Journal of Sciences, Islamic Republic of Iran 32(2): 121 - 130 (2021) http://jsciences.ut.ac.ir
University of Tehran, ISSN 1016-1104

Lack of Association between C1236T, G2677T/A and C3435T Variants of the *ABCB1* Gene and Imatinib Response in Iranian Chronic Myeloid Leukemia Patients

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Received: 17 January 2020 / Revised: 10 January 2021 / Accepted: 3 February 2021

Abstract

Imatinib introduction caused to improve the clinical outcomes of chronic myeloid leukemia (CML) patients. Despite the significant effects of Imatinib, pharmacogenetic variables induced treatment resistant is also observed. Imatinib is known as a P-glycoprotein (P-gp) efflux pump substrate encoded by the *ABCB1* gene. The *ABCB1* C1236T, G2677T/A and C3435T variants are possibly correlated with interindividual variation in pharmacokinetic response to Imatinib therapy. The present study aimed to examine the effect of *ABCB1* gene variants on the therapeutic response of Imatinib in CML patients. Sixty-nine Iranian CML patients treated with Imatinib or Nilotinib were selected and divided into two groups of sensitive and resistant to Imatinib. C1236T and G2677T/A variants were genotyped by high resolution melting (HRM) analysis, and C3435T variant was genotyped using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Then, the results were compared between the two groups of patients. Our results showed that there were no significant differences between C1236T, G2677T/A and C3435T variants of *ABCB1* gene and clinical response to Imatinib in the Iranian CML patients. According to the results of this study, genotyping of *ABCB1* C1236T, G2677T/A and C3435T variants couldn't help to predict the

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outcomes of Imatinib treatment in CML patients. So, these variants are not useful to make decisions about treatment, but it is suggested to do further investigations.

Keywords: *ABCB1*; Chronic myeloid leukemia; Imatinib mesylate; Variants.

Introduction

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder that originates from the transformation of a primitive hematopoietic cell that suffers a t(9;22) resulted to produce BCR-ABL1, a constitutively active tyrosine kinase that drives a wide variety of physiological alterations [1, 2].

The standard treatment of CML is a tyrosine kinase inhibitor (TKI) like Imatinib (Gleevec), Nilotinib (Tasigna), Dasatinib (Sprycel), or Bosutinib (Bosulif). Imatinib mesylate (IM) was introduced as a first-line treatment for chronic myeloid leukemia (CML) almost 10 years ago and radically improved the outcome of CML patients. [3, 4]. It blocks the ATP binding site of the BCR-ABL1 protein and consequently inhibits tyrosine kinase activity [5] lead to complete hematologic response (CHR), complete cytogenetic response (CCyR) and major molecular response (MMR) in many patients [6].

Despite the high efficacy of Imatinib, drug resistance may occur in 20%-30% of the patients through BCR-ABL1 dependent and independent pathways [7].

In BCR-ABL1 independent mechanisms, variants of efflux transporter genes are the key factors contributing in Imatinib pharmacokinetics. Efflux transporters limit the accumulation of anticancer drugs in cancer cells. Overexpression of these transporters has frequently been implicated in the mechanism of resistance to different drugs including Imatinib [8]. P-glycoprotein (P-gp) is one of the most vital efflux transporters encoded by the *ABCB1* gene, also known as multidrug resistance 1 (*MDR1*), on chromosome 7q21.1 consisted from 28 exons and 28 introns. The ABCB1 gene is highly polymorphic and to date, more than 60 single nucleotide polymorphisms (SNPs) have been detected in its coding region. It is reported that some of these variants may cause interpersonal variations in response to Imatinib [3]. Genetic variants in the *ABCB1* gene can alter function of P-gp and bioavailability of Imatinib. Therefore, these variants can be effective determinants involved in Imatinib resistance [8]. In particular, three common variants have been found in CML patients with strong linkage disequilibrium (LD): two silent variants of c.1236 C > T (T= NG_011513.1:g.167964=, C= NG_011513.1:g.167964T > C) and c.3435 C>T (T= NG_011513.1:g.208920=, C= NG_011513.1:g.208920T > C) on the exons of 12 and 26 and one nonsynonymous variant c.2677 G>T/A (T= NG_011513.1:g.186947=, A= NG_011513.1:g.186947T > A, G= NG_011513.1:g.186947T > G) on exon 21. G2677T/A variant causes the substitution of alanine amino acid with serine or threonine amino acids (Ala 893 Ser/Thr) [9], but the silent variants of C1236T and C3435T are effective in splicing donor site inactivation and shorter mRNA transcription. So, they impact on protein translation, protein function and folding and modifying the substrate specificity [10].

