of drugs into the cell.[6] The findings of 2 studies[7,8] showed that the levels of plasma concentration of IM are important for clinical outcomes in patients with CML; besides, active-transport processes could mediate the concentration of IM into mononuclear cells. IM is a substrate for influx transporters such as *SLC22A1* (solute carrier 22A1, organic cation transporter1, OCT1) (GenBank accession number; NC_000006.12) and *SLCO1B3* (solute carrier organic anion transporter family member 1B3, organic anion transporting polypeptide 1B3 [*OATP1B3*]) (NC_000012.12).[9]

White et al[10] found that the activity rate of *SLC22A1* is associated with the achievement of major molecular response (MMR), so MMR was observed with an increase of transporter activity. These investigators also showed that despite the decrease of *SLC22A1* activity, the MMR was achieved with an elevated IM dose. Further, in patients achieving complete cytogenetic response (CCyR), the *SLC22A1* mRNA expression was significantly higher than in patients with partial cytogenetic response (PCyR) and patients with resistance to IM.[11] It has been reported[12] that *SLC22A1* *c.480C > G* (p. L160F, db SNP ID number; rs683369) single nucleotide polymorphism (SNP) affects IM pharmacokinetics, event-free survival (EFS) duration, and rates of exposure to the drug. Another *SLC22A1* polymorphism, *c.1222A > G* (p.M408V, dbSNP ID number;rs628031), influences the prevalence of poor response, duration of EFS, and overall survival (OS).[13]

Another transporter, known as *SLCO1B3*, has a key role in the uptake of IM into hepatocytes and intracellular IM accumulation in leukocytes.[14] *SLCO1B3* has 2 major SNPs, including c.334T > G (p.Ser112Ala,dbSNP ID number; rs4149117) in exon 3 and c.699 G > A (p.Met233Ile,dbSNP ID number; rs7311358) in exon 6.[15] Some studies, such as de Lima et al,[16] have found that the aforementioned polymorphisms are associated with nonresponse to IM. Other study reports, such as Sayyed et al,[17] established that cigarette smoke inhibits the activity of drug transporters and alters their expression. Cigarette smoke directly inhibits activity of transporters, reduces the uptake function of the drug into the cell, and causes drug resistance.

The biological response to carcinogens activates several metabolic pathways that are involved in exclusion, such as transporters; detoxification agents, such as drug-metabolizing enzymes; and DNA repair. The polymorphisms of genes belonging to these pathways can modulate gene activity; however, carcinogens, including smoke, can modify the association of these polymorphisms with cancer risk and chemotherapy resistance.[18] Several studies, such as Björk et al,[19] have reported that benzene in cigarette smoke is associated with leukemia risk, and a relationship exists between cytogenetic abnormalities and AML risk in subjects who smoke. Some study reports, such as Kim HN et al,[20] have revealed that the association of GSTT1 polymorphisms with AML risk is dependent on smoking status.

To our knowledge, no study in the literature has investigated the joint effect of the smoking (as a synergistic factor) and genetic polymorphisms in *SLC22A1* and *SLCO1B3* genes on treatment response and CML risk. Moreover, we have also analyzed the impact of SNP combinations on response to IM and CML susceptibility.

Materials and Methods

Study Population

In this study, peripheral blood specimens were collected from 132 Ph + CML patients undergoing IM therapy (300–800 mg/day) at Arad Hospital and Saba Oncology Clinic in Tehran, Iran. This study was approved by the Research Ethics Committee of the Pasteur Institute of Iran, and specimens were used according to ethical standards (ethical approval no. IR.PII.REC.1397.56). Written informed consent was obtained from all patients and control individuals.

The median duration of IM treatment was 46 months (range, 10–175 months). Based on the response to IM therapy, the patients were classified into 2 groups: the responder group consisted of 58 patients who acquired a CCyR within 12 months from IM therapy, and the nonresponder group included 74 patients who had no CCyR. Moreover, venous blood specimens were obtained from 109 sex- and age-matched healthy individuals with the same ethnicity

DOI: 10.1093/labmed/lmab023

and without medical history or hematological evidence of leukemia or other chronic diseases. Smoking criteria were described as follows: *active smokers* were people have smoked at least pack of cigarettes daily; *passive smokers*, those exposed to cigarette smoke or have smoked 1 to 2 cigarettes daily; and *never smokers*, those who have never smoked. The characteristics of the participants are shown in **Table 1**.

Assessment of Response

Disease-phase definitions include chronic phase (CP), accelerated phase (AP), and blastic phase (BP); also, CCyR were determined according to World Health Organization (WHO) criteria[21] and European Leukemia Net (ELN) criteria.[22,23] The patients were evaluated at regular intervals for cytogenetic response using standard chromosome banding analysis (CBA) of bone-marrow-cell metaphases, as previously described.[24,25] CCyR was described as 0% Ph + chromosome in at least 20 metaphases.

SNPs Genotyping

Genomic DNA was extracted from blood using the salting-out extraction technique.[26] Genotype analysis was conducted by the PCR-restriction fragment length polymorphism (PCR-RFLP) and sequencing methods. Suitable enzymes were used to digest PCR products according to manufacturer instructions (Thermo Fisher Scientific Inc.). To confirm the quality of genotyping, 10% of the specimens were randomly sequenced; the results of both methods were consistent. Primer sequences, restriction enzymes, and PCR conditions are shown in Supplementary Table S1.

Statistical Analysis

Hardy-Weinberg equilibrium was calculated by comparing the observed and expected genotype frequencies for all SNPs using χ^2 testing. The distribution of baseline features between groups was compared for qualitative variables using χ^2 testing and for a quantitative variable (age) using *t* testing. In this study we used different genetic models for determining the association between all genotypes and alleles, with disease risk and response to IM determined using logistic regression test. The odds ratios (ORs), along with 95% confidence intervals (CIs), were also estimated.

Linkage disequilibrium (LD) analysis and calculation of haplotype frequencies were performed using the software HaploView ver. 4.2 AVAILABILITY: (https://www.broadinstitute.org/haploview/haploview). The association between combined polymorphisms, and also polymorphisms–smoking interaction with CML and IM resistance risk, was determined using logistic regression analysis. Because several comparisons can lead to false-positive results, Bonferroni correction of *P* values was carried out. *P* values of less than .05 were considered statistically significant. Statistical analysis was performed using SPSS software, version 22 (IBM Corporation).

Results

Baseline Characteristics of the Studied Subjects

The study-subjects cohort consisted of 132 patients with CML and 109 controls, of whom 56.1% of patients were in the IM nonresponder group and 43.9% in the IM responder group (**Table 1**). There was no significant difference in mean age in male or female participants between cases and controls ($P > .05$). However, the mean age difference was significant for male responders compared with nonresponders (39.55 vs 47.06; $P = .02$), as well as for male nonresponders compared with female nonresponders (39.55 vs 48.12; $P = .20$; data not shown). Smoking status was significantly different between cases and controls and also among drug response groups ($P < .001$). Moreover, there was a significant difference regarding smoking in females and male participants, between patients and controls ($P < .001$), as well as between response groups ($P = .001$; $P = .005$), respectively.