To date, the association between *ABCB1* gene variants and Imatinib resistance has been widely studied [11-16]. However, the reports are inconsistent and there is no agreement on the role of *ABCB1* gene variants and clinical response in CML patients. Besides, in the Iranian population, no efficient information has been reported on the pharmacogenetic status of CML to *ABCB1* gene variants. So, in this study, we investigated the association of *ABCB1* C1236T, G2677T/A and C3435T variants with clinical outcomes in CML patients with Imatinib sensitivity and resistance.

Materials and Methods

Sample collection

A total of 69 Philadelphia (Ph) chromosome-positive CML patients (33 males and 36 females) with a mean age of 55 years were selected from the referred patients to Cancer Research Center in Isfahan province of Iran, from August 2018 to December 2019. Patients receiving Imatinib (300-600 mg/day) for at least 12 months were included and in the case of using effective drugs on Imatinib metabolisms such as Phenobarbital and Phenytoin they were excluded. All the patients were asked to fill the written informed consent and two ml of peripheral blood sample was collected from each one kept in EDTA sterile tubes.

Table 1. Primer sequences used for the analysis of C1236T, G2677T/A and C3435T variants

Variant ID	Nucleotide change	Amino acid change	Primer sequence (5' → 3')	Product length (bp)
rs1128503	C1236T	Gly412Gly	F: CCTGTGTCTGTGAATTGCC R: TGCATCAGCTGGACTGTTG	147
rs2032582	G2677T/A	Ala893Ser/Thr	F: GTCTGGACAAGCACTGAAAG R: GCATAGTAAGCAGTAGGGAG	137
rs1045642	C3435T	Ile1145Ile	F: GACAGTTCCTCAAGGCATAC R: AGGAAGTGTGGCCAGATG	546

F: Forward, R: Reverse, bp: base pair

Genotyping

DNA was extracted from the blood samples of each patient using GeNet Bio extraction kit (GeNet Bio, Korea). High resolution melting (HRM) analysis was used to genotyping of C1236T and G2677T/A variants and polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method for C3435T variant. Primers used in this study are shown in Table 1.

HRM method is simple, rapid, effective and more economical compared to other modern methods of genotyping and variant scanning. It is a novel, homogeneous, close-tube, post-PCR method, enabling genomic researchers to analyze genetic variations. HRM is an appropriate alternative to Sanger sequencing, restriction enzyme analysis and hydrolysis probes for variant screening in clinical samples. HRM analysis was performed in a 36-well Rotor on the Corbett Research Rotor Gene-6000 (Qiagen–Germany). The reaction mixture components contained 60ng of genomic DNA, 0.5µl of each primer (10pM), 4µl of 5X HRM master mix (Solis BioDyne, Estonia) and RNase free water to reach the final volume of 20µl. PCR reactions were performed under the following conditions: an initial step at 95°C for 12 minutes to activate HOT FIREPOL DNA polymerase, Continued by 40 cycles at 95°C for 15 seconds, 61°C for 20 seconds and 72°C for 20 seconds. Then, HRM analysis was completed with ramping from 80 to 95°C and 70 to 85°C for C1236T and G2677T/A variants, respectively and rising by 0.2°C with 2 seconds of holding after each step. The results were analyzed by Q 5plex HRM software V.2.3.4.

C3435T variant is near to another variant and interferes with the analysis of the HRM melting curve. So, C3435T variant was genotyped by PCR-RFLP method; in a final volume of 25µl ,containing 75ng of genomic DNA, 0.5µl of each forward and reverse primers at 10pM, 2.5µl of 10X buffer, 0.75µl of MgCl2 at 50mM, 0.5µl of dNTPs at 10mM and 1.25 U of taq DNA polymerase . PCR reactions were performed with these cycling conditions: initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 1 min. The final extension was performed at 72°C for 7 min and in the end PCR products (546 bp) were digested with the MboI restriction enzyme (Fermentas, Lithuania, ER0811) at 37°C for 1h. Digested DNA fragments were electrophoresed on 3% agarose gel, stained with GelRed and visualized under UV light.