Allelic and Genotypic Frequencies of SNPs

All of the SNPs were in agreement with Hardy-Weinberg equilibrium (HWE) in the CML patients and controls. The only exception was *SLC22A1c.480G > C* polymorphism, which was inconsistent with HWE only in patients.

The distribution of the genotypes in cases and controls, as well as in IM response groups, is shown in **Table 2**. We used different genetic models to evaluate the relationship between SNPs with CML risk and response to IM. The frequencies of genotypes and alleles of *SLCO1B3* (*c.334T > G*, *c.699G > A*) and *SLC22A1* (*c.480G > C*, *c.1222A > G*) polymorphisms were similar among patients with CML and

Table 1. Baseline Features of the Studied Subject Individuals

Features		Control Individuals	Patients	*P* Value[a]	IM Responders[b]	IM Nonresponders	*P* Value[a]
Individuals, no.		109	132		58	74	
Age (y)							
Sex, mean (SD)		43.27 (15.23)	44.6 (15.08)	.65	46.02 (14.44)	43.49 (15.57)	.34
Male		42.44 (14.6)	43.05 (14.2)	.81	47.06 (13.6)	39.55 (13.94)	**.02**
Female		45.12 (15.9)	46.63 (16.05)	.66	44.43 (15.8)	48.12 (16.29)	.40
Sex, no. (%)							
Male		57 (52.3%)	75 (56.8%)	.48	35 (60.3%)	40 (54.1%)	.47
Female		52 (47.7%)	57 (43.2%)		23 (39.7%)	34 (45.9%)	
Smoking status, no (%)							
Total	Active	10 (9.2%)	35 (26.5%)	**<.001**	9 (15.5%)	26 (35.1%)	**<.001**
	Passive	9 (8.3%)	35 (26.5%)		9 (15.5%)	26 (35.1%)	
	Never	90 (82.6%)	62 (47%)		40 (69.0%)	22 (29.7%)	
Males	Active	9 (15.8%)	33 (44.0%)	**<.001**	9 (25.7%)	24 (60.0%)	**.005**
	Passive	5 (8.8%)	11 (14.7%)		5 (14.3%)	6 (15.0%)	
	Never	43 (75.4%)	31 (41.3%)		21 (60.0%)	10 (25.0%)	
Females	Active	1 (1.9%)	2 (3.5%)	**<.001**	0	2 (5.9%)	**.001**
	Passive	4 (7.7%)	24 (42.1%)		4 (17.4%)	20 (58.8%)	
	Never	47 (90.4%)	31 (54.4%)		19 (82.6%)	12 (35.3%)	
Follow- up duration (mo)							
Mean (SD)					62.67 (36.986)	57.70 (30.518)	
IM treatment duration (mo)							
Mean (SD)					58.88 (35.635)	50.65 (23.105)	

IM; imatinib mesylate.
[a]P *<.05 (bolded) was considered statistically significant.*

healthy individuals (*P*> .05), indicating no relationship between these SNPs and CML risk (**Table 2**).

The frequency of the major allele C and the minor allele G for *SLC22A1c.480G > C* was 0.88 and 0.12. respectively; for *SLC22A1 c.1222A > G*, the frequency of the major allele G and the minor allele A were 0.70 and 0.30, respectively. In the case of *SLCO1B3c.699A > G*, the frequency of major allele A and minor allele G was 0.93 and 0.07, respectively; also, for *SLCO1B3c.334T > G*, the frequency of major and minor alleles was 0.87 and 0.13, respectively. There was no significant difference in the genotype and allele frequency of *SLCO1B3c.699G > A* and *SLC22A1c.480G > C* polymorphisms between IM responders and IM nonresponders (*P*> .05).

The *SLCO1B3c.334TG* (codominant model) and *SLCO1B3c.334 TG/TT* genotypes (dominant model) were associated with IM resistance: patients with 334TG and 334 TG/TT genotypes had a higher risk of resistance (*P* = .01, OR = 6.02; *P* = .007, OR = 4.37). There was also an increased risk of IM resistance in patients with the 334T allele (*P* = .03; OR = 2.86). *SLC22A1c.1222 AA* (recessive model) and *SLC22A1c. 1222* AG genotypes (codominant model) were associated with IM response—those with the *SLC22A1c.1222 AA* genotype had decreased IM resistance risk (*P* = .04; OR = 0.25) and those with the *SLC22A1c.1222 AG* genotype had increased resistance risk to IM (*P* = .03; OR = 2.20; **Table 2**).

In female and male groups, there was no relationship between *SLCO1B3* (*c.334T > G*, *c.699G > A*) and *SLC22A1* (*c.480G > C*, *1222A > G*) SNPs with CML risk and IM response. The only exception was the AG genotype from *SLC22A1 c.1222A > G* SNP, which was associated with increase of IM resistance risk in female participants, following the codominant model (*P* = .03; OR = 8.68; Supplementary Tables S2, S3, S4, S5).

Haplotyping

Haplotype analysis revealed a strong linkage disequilibrium between *SLCO1B3 c.334T > G*, *c.699G > A* polymorphisms (D′ = 1; r^2=0.53; LOD = 25.5), and also between *SLC22A1 c.480G > C*, *c.1222A > G* polymorphisms (D′ = 0.88; r^2 = 0.2;, LOD = 16.4; **Figure 1**, **Table 2**). The *SLCO1B3 c.334G- c.699A* haplotype was associated with decreased risk of IM response failure—its frequency was significantly lower in IM nonresponders than in responders (85.1% vs 93.1%; *P* = .04, OR = 0.43). None of the haplotypes were

Table 2. Association Analyses Between SLC22A1, SLCO1B3 SNP Genotypes and Haplotypes with Imatinib Response and CML Risk

SNP Model	Genotype Allele	CCyR, no. (%)[a]	Non-CCyR[b]	OR (95% CI)[c]		Control Individuals[d]	Patients[e]	*P* Value	OR (95% CI)
SLC22A1c.480G > C									
Codominant	CC	46 (79.3)	62 (83.8)		1 [reference]	82 (75.9)	108 (81.8)		1 [reference]
	GC	11 (19.0)	9 (12.2)	.51	0.58 (0.20–1.69)	23 (21.3)	20 (15.2)	.38	0.61 (0.29–1.25)
	GG	1 (1.7)	3 (4.0)		1.89 (0.16–21.83)	3 (2.8)	4 (3.0)		1.17 (0.23–6.02)
Missing data						1			
Dominant	CC	46 (79.3)	62 (83.8)		1 [reference]	82 (75.9)	108 (81.8)		1 [reference]
	GC/GG	12 (20.7)	12 (16.2)	.48	0.70 (0.26–1.87)	26 (24.1)	24 (18.2)	.24	0.67 (0.34–1.32)
Recessive	CC/GC	57 (98.3)	71 (96.0)		1 [reference]	105 (97.2)	128 (97.0)		1 [reference]
	GG	1 (1.7)	3 (4.0)	.58	2.00 (0.17–22.99)	3 (2.8)	4 (3.0)	.76	1.28 (0.25–6.54)
Allele	C	103 (89.0)	133 (90.0)	[reference]	1	187 (86.6)	236 (89.4)	[reference]	1
	G	13 (11.0)	15 (10.0)	.69	0.84 (0.35–1.99)	29 (13.4)	28 (10.6)	.36	0.75(0.41–1.37)
SLC22A1c.1222A > G									
Codominant	GG	30 (51.7)	36 (48.6)		1 [reference]	52 (47.7)	66 (50.0)		1 [reference]
	AG	21 (36.2)	32 (43.2)	.03[c]	2.20 (0.92–5.27)	46 (42.2)	53 (40.2)	.46	1.01 (0.57–1.81)
	AA	7 (12.1)	6 (8.1)		0.33 (0.08–1.26)	11 (10.1)	13 (9.8)		0.54 (0.20–1.47)
Dominant	GG	30 (51.7)	36 (48.6)		1 [reference]	52 (47.7)	66 (50.0)		1 [reference]
	AG/AA	28 (48.3)	38 (51.4)	.36	1.43 (0.66–3.08)	57 (52.3)	66 (50.0)	.73	0.91 (0.52–1.57)
Recessive	GG/AG	51 (87.9)	68 (91.9)		1 [reference]	98 (89.9)	119 (90.2)		1 [reference]
	AA	7 (12.1)	6 (8.1)	.04[c]	0.25 (0.07–0.94)	11 (10.1)	13 (9.8)	.21	0.54 (0.21–1.41)
Allele	G	81 (69.8)	104 (70.3)		1 [reference]	150 (68.8)	185 (70.1)		1 [reference]
	A	35 (30.2)	44 (29.7)	.83	0.94 (0.52–1.69)	68 (31.2)	79 (29.9)	.41	0.838 (0.55–1.28)
SLCO1B3c.699G > A									
	AA	54 (93.1)	64 (86.5)		1 [reference]	88 (80.7)	118 (89.4)		1 [reference]
	GA	4 (6.9)	10 (13.5)	.13	2.85 (0.74–11.08)	21 (19.3)	14 (10.6)	.08	0.50 (0.23–1.10)
Allele	A	112 (96.6)	138 (93.2)		1 [reference]	197 (90.4)	250 (94.7)		1 [reference]
	G	4 (3.4)	10 (6.8)	.14	2.67 (0.72–9.83)	21 (9.6)	14 (5.3)	.10	0.53 (0.25–1.13)
SLCO1B3c.334T > G									
Codominant	GG	51 (87.9)	52 (70.3)		1 [reference]	79 (72.5)	103 (78)		1 [reference]
	TG	6 (10.3)	22 (29.7)	.01[c]	6.02 (1.88–19.26)	28 (25.7)	28 (21.2)	.41	0.70 (0.36–1.34)
	TT	1 (1.7)	0		NA	2 (1.8)	1 (0.8)		0.34 (0.02–4.68)
Dominant	GG	51 (87.9)	52 (70.3)		1 [reference]	79 (72.5)	103 (78.0)		1 [reference]
	TG/TT	7 (12.1)	22 (29.7)	.007[c]	4.37 (1.49–12.84)	30 (27.5)	29 (22.0)	.22	0.67 (0.35–1.28)
Recessive	GG/TG	57 (98.3)	74 (100)		1 [reference]	107 (98.2)	131 (99.2)		1 [reference]
	TT	1 (1.7)	0	1	NA	2 (1.8)	1 (0.8)	.44	0.37 (0.03–5.09)
Allele	G	108 (0.93)	126 (85.1)		1 [reference]	186 (85.3)	234 (88.6)		1 [reference]
	T	8 (6.9)	22 (14.9)	.03[c]	2.86 (1.11–7.36)	32 (14.7)	30 (11.4)	.20	0.68 (0.38–1.22)

Table 2. Continued

Gene	Haplotype	CCyR N (%)	Non-CCyR N (%)	*P* Value	OR (95% CI)	Controls, no. (%)	Patients, no. (%)	*P* Value	OR (95% CI)
SLC22A1	C-G	81 (69.8)	103 (69.5)	.97	0.99 (.58–1.68)	184 (69.6)	147 (67.3)	.59	1.11 (0.75–1.63)
	C-A	22 (19.0)	30.1 (20.3)	.79	1.09 (0.59–2.01)	52 (19.8)	42 (19.4)	.91	1.03 (0.65–1.61)
	G-A	13 (11.1)	13.9 (9.4)	.64	0.83 (0.37–1.84)	27 (10.1)	26 (11.8)	.55	0.84 (0.47–1.50)
SLCO1B3	G-A	108 (93.1)	126 (85.1)	**.04**[c]	0.43 (0.18–0.992)	234 (86.6)	186 (85.3)	.27	1.34 (0.79–2.29)
	T-A	4 (3.4)	12 (8.1)	.12	2.47 (0.77–7.87)	14 (5.3)	21 (9.6)	.07	0.53 (0.26–1.06)
	T-G	4 (3.4)	10 (6.8)	.23	2.03 (0.62–6.64)	16 (6.1)	11 (20.7)	.63	1.21 (0.55–2.67)

SNP, single nucleotide polymorphism; CCyR, complete cytogenetic response;OR, odds ratio; CI, confidence interval; SLC22A1, solute carrier 22A1 (GenBank accession number; NC_000006.12); SLCO1B3, solute carrier organic anion transporter family member 1B3(NC_000012.12); NA, nonapplicable.
[a] *n = 58.*
[b] *n = 74.*
[c] *P <.05 was considered statistically significant. Logistic regression model adjusted for age, sex, and smoking status.*
[d] *n = 109.*
[e] *n = 132.*
[f] *D' =0.88; LOD = 16.4; r^2=0.23.*
[g] *D' =1; LOD = 25.5; r^2 =0.53.*

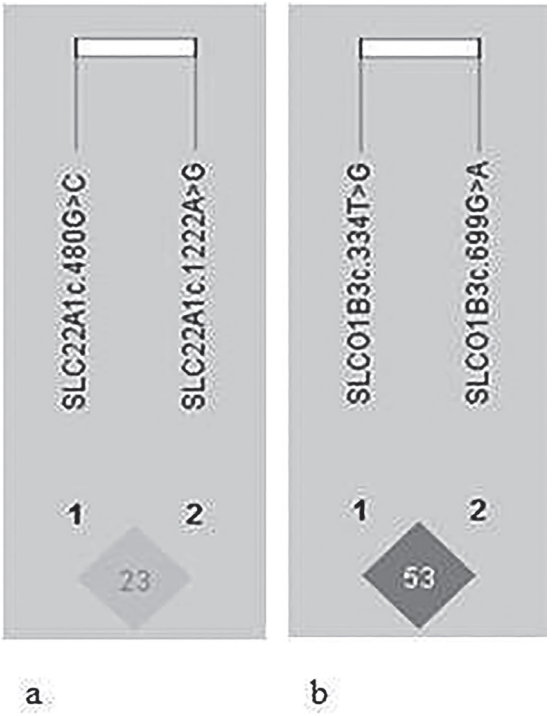

Figure 1

Haploview linkage disequilibrium (LD) map of studied-genes polymorphisms: The plots show the r^2 values. A, LD Plot of SLC22A1 (c.480G > C, c.1222A > G) Polymorphisms (GenBank accession number; NC_000006.12). B, LD Plot of SLCO1B3 (c.334T > G, c.699G > A) Polymorphisms (SLCO1B3; NC_000012.12).

associated with CML risk. The haplotype frequency of *SLCO1B3* and *SLC22A1* polymorphisms are shown in **Table 2**.