Sequencing is the gold standard method for determining the variants, so in this study some heterozygous, mutant and wild type homozygous samples of C1236T and G2677T/A and C3435T variants were amplified with the specific primer pairs and then sequenced on an ABI 3730XL automatic sequencer (Applied Biosystems, USA).

Statistical analysis

Data analysis was performed using SPSS software package version 26 (SPSS Inc., Chicago, IL, USA). The deviation from Hardy-Weinberg equilibrium (HWE) was evaluated by x^2 test for different allele frequencies. Binary logistic regression and chi-square analysis were used to investigate the association between each *ABCB1* variant and clinical response to Imatinib among the sensitive and resistant groups of CML patients. Odds ratio (OR) with 95% of confidence interval (CI) and two-sided *p*-value were calculated for all variables. The *p*-value of $\leq$ 0.05 was considered statistically significant.

Ethical statement

The study was approved by the ethics committee of Isfahan University of Medical Sciences, Isfahan, Iran (ethical code: IR.MUI.MED.REC.1398.223). Written informed consents were acquired from all patients.

Results

Genotyping

Genotypes of C1236T and G2677T/A variants was detected by HRM and PCR-RFLP method was used for C3435T variant. The results of HRM and RFLP methods for wild type and mutant homozygous as well

as heterozygous patients have shown in Figures 1 and 2, respectively. In HRM analysis, three genotypes of C1236T variant (CC, CT and TT) and four genotypes of G2677T/A variant (GG, TG, TT and AT) were found (Fig. 1). There were not any sample with 2677GA and 2677AA genotypes in our samples. Using PCR-RFLP, the wild type and mutant homozygous and heterozygous genotypes were separated by different lengths of DNA fragments on agarose gel (Fig. 2). The presence of the C allele induced the three fragments of 164 bp, 172 bp, 210 bp; whereas in the case of T allele, two fragments with 164 bp and 382 bp sizes were generated. The sequencing of random samples with different genotypes confirmed the results of HRM and RFLP experiments (Fig. 3).