Assessment of Combined Genotypes

We considered AA genotype to be a reference genotype for *SLCO1B3c.699A > G*, an overdominant model for *SLC22A11222A > G* and dominant model for *SLC22A1c.480G > C, SLCO1B3c.334T > G* polymorphisms, to evaluate the joint effect of double SNP combination on CML susceptibility and CCyR to IM. An increased risk of non-CCyR to IM was observed in patients carrying the double combination of *SLCO1B3 c.334TG/TT* genotypes with any of *SLC22A1 c.480CC* and *SLC22A1c.1222AG* genotypes (*P* = .006, OR = 8.84; *P* = .05, OR = 7.73, respectively; **Table 3**). In female and male groups, there were no significant association between the double combined genotypes of the SNPs with increased risk of IM resistance and CML development (Supplementary Tables S6–S8).

Gene-Smoking Interaction

The effect of the interaction between *SLC22A1, SLCO1B3* SNPs with smoking on the risk of IM resistance and CML risk are shown in **Tables 4** and **5**, respectively. The reference group was nonsmoker subjects with any of the *SLC22A1c.480 CC*, *SLC22A1c.1222GG/AA,*

Table 3. Combined SLC22A1 and SLCO1B3 SNP Genotypes and Imatinib Response

SNP–SNP Combination		CCyR, no. (%)[a]	Non-CCyR, no. (%)[b]	Adjusted OR (95% CI)	*P* Value[b]	*P* Value, Bonferroni-Corrected
SLC22A1c.1222A > G	SLC22A1c.480G > C					
GG/AA	CC	32 (55.2%)	39 (52.7%)	1 [reference]		>.99
	GG/GC	5 (8.6%)	3 (4.1%)	0.33 (0.06–1.72)	.33	>.99
AG	CC	14 (24.1%)	23 (31.1%)	2.61 (1.00–6.85)	.05	.31
	GG/GC	7 (12.1%)	9 (12.2%)	1.69 (0.48–5.98)	.48	>.99
SLCO1B3c.699G > A	SLC22A1c.480G > C					
AA	CC	42 (72.4%)	53 (71.6%)	1 [reference]		
	GG/GC	12 (20.7%)	11 (14.9%)	0.64 (0.23–1.78)	.39	>.99
GA	CC	4 (6.9%)	9 (12.2%)	2.06 (0.50–8.49)	.32	>.99
	GG/GC	0	1 (1.4%)	NA	>.99	>.99
SLCO1B3c. 334T > G	SLC22A1c.480G > C					
GG	CC	42 (72.4%)	41 (55.4%)	1 [reference]		
	GG/ GC	9 (15.5%)	11 (14.9%)	1.29 (0.43–3.85)	.65	>.99
TG/TT	CC	4 (6.9%)	21 (28.4%)	8.84 (2.33–33.49)	.001	.006[d]
	GG/ GC	3 (5.2%)	1 (1.4%)	0.29 (0.02–3.83)	.35	>.99
SLCO1B3c. 699G > A	SLC22A1c.1222A > G					
AA	GG/AA	35 (60.3%)	35 (47.3%)	1 [reference]		
	AG	19 (32.8%)	29 (39.2%)	3.20 (1.28–8.05)	.01	.78
GA	GG/AA	2 (3.4%)	7 (9.5%)	6.85 (1.02–45.93)	.048	.29
	AG	2 (3.4%)	3 (4.1%)	3.52 (0.41–30.37)	.25	>.99
SLCO1B3c. 334T > G	SLC22A1c.1222A > G					
GG	GG/AA	34 (58.6%)	29 (39.2%)	1 [reference]		
	AG	17 (29.3%)	23 (31.1%)	2.86 (1.09–7.47)	.03	.19
TG/TT	GG/AA	3 (5.2%)	13 (17.6%)	6.54 (1.34–31.83)	.02	.12
	AG	4 (6.9%)	9 (12.2%)	7.73 (1.69–35.36)	.008	.048[d]
SLCO1B3c. 334T > G	SLC01B3c. 699G > A					
GG	AA	51 (87.9%)	52 (70.3%)	1 [reference]		
	GA	0	0	NA	NA	
TG/TT	AA	3 (5.2%)	12 (16.2%)	5.48 (1.23–24.35)	.02	.15
	GA	4 (6.9%)	10 (13.5%)	3.55 (0.89–14.20)	.07	.44

SNP, single nucleotide polymorphism; CCyR, complete cytogenetic response; OR, odds ratio; CI, confidence interval; SLC22A1, solute carrier 22A1 (GenBank accession number; NC_000006.12); SLCO1B3, solute carrier organic anion transporter family member 1B3(NC_000012.12);
[a] *n = 58.*
[b] *n = 74.*
[c] *Logistic regression model adjusted for age, sex, and smoking status.*
[d] *P <.05 was considered statistically significant.*

SLCO1B3c.699AA, and *SLCO1B3c.334GG* genotypes. We considered the sum of active and passive groups as smokers. The risk of IM resistance increased in the smoker subjects with the *SLC22A1c.480CC*; *SLC22A1c.1222AA/GG,AG*; *SLCO1B3c.699AA*; and *SLCO1B3c.334GG,TG/TT* genotypes (*P* <.001; OR = 7.03, 12.67, 16.74, 5.58, 8.13, and 22.09, respectively). Moreover, nonsmoker patients with the SLCO1B3c.334TG/TT genotype had an increased risk of IM resistance (*P* = .04; OR = 5.86). We also observed that the *SLC22A1c.480CC*; *SLC22A1c.1222AA/GG,AG*; and *SLCO1B3c.334GG,TG* genotypes in male smoker participants were associated with increased resistance to IM (*P* = .01, OR = 6.09; *P* = .03, OR = 8.33; *P* = .03, OR = 9.36; *P* = .02, OR = 5.98; and *P* = .04, OR = 24.10, respectively). The female smoker participants with genotypes including *SLC22A1c.480CC*, *SLC22A1c.1222AA/GG*, *SLCO1B3c.699AA*, and *SLCO1B3c.334GG* genotypes had an increased risk of resistance to IM (*P* = .004, OR = 11.74; *P* <.001, OR = 27.54; *P* = .004, OR = 9.42; and *P* = .004, OR = 11.22, respectively; **Table 4**).