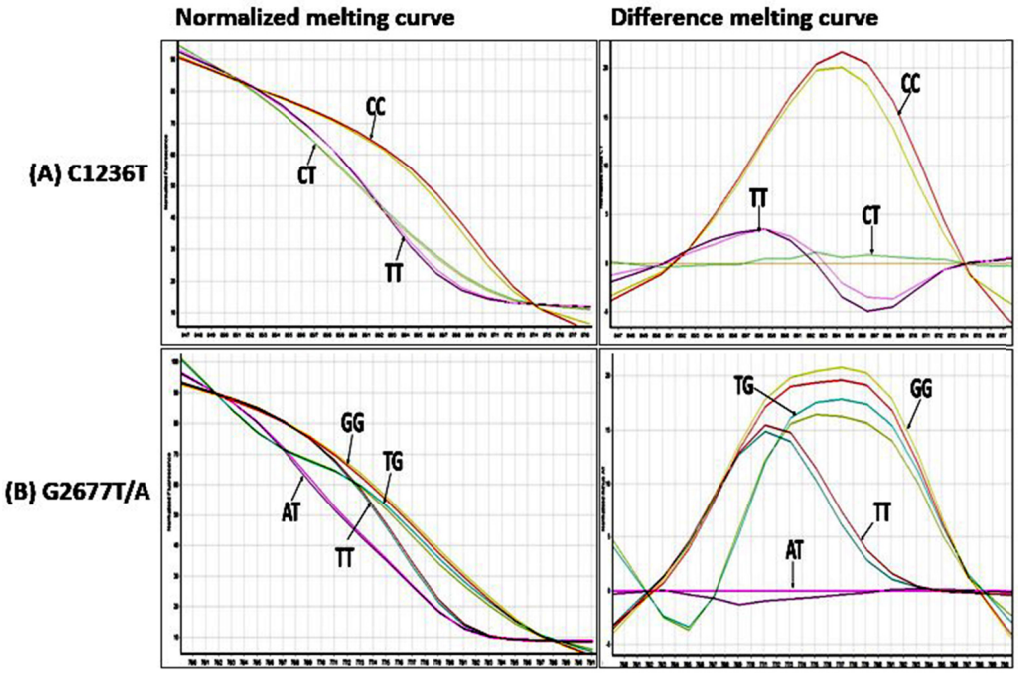

Figure 1. Normalized and difference high resolution melting curves for C1236T and G2677T/A variants of ABCB1 gene
(A) Normalized melting curve (left) and difference high resolution melting curve (right) for C1236T variant
(B) Normalized melting curve (left) and difference high resolution melting curve (right) for G2677T/A variant

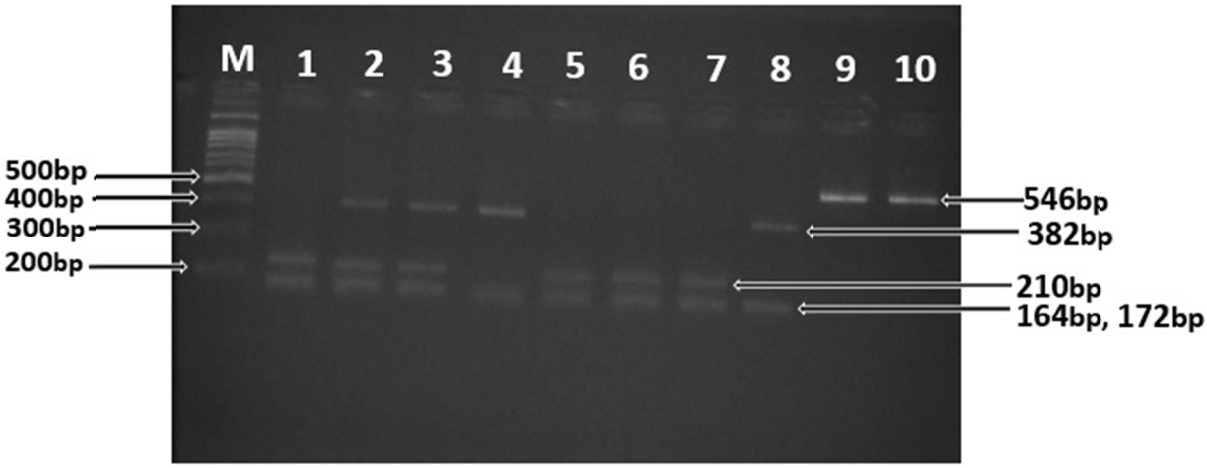

Figure 2. Detection of the C3435T variant by PCR-RFLP assay
Lane M: 100 bp DNA ladder, Lane 1, 5, 6, 7: CC homozygous (164, 172, 210 bp), Lane 2, 3: CT heterozygous (164, 172, 210, 382 bp), Lane 4, 8: TT homozygous (164, 382 bp), Lane 9, 10: undigested DNA (546 bp)