Regarding CML risk, the smoker subjects carrying *SLC22A1c.480CC*; *SLC22A1c.1222AA/GG, AG*; *SLCO1B3c.699AA*; and *SLCO1B3c.334GG* genotypes had an increased risk for CML development (*P* <.001, OR = 5.48; *P* < .001, OR = 8.67; *P* = .01, OR = 4.09; *P* <.001, OR = 5.59; and *P* <.001, OR = 6.18, respectively). The smoker females with *SLC22A1c.480CC*, *SLC22A1c.1222AA/GG*,

Table 4. Gene-Smoking Interaction and Imatinib Response in Patient Groups

SNP	Current or Ever Smoking	CCyR, no. (%)	Non-CCyR, no. (%)	Adjusted OR (95% CI)	*P* Value[a,b]	*P* Value,' Bonferroni corrected[b]
Patients[c]						
SLC22A1c.480G > C						
CC	N	33 (56.9%)	18 (24.3%)	1 [reference]		
	Y	13 (22.4%)	44 (59.5%)	7.03 (2.93–16.87)	<.001	<.001[b]
GC/GG	N	7 (12.1)	4 (5.4%)	1.06 (0.26–4.28)	.93	>.99
	Y	5 (8.6%)	8 (10.8%)	3.49 (0.96–12.70)	.06	.23
SLC22A1c.1222A > G						
GG/AA	N	24 (41.4%)	6 (8.1%)	1 [reference]		
	Y	13 (22.4%)	36 (48.6%)	12.67 (4.07–39.43)	<.001	<.001[b]
AG	N	16 (27.6%)	16 (21.6%)	4.22 (1.32–13.45)	.01	.06
	Y	5 (8.6%)	16 (21.6%)	16.74 (4.10–68.43)	<.001	<.001[b]
SLCO1B3c.699G > A						
AA	N	36 (62.1%)	19 (25.7%)	1 [reference]		
	Y	18 (31.0%)	45 (60.8%)	5.58 (2.45–12.74)	<.001	<.001[b]
GA	N	4 (6.9%)	3 (4.1%)	1.69 (0.33–8.63)	.53	>.99
	Y	0	7 (9.5%)	NA	>.99	>.99
SLCO1B3c.334T > G						
GG	N	35 (60.3%)	14 (18.9%)	1 [reference]		
	Y	16 (27.6%)	38 (51.4%)	8.13 (3.14–21.02)	<.001	<.001[b]
TG/TT	N	5 (8.6%)	8 (10.8%)	5.86 (1.50–22.89)	.01	.04[b]
	Y	2 (3.4%)	14 (18.9%)	22.09 (4.22–115.73)	<.001	<.001[b]
Female[d]						
SLC22A1c.480G > C						
CC	N	16 (69.6%)	9 (26.5%)	1 [reference]		
	Y	3 (13.0%)	19 (55.9%)	11.74 (2.66–51.77)	.001	.004[b]
GC/GG	N	3 (13.0%)	3 (8.8%)	2.03 (0.32–12.88)	.45	>.99
	Y	1 (4.3%)	3 (8.8%)	5.03 (0.45–56.58)	.19	.76
SLC22A1c.1222A > G						
GG/AA	N	12 (52.2%)	2 (5.9%)	1 [reference]		
	Y	4 (17.4%)	18 (52.9%)	27.54 (4.28–177.29)	<.001	<.001[b]
AG	N	7 (30.4%)	10 (29.4%)	8.75 (1.45–52.69)	.02	.07
	Y	0	4 (11.8%)	NA	>.99	>.99
SLCO1B3c.699G > A						
AA	N	19 (82.6%)	10 (29.4%)	1 [reference]		
	Y	4 (17.4%)	20 (58.8%)	9.42 (2.52–35.27)	.001	.004[b]
GA	N	0	2 (5.9%)	NA	>.99	>.99
	Y	0	2 (5.9%)	NA	>.99	>.99
SLCO1B3c.334T > G						
GG	N	19 (82.6%)	9 (26.5%)	1 [reference]		
	Y	3 (13.0%)	16 (47.1%)	11.22 (2.59–48.66)	.001	.004[b]
TG/TT	N	0	3 (8.8%)	NA	>.99	>.99
	Y	1 (4.4%)	6 (17.6%)	12.47 (1.28–121.70)	.03	.12
Male[e]						
SLC22A1c.480G > C						
CC	N	17 (48.6%)	9 (22.5%)	1 [reference]	[reference]	
	Y	10 (28.6%)	25 (62.5%)	6.09 (1.86–19.88)	.003	.01[b]
GC/GG	N	4 (11.4%)	1 (2.5%)	1.04 (0.09–12.25)	.97	>.99
	Y	4 (11.4%)	5 (12.5%)	2.89 (0.55–15.03)	.21	.83
SLC22A1c.1222A > G						
GG/AA	N	12 (34.3%)	4 (10.0%)	1 [reference]		
	Y	9 (25.7%)	18 (45.0%)	8.33 (1.82–38.13)	.008	.03[b]
AG	N	9 (25.7%)	6 (15.0%)	2.66 (0.52–13.70)	.24	.97
	Y	5 (14.3%)	12 (30.0%)	9.36 (1.78–49.24)	.008	.03[b]
SLCO1B3c.699G > A						

DOI: 10.1093/labmed/lmab023

Table 4. Continued

SNP	Current or Ever Smoking	CCyR, no. (%)	Non-CCyR, no. (%)	Adjusted OR (95% CI)	*P* Value[a,b]	*P* Value,' Bonferroni corrected[b]
AA	N	17 (48.6%)	9 (22.5%)	1 [reference]		
	Y	14 (40.0%)	25 (62.5%)	3.80 (1.27–11.38)	.02	.07
GA	N	4 (11.4%)	1 (2.5%)	0.33 (0.03–3.82)	.38	>.99
	Y	0	5 (12.5%)	NA	>.99	>.99
SLCO1B3c.334T > G						
GG	N	16 (45.7%)	5 (12.5%)	1 [reference]		
	Y	13 (37.1%)	22 (55.0%)	5.98 (1.68–21.33)	.006	.02[b]
TG	N	5 (14.3%)	5 (12.5%)	2.74 (0.52–14.42)	.23	.94
	Y	1 (2.9%)	8 (20.0%)	24.10 (2.25–258.24)	.009	.04[b]

SNP, single nucleotide polymorphism; CCyR, complete cytogenetic response; OR, odds ratio; CI, confidence interval; SLC22A1, solute carrier 22A1 (GenBank accession number; NC_000006.12); SLCO1B3, solute carrier organic anion transporter family member 1B3(NC_000012.12).
[a]*P-value logistic regression model adjusted for age status.*
[b]*P <.05 was considered statistically significant.*
[c]*n = 132.*
[d]*n = 57.*
[e]*n = 75.*

SLCO1B3c.699AA, and *SLCO1B3c.334GG* genotypes had an increased risk of CML development (*P* = .004, OR = 14.49; *P* < .001, OR = 13.19; *P* = .004, OR = 7.71; and *P* = .02, OR = 5.8, respectively). Also, the carriers of *SLC22A1c.480CC*; *SLC22A1c.1222AA/GG,AG*; *SLCO1B3c.699AA*, and *SLCO1B3c.334GG* genotypes in male smokers had an increased risk of CML (*P* = .004, OR = 3.92; *P* <.001, OR = 6.75; *P* = .036, OR = 4.23; *P* <.001, OR = 4.77; and *P* <.001, OR = 6.66, respectively; **Table 5**).

Discussion

Many studies have been carried out to identify pharmacogenetic factors that predict IM treatment outcomes in patients with CML. To our knowledge, this is the first study that evaluates the association of 4 polymorphisms in *SLC22A1* and *SLCO1B3*, along with SNP combinations and SNP-smoking interaction, with the risk of CML development and IM resistance in the Iranian population. In this study, minor allele frequency (MAF) of the *SLC22A1c.480G > C*, *c.1222A > G*, *SLCO1B3c.699G > A*, and *c.334T > G* polymorphisms, compared with other populations (1000 Genomes Project Phase 3) was closer to American, African, South Asian, and European populations, respectively (Ensembl.org).

An important finding of our study was the lower mean age of male nonresponders than male responders. Some studies, such as Singh et al,[27] have shown that younger patients have a better response to the drug, compared with older patients. However, the results of a more recent study[28] showed no difference in achieving the optimal response among elderly patients than younger ones. Based on our findings, a significant association was observed between *SLC22A1c.1222AA* with higher CCyR achievement. A significant association between *SLC22A1c.1222A > G* with IM response was revealed by another study report[29] as well.

In contrast to our results, there are conflicting findings in the literature. For instance, Makhtar et al[30] found that the *SLC22A1c.1222AA* genotype, together with 8-bp insertion and 3-bp deletion, and M420del alleles increased the risk of resistance to IM. Also, Vaidya et al[31] and Takahashi et al[32] reported the association of the GG genotype of *SLC22A1c.1222* SNP with better response to IM.

Some study reports found no relationship between this polymorphism and IM response.[33,34] These findings may be due to the different ethnicities of the studied populations.[35] Our findings showed no association between *SLC22A1c.408G > C* polymorphism and IM treatment response, which is consistent with the findings of 2 studies[36,37] and contradicts other findings.[30,38]

We analyzed 2 known polymorphisms in the *SLCO1B3* gene, namely, c.699G > A and C.334T > G, that are related

Table 5. Gene-Smoking Interaction and CML Risk in All Subjects, the Female Group, and the Male Group

SNP	Current or Ever Smoking	Controls	Patients	Adjusted OR (95% CI)	*P* Value[a,b]	*P* Value, Bonferroni Corrected[b]
Subjects[c]						
SLC22A1c.480G > C						
CC	N	68 (63.1%)	51 (38.5%)	1 [reference]		
	Y	14 (13.1%)	57 (43.2%)	5.48 (2.73–10.97)	<.001	>.99
GC/GG	N	21 (19.41%)	11 (8.3%)	0.69 (0.30–1.56)	.37	>.99
	Y	5 (4.6%)	13 (9.8%)	3.44 (1.15–10.32)	.03	.11
SLC22A1c.1222A > G						
GG/AA	N	53 (48.6%)	30 (22.7%)	1 [reference]		
	Y	10 (9.2%)	49 (37.1%)	8.67 (3.84–19.59)	<.001	<.001
AG	N	37 (33.9%)	32 (24.2%)	1.55 (0.80–2.98)	.19	.77
	Y	9 (8.3%)	21 (15.9%)	4.09 (1.64–10.20)	.003	<.01
SLCO1B3c.699G > A						
AA	N	73 (67.0%)	55 (41.7%)	1 [reference]		
	Y	15 (13.8%)	63 (47.7%)	5.59 (2.86–10.93)	<.001	<.001
GA	N	17 (15.6%)	7 (5.3%)	0.55 (0.21–1.41)	.21	.85
	Y	4 (3.7%)	7 (5.3%)	2.34 (0.65–8.46)	.19	.78
SLCO1B3c.334T > G						
GG	N	67 (61.5%)	49 (37.1%)	1 [reference]		
	Y	12 (11.0%)	54 (40.9%)	6.18 (2.96–12.89)	<.001	<.001
TG/TT	N	23 (21.1%)	13 (9.8%)	0.77 (0.36–1.68)	.52	>.99
	Y	7 (6.4%)	16 (12.1%)	3.15 (1.20–8.30)	.02	<.08
Female[d]						
SLC22A1c.480G > C						
CC	N	33 (63.5%)	25 (43.9%)	1 [reference]		
	Y	2 (3.8%)	22 (38.6%)	14.49 (3.11–67.45)	.001	.004
GC/GG	N	14 (26.9%)	6 (10.5%)	0.57 (0.19–1.69)	.31	>.99
	Y	3 (5.8%)	4 (7.0%)	1.72 (0.35–8.46)	.51	>.99
SLC22A1c.1222A > G						
GG/AA	N	25 (48.1%)	14 (24.6%)	1 [reference]		
	Y	3 (5.8%)	22 (38.6%)	13.19 (3.34–52.13)	<.001	<.001
AG	N	22 (42.3%)	17 (29.8%)	1.42 (0.56–3.60)	.46	>.99
	Y	2 (3.8%)	4 (7.0%)	3.54 (0.57–21.85)	.17	.69
SLCO1B3c.699G > A						
AA	N	38 (73.1%)	29 (50.9%)	1 [reference]		
	Y	4 (7.7%)	24 (42.1%)	7.71 (2.40–24.75)	.001	.004
GA	N	9 (17.5%)	2 (3.5%)	0.28 (0.05–1.41)	.12	.49
	Y	1 (1.9%)	2 (3.5%)	2.56 (0.22–29.78)	.45	>.99
SLCO1B3c.334T > G						
GG	N	35 (67.3%)	28 (49.1%)	1 [reference]		
	Y	4 (7.7%)	19 (33.3%)	5.80 (1.76–19.07)	.004	.02
TG/TT	N	12 (23.1%)	3 (5.3%)	0.29 (0.07–1.17)	.08	.33
	Y	1 (1.9%)	7 (12.3%)	8.46 (0.98–73.18)	.05	.21
Male[e]						
SLC22A1c.480G > C						
CC	N	35 (62.5%)	26 (37.4%)	1 [reference]		
	Y	12 (21.4%)	35 (46.7%)	3.92 (1.71–8.98)	.001	.004
GC/GG	N	7 (12.5%)	5 (6.7%)	0.95 (0.26–3.39)	.99	>.99
	Y	2 (3.6%)	9 (12%)	6.01 (1.19–30.31)	.03	.12
SLC22A1c.1222A > G						
GG/AA	N	28 (49.1%)	16 (21.3%)	1 [reference]		
	Y	7 (12.3%)	27 (36.0%)	6.75 (2.40–18.96)	<.001	<.001
AG	N	15 (26.3%)	15 (20.0%)	1.75 (0.68–4.49)	.25	.99
	Y	7 (12.3%)	17 (22.7%)	4.23 (1.45–12.40)	.009	.04
SLCO1B3c.699G > A						

DOI: 10.1093/labmed/lmab023

Table 5. Continued

SNP	Current or Ever Smoking	Controls	Patients	Adjusted OR (95% CI)	*P* Value[a,b]	*P* Value, Bonferroni Corrected[b]
AA	N	35 (61.4%)	26 (34.7%)	1 [reference]		
	Y	11 (19.3%)	39 (52.0%)	4.77 (2.06–11.06)	<.001	<.001
GA	N	8 (14.0%)	5 (6.7%)	0.85 (0.25–2.91)	.79	>.99
	Y	3 (5.3%)	5 (6.7%)	2.25 (0.49–10.26)	.30	>.99
SLCO1B3c.334T > G						
GG	N	32 (56.1%)	21 (28.0%)	1 [reference]		
	Y	8 (14.0%)	35 (46.7%)	6.66 (2.59–17.14)	<.001	<.001
TG/TT	N	11 (19.3%)	10 (13.0%)	1.39 (0.50–3.85)	.53	
	Y	6 (10.5%)	9 (12.0%)	2.29 (0.71–7.41)	.17	.66