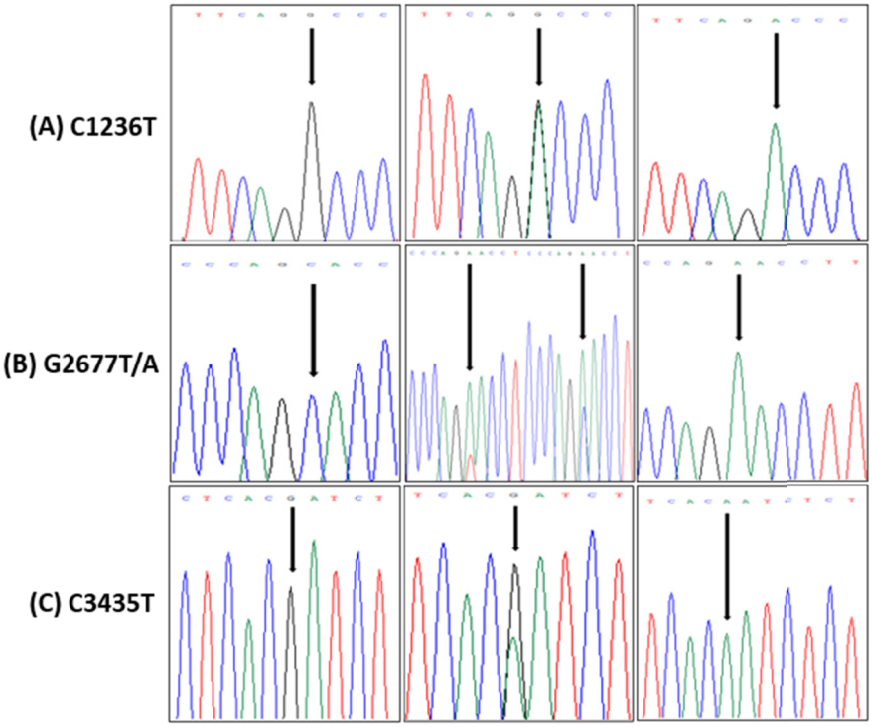

Figure 3. Sanger sequencing results. (A) C1236T variant (B) G2677T/A variant (C) C3435T variant

***ABCB1* genotype and haplotype frequencies**

ABCB1 C1236T, G2677T/A and C3435T variants were successfully genotyped in 69 CML patients. Genotype frequencies of the samples are shown in Figure 4. There was no deviation of Hardy-Weinberg equilibrium for allele and genotype frequencies distribution. Different haplotypes of TTT, CTC, CGC, TAT, CGT, CTT, TTC, TGC, TGT and TAC were identified in the CML patients with frequencies of 34.8%, 17.4%, 20.3%, 13%, 5.1%, 4.3%, 2.9%, 0.7%, 0.7% and 0.7%, respectively.

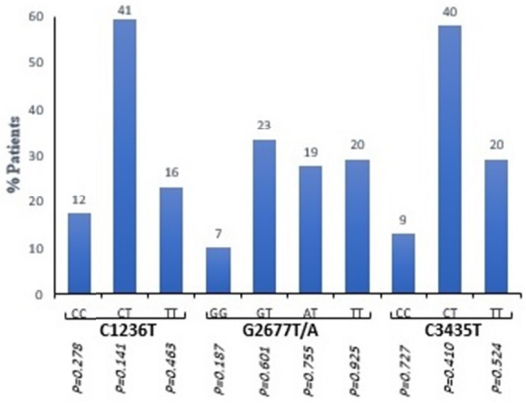

Figure 4. Genotype frequencies of ABCB1 C1236T, G2677T/A and C3435T variants in CML patients
* The numbers above the charts are related to the patients.

Association between C1236T, G2677T/A and C3435T genetic variants of ABCB1 gene and hematological and molecular responses to Imatinib in CML patients

In this study, the association between different alleles, genotypes, diplotypes and haplotypes of C1236T, G2677T/A and C3435T variants of *ABCB1* gene and hematological and molecular responses of CML patients to Imatinib were investigated. Of all patients, 42 cases had Imatinib sensitivity and 27 ones were resistant. Being resistant was determined by complete hematological responses as $< 450 \times 10^9/$ L platelet , $< 10 \times 10^9/$L white blood cell (WBC) and < 5% basophil count and major molecular response was defined as BCR-ABL1 ratios ≤ 0.1% [9]. Several patients were screened for BCR-ABL1 kinase domain mutations and the results were negative.

The distribution of allele and genotype frequencies of *ABCB1* variants in the sensitive and resistant patients is indicated in Table 2. In the sensitive patients, genotypes of 1236CC, 2677GG and 3435CT were slightly more frequent (21.4% vs. 11.1% for 1236CC, 14.3% vs. 3.7%

Table 2. Distribution of allele and genotype frequencies of ABCB1 variants in two groups of CML patients

Variant	Genotype	Patients n=69 (100%)	Sensitive to Imatinib n=42 (60.9%)	Resistant to Imatinib n=27 (39.1%)	x^2	p value*	OR (95% CI)
C1236T	CC	12 (17.4)	9 (21.4)	3 (11.1)	1.218	0.278	0.458 (0.112-1.874)
	CT	41 (59.4)	22 (52.4)	19 (70.4)	2.206	0.141	2.159 (0.775-6.013)
	TT	16 (23.2)	11 (26.2)	5 (18.5)	0.543	0.463	0.640 (0.195-2.105)
	C	65 (47.1)	40 (47.6)	25 (46.3)		0.380	0.625 (0.219-1.787)
	T	73 (52.9)	44 (52.4)	29 (53.7)		0.538	0.659 (0.175-2.480)
G2677T/A	GG	7 (10.1)	6 (14.3)	1 (3.7)	2.019	0.187	0.231 (0.026-2.034)
	TG	23 (33.3)	13 (30.9)	10 (37.1)	0.274	0.601	1.312 (0.474-3.635)
	AT	19 (27.5)	11 (26.2)	8 (29.6)	0.097	0.755	1.187 (0.405-3.477)
	TT	20 (29)	12 (28.6)	8 (29.6)	0.009	0.925	1.053 (0.364-3.048)
	G	37 (26.8)	25 (29.8)	12 (22.2)		0.158	0.524 (0.213-1.287)
	T	82 (59.4)	48 (57.1)	34 (63)		0.780	1.417 (0.123-16.259)
	A	19 (13.8)	11 (13.1)	8 (14.8)		0.908	1.063 (0.376-3.002)
C3435T	CC	9 (13)	5 (11.9)	4 (14.8)	0.123	0.727	1.287 (0.313-5.293)
	CT	40 (58)	26 (61.9)	14 (51.9)	0.682	0.410	0.663 (0.249-1.763)
	TT	20 (29)	11 (26.2)	9 (33.3)	0.407	0.524	1.409 (0.491-4.048)
	C	58 (42)	36 (42.9)	22 (40.7)		0.393	0.662 (0.257-1.707)
	T	80 (58)	48 (57.1)	32 (59.3)		0.378	0.333 (0.029-3.831)

* $p \leq 0.05$ statistically significant. It was calculated by Binary logistic regression analysis.
OR odds ratio, CI confidence interval

for 2677GG and 61.9% vs. 51.9% for 3435CT); while the frequency of 1236CT genotype was slightly higher in resistant patients (70.4% vs. 52.4%). However, these differences were not statistically significant. Table 3 shows the diplotypes distribution of *ABCB1* variants in sensitive and resistant patients indicating no correlation between *ABCB1* diplotypes and increasing risk of Imatinib resistance. Although 1236CC/3435CT and 2677GG/3435CT diplotypes were found only in the sensitive patients but it was not statistically significant. More investigation of *ABCB1* haplotypes influence on therapeutic response to Imatinib revealed that the most frequent haplotype among the patients was TTT and there were no significant differences in the frequency of the *ABCB1* haplotypes between Imatinib sensitive and resistant patients (Table 4).

Discussion

Imatinib dramatically improves clinical and

Table 3. Diplotype distribution of ABCB1 variants in CML patients treated with Imatinib

Diplotypes	Patients n=69 (100%)	Sensitive to Imatinib n=42 (60.9%)	Resistant to Imatinib n=27 (39.1%)	p value	OR (95% CI)
1236CT/2677AT	18 (8.7)	10 (7.9)	8 (9.9)	0.592	1.347 (0.453-4.005)
1236CT/2677TG	21 (10.1)	11 (8.7)	10 (12.3)	0.341	1.658 (0.585-4.694)
1236TT/2677TT	14 (6.8)	9 (7.1)	5 (6.2)	0.769	0.833 (0.246-2.820)
1236CC/2677GG	6 (2.9)	5 (4)	1 (1.2)	0.380	0.365 (0.039-3.458)
others	10 (4.8)	7 (5.6)	3 (3.7)		
1236CC/3435CC	7 (3.4)	4 (3.2)	3 (3.7)	0.831	1.187 (0.244-5.775)
1236CC/3435CT	5 (2.4)	5 (4)	0 (0)	0.999	—
1236CT/3435CT	31 (15)	18 (14.3)	13 (16)	0.666	1.238 (0.469-3.270)
1236CT/3435TT	8 (3.9)	3 (2.4)	5 (6.2)	0.163	2.955 (0.644-13.560)
1236TT/3435TT	12 (5.8)	8 (6.3)	4 (4.9)	0.652	0.739 (0.199-2.744)
others	6 (2.9)	4 (3.2)	2 (2.5)		
2677TT/3435TT	14 (6.8)	9 (7.1)	5 (6.2)	0.769	0.833 (0.246-2.820)
2677AT/3435CT	16 (7.7)	10 (7.9)	6 (7.4)	0.879	0.914 (0.289-2.894)
2677TG/3435CT	17 (8.2)	10 (7.9)	7 (8.6)	0.842	1.120 (0.367-3.418)
2677GG/3435CT	4 (1.9)	4 (3.2)	0 (0)	0.999	—
others	18 (8.7)	9 (7.1)	9 (11.1)		