SNP, single nucleotide polymorphism; CCyR, complete cytogenetic response; OR, odds ratio; CI, confidence interval; SLC22A1: solute carrier 22A1 (GenBank accession number; NC_000006.12); SLC22A1: solute carrier 22A1 (GenBank accession number; NC_000006.12).
[a]*Logistic regression model adjusted for age status.*
[b]*P <.05 was considered statistically significant.*
[c]*N = 241.*
[d]*n = 57.*
[e]*n = 75.*

to IM response. Although no association was found between the c.699G > A SNP and CCyR, a strong association was observed between the c.334T > G SNP and CCyR because the patients with the c.334TT/TG genotype (dominant genetic model) showed statistically significant lack of response (*P* = .007). Nair et al[39] showed the relationship between *SLCO1B3c.334TT* genotype and failure of CCyR, and de Lima et al[16] reported an increased risk of IM resistance in patients with *SLCO1B3c.334TT* and *c.699GG* genotypes.

We were intrigued to discover that, in our patients and the Nair et al study,[39] the frequency of the TT genotype was very low (0.7% and 1.96%, respectively), whereas in the de Lima et al[16] study, was approximately 56%, which is similar to genotypic variation in other populations.[15] Inconsistent with our findings, some study reports showed no association between *SLCO1B3c. 334T > G* with clinical response to IM.[14] Other study results found a relationship between this polymorphism with IM clearance[40] and its intracellular concentration,[9] whereas in a recent study report, no impact of pharmacogenetic items, such as *SLCO1B3c.699G > A, c.334T > G*, was found in IM pharmacokinetics in Chinese patients with CML.[41] In our study findings, haplotype analysis showed no association between haplotypes with response to IM, except the 334G-699A haplotype in *SLCO1B3*, as frequency of 334G-699A haplotype in nonresponder patients was lower than in responders, which suggests a protective role on IM resistance risk.

In accordance with our results, Kim DHD et al[38] found no association between *SLC22A1c.480G > C*, *1222A > G*, and *156T > C* haplotypes and major cytogenetic response (MCyR), CCyR, loss of response (LOR), or treatment failure. Moreover, in our study results, we showed a strong complete linkage disequilibrium (D′ = 1, r^2=0.53, LOD = 25.5) between *SLCO1B3* SNPs, similar to the findings of other studies.[9,15,16] In the present study, we found no statistically significant association between these SNPs and their combinations with CML risk. These findings are in concordance with those of de Lima et al,[16] regarding the association of *SLCO1B3* polymorphisms and CML risk.

We observed considerable differences between the male and female groups. First, the mean age of nonresponder females was significantly higher than that in nonresponder males (48.1 years vs 39.5 years). Secondly, the female group with the *SLC22A1c.1222AG* genotype has significantly increased risk of IM resistance, unlike the group of males with that genotype. Although these results have not been reported so far, these differences between females and males appear to be due to the effect of female hormones, such as progesterone, on drug transporters.

Some study reports, such as Vasconselos et al,[42] indicate that an efflux transporter, P-glycoprotein (Pgp), has low activity in young females and that synthetic progestins inhibit Pgp, in vitro and ex vivo. However, there is no report of such effect on influx transporters.

Our study is the first in the literature to show the joint effect of influx-transporter genes and smoking on CML risk and IM resistance. In general, few studies have been conducted on

this issue. In our study findings, neither the polymorphisms by themselves nor their combinations had any role in CML risk; however, the association of transporter polymorphism with CML risk was dependent on smoking status. This result is compatible with those of Kim HN et al,[20] namely, that the relationship of the GSTT1 polymorphism with AML risk is dependent on smoking status. In 1 study report,[43] it was revealed that there was a higher probability of survival and lower rate of disease progression in nonsmokers than smokers among patients with CML, as well as a similar molecular response rate in the 2 groups.

Another study report[3] has discussed the association of the smoking–metabolizing genes interaction with CML risk. Taken together, previous data[17] have suggested that cigarette smoke can inhibit expression and/or activity of *SLC22A1* and *SLCO1B3*. Such changes may be attributed to cigarette smoke–induced alteration of pharmacokinetics. It seems that cigarette smoke changes the expression and activity of the transporter and reduces the uptake function of the IM into the cells by influx transporters and causes drug resistance.

In conclusion, our findings have revealed the impact of *SLC22A1*, *SLCO1B3* polymorphisms on cytogenetic response to IM, and also the influence of SNP combinations and the joint effect of SNPs and smoking as a synergistic factor affecting treatment response and CML risk. Also, we demonstrated the usefulness of the pharmacogenetic-environmental approach for predicting the clinical outcome of IM therapy, which may help in personalized treatment in patients with CML. LM

Acknowledgments

We are grateful for the help and collaboration of the patients, as part of their participation in the study.

Personal and Professional Conflicts of Interest

None reported.

Funding

This work was supported by the Pasteur Institute of Iran (grant no. 1033).

References

1. Rostami G, Hamid M, Yaran M, Khani M, Karimipoor M. Incidence and clinical importance of BCR-ABL1 mutations in Iranian patients with chronic myeloid leukemia on imatinib. *J Hum Genet*. 2015;60:253–258.
2. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *Am J Hematol*. 2018;93(3):442–459.
3. Rostami G, Assad D, Ghadyani F, et al. Influence of glutathione S-transferases (GSTM1, GSTT1, and GSTP1) genetic polymorphisms and smoking on susceptibility risk of chronic myeloid leukemia and treatment response. *Mol Genet Genomic Med*. 2019;7(7):e00717.
4. Cargnin S, Ravegnini G, Soverini S, Angelini S, Terrazzino S. Impact of SLC22A1 and CYP3A5 genotypes on imatinib response in chronic myeloid leukemia: a systematic review and meta-analysis. *Pharmacol Res*. 2018;131:244–254.
5. Jaruskova M, Curik N, Hercog R, et al. Genotypes of SLC22A4 and SLC22A5 regulatory loci are predictive of the response of chronic myeloid leukemia patients to imatinib treatment. *J Exp Clin Cancer Res*. 2017;36:55.
6. He L, Vasiliou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. *Hum Genomics*. 2009;3:195.
7. Picard S, Titier K, Etienne G, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. *Blood*. 2006;109(8):3496–3499.
8. Widmer N, Decosterd LA, Csajka C, et al. Population pharmacokinetics of imatinib and the role of α1-acid glycoprotein. *Br J Clin Pharmacol*. 2006;62(1):97–112.
9. Nambu T, Hamada A, Nakashima R, et al. Association of *SLCO1B3* polymorphism with intracellular accumulation of imatinib in leukocytes in patients with chronic myeloid leukemia. *Biol Pharm Bull*. 2011;34(1):114–119.
10. White DL, Saunders VA, Dang P, et al. Most CML patients who have a suboptimal response to imatinib have low OCT-1 activity: higher doses of imatinib may overcome the negative impact of low OCT-1 activity. *Blood*. 2007;110(12):4064–4072.
11. Zhong J-S, Meng F-Y, Xu D, Zhou H-S, Dai M. Correlation between imatinib trough concentration and efficacy in Chinese chronic myelocytic leukemia patients. *Acta Haematol*. 2012;127(4):221–227.
12. Di Paolo A, Polillo M, Capecchi M, et al. The c. 480C>G polymorphism of hOCT1 influences imatinib clearance in patients affected by chronic myeloid leukemia. *Pharmacogenomics J*. 2014;14:328–335.
13. Koren-Michowitz M, Buzaglo Z, Ribakovsky E, et al. OCT 1 genetic variants are associated with long term outcomes in imatinib treated chronic myeloid leukemia patients. *Eur J Haematol*. 2014;92(4):283–288.
14. Bedewy AML, El-Maghraby SM. Do SLCO1B3 (T334G) and CYP3A5*3 polymorphisms affect response in Egyptian chronic myeloid leukemia patients receiving imatinib therapy? *Hematol*. 2013;18(4):211–216.
15. Smith NF, Marsh S, Scott-Horton TJ, et al. Variants in the *SLCO1B3* gene: interethnic distribution and association with paclitaxel pharmacokinetics. *Clin Pharmacol Ther*. 2007;81(1):76–82.
16. de Lima LT, Bueno CT, Vivona D, et al. Relationship between SLCO1B3 and ABCA3 polymorphisms and imatinib response in chronic myeloid leukemia patients. *Hematol*. 2015;20(3):137–142.
17. Sayyed K, Le Vee M, Abdel-Razzak Z, et al. Alteration of human hepatic drug transporter activity and expression by cigarette smoke condensate. *Toxicology*. 2016;363:58–71.
18. Marsh S, McLeod H. Cancer pharmacogenetics. *Br J Cancer*. 2004;90(1):8–11.
19. Björk J, Albin M, Mauritzson N, Strömberg U, Johansson B, Hagmar L. Smoking and acute myeloid leukemia: associations with morphology and karyotypic patterns and evaluation of dose–response relations. *Leuk Res*. 2001;25(10):865–872.
20. Kim HN, Kim NY, Yu L, et al. Association of *GSTT1* polymorphism with acute myeloid leukemia risk is dependent on smoking status. *Leuk Lymphoma*. 2012;53(4):681–687.
21. Haznedaroğlu İC, Kuzu I, İlhan O. WHO 2016 definition of chronic myeloid leukemia and tyrosine kinase inhibitors. *Turk J Haematol*. 2020;37(1):42–47.

22. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. *J Clin Oncol*. 2009;27(35):6041–6051.
23. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122(6):872–884.
24. Schoch C, Schnittger S, Bursch S, et al. Comparison of chromosome banding analysis, interphase-and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. *Leukemia*. 2002;16:53–59.
25. Sumner AT, Evans HJ, Buckland RA. New technique for distinguishing between human chromosomes. *Nature New Biology*. 1971;232:31–32.
26. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
27. Singh M, Gupta AK, Singh JK, et al. Effect of age and sex under imatinib mesylate therapy on chronic myeloid leukaemia patients: a pilot study from India. *Int J Pharm Sci Res*. 2017;8(4):1727–1733.
28. Belohlavkova P, Steinerova K, Karas M, et al. First-line imatinib in elderly patients with chronic myeloid leukaemia from the CAMELIA registry: age and dose still matter. *Leuk Res*. 2019;81:67–74.
29. Watkins DB, Hughes TP, White DL. OCT1 and imatinib transport in CML: is it clinically relevant? *Leukemia.* 2015;29:1960–1969.
30. Makhtar SM, Husin A, Baba AA. Genetic variations in influx transporter gene *SLC22A1* are associated with clinical responses to imatinib mesylate among Malaysian chronic myeloid leukaemia patients. *J Genet*. 2018;97(4):835–842.
31. Vaidya S, Ghosh K, Shanmukhaiah C, Vundinti BR. Genetic variations of hOCT1 gene and CYP3A4/A5 genes and their association with imatinib response in Chronic Myeloid Leukemia. *Eur J Pharmacol*. 2015;765:124–130.
32. Takahashi N, Miura M, Scott SA, et al. Influence of CYP3A5 and drug transporter polymorphisms on imatinib trough concentration and clinical response among patients with chronic phase chronic myeloid leukemia. *J Hum Genet*. 2010;55(11):731–737.
33. Maffioli M, Camós M, Gaya A, et al. Correlation between genetic polymorphisms of the *h*OCT1 and *MDR1* genes and the response to imatinib in patients newly diagnosed with chronic-phase chronic myeloid leukemia. *Leuk Res*. 2011;35(8):1014–1019.
34. White DL, Saunders VA, Dang P, Engler J, Hughes TP. OCT-1 activity measurement provides a superior imatinib response predictor than screening for single-nucleotide polymorphisms of OCT-1. *Leukemia*. 2010;24:1962–1965.
35. Umamaheswaran G, Praveen RG, Arunkumar AS, Das AK, Shewade DG, Adithan C. Genetic analysis of *OCT1* gene polymorphisms in an Indian population. *Indian J Hum Genet*. 2011;17(3):164–168.
36. Gromicho M, Magalhães M, Torres F, et al. Instability of mRNA expression signatures of drug transporters in chronic myeloid leukemia patients resistant to imatinib. *Oncol Rep*. 2013;29(2):741–750.
37. Belohlavkova P, Vrbacky F, Voglova J, et al. The significance of enzyme and transporter polymorphisms for imatinib plasma levels and achieving an optimal response in chronic myeloid leukemia patients. *Arch Med Sci*. 2018;14(6):1416–1423.
38. Kim DHD, Sriharsha L, Xu W, et al. Clinical relevance of a pharmacogenetic approach using multiple candidate genes to predict response and resistance to imatinib therapy in chronic myeloid leukemia. *Clin Cancer Res*. 2009;15(14):4750–4758.
39. Nair D, Dhangar S, Shanmukhaiah C, Vundinti BR. Association of genetic polymorphisms of the ABCG2, ABCB1, SLCO1B3 genes and the response to imatinib in chronic myeloid leukemia patients with chronic phase. *Meta Gene*. 2017;11:14–19.
40. Yamakawa Y, Hamada A, Nakashima R, et al. Association of genetic polymorphisms in the influx transporter SLCO1B3 and the efflux transporter ABCB1 with imatinib pharmacokinetics in patients with chronic myeloid leukemia. *Ther Drug Monit*. 2011;33(2):244–250.
41. Wang Q, Jiang Z-P, Yu E-Q, et al. Population pharmacokinetic and pharmacogenetics of imatinib in Chinese patients with chronic myeloid leukemia. *Pharmacogenomics*. 2019;20(4):251–260.
42. Vasconcelos FC, Bonecker ST, de Souza PS, et al. Age, gender and efflux transporter activity influence imatinib efficacy in chronic myeloid leukemia patients. *Leuk Res*. 2019;82:33–35.
43. Lauseker M, Hasford J, Saussele S, et al. Smokers with chronic myeloid leukemia are at a higher risk of disease progression and premature death. *Cancer*. 2017;123(13):2467–2471.