* $p \leq 0.05$ statistically significant. It was calculated by Binary logistic regression analysis.
OR odds ratio, CI confidence interval

Table 4. Distribution of haplotype frequency of ABCB1 variants in Imatinib treated CML patients

Haplotype	Patients n=69 (100%)	Sensitive to Imatinib n=42 (60.9%)	Resistant to Imatinib n=27 (39.1%)	p value	OR (95% CI)
TTT	34.8	34.5	35.2	0.907	1.065 (0.371-3.054)
CTC	17.4	16.7	18.5	0.753	1.176 (0.428-3.233)
CGC	20.3	21.4	18.5	0.631	0.784 (0.291-2.114)
TAT	13	11.9	14.8	0.592	1.347 (0.453-4.005)
CGT	5.1	5.9	3.7	0.549	0.592 (0.106-3.295)
CTT	4.3	3.6	5.6	0.571	1.625 (0.303-8.712)
TTC	2.9	2.4	3.7	0.649	1.600 (0.212-12.093)
TGC	0.7	1.2	0	1.000	—
TGT	0.7	1.2	0	1.000	—
TAC	0.7	1.2	0	1.000	—

* $p \leq 0.05$ statistically significant. It was calculated by Binary logistic regression analysis.
OR odds ratio, CI confidence interval

prognostic outcomes in CML patients [17]. Despite the high efficacy, resistance to this drug is observed in approximately 25% of the patients [2]. Introduction of second-generation tyrosine kinases such as Nilotinib and Dasatinib which are more potent and selective in BCR-ABL1 inhibition has been developed the therapeutic response in resistant CML patients [18]. Imatinib resistance can be partially explained by mutations in the BCR-ABL1 kinase domain, efflux and influx transporters and metabolizing enzymes [8]. Therefore, assessments of these biomarkers help to predict imatinib responses. One of the most important factors that play a significant role in Imatinib resistance is *ABCB1* gene as an efflux transporter [19] that is highly polymorphic which is significantly affectted by C1236T, G2677T/A and C3435T variants on its expression and function and substrate distribution [9].

Numerous pharmacogenetic studies have done to investigate the association between *ABCB1* variants and Imatinib resistance. However, the results are inconsistent; some studies demonstrated a relationship between variants of *ABCB1* and responses to Imatinib [20-23], whereas the others failed to identify any association [14, 15, 24, 25].

In the present study, Imatinib sensitive and resistant CML patients were selected and after determining the frequency of alleles, genotypes, diplotypes and haplotypes of C1236T, G2677T/A and C3435T variants of *ABCB1*, their correlation with clinical outcomes was evaluated.

There is no efficient information on the pharmacogenetic status of Iranian CML patients regarding *ABCB1* variants. In the recent study conducted in Iran reported that the 1236CC genotype was associated with the cytogenetic responses to Imatinib [26]; while our study failed to identify an association between variants of *ABCB1* and molecular and hematological responses to Imatinib. In addition, different frequencies of *ABCB1* variants have been observed within within various ethnic groups [9, 12]. Our results showed that the most frequent genotypes were 1236CT, 2677GT and 3435CT and T mutant allele had the higher frequency in each variants. A study on randomly selected healthy people by Saidijam *et al* [27] in Hamadan, Iran, indicated the C1236, G2677 and 3435T alleles are the most frequent alleles in that population. Moreover, they showed that the frequency of 2677GA genotype was higher than the other genotypes at G2677T/A loci, while 2677GA genotype was not identified in our study. Kassogue *et al* [28] described that the most frequent genotypes were 1236CC, 2677GG and 3435CC in Moroccan CML patients. Furthermore, a study conducted in China reported that the 1236TT, 2677GT and 3435CT genotypes were the most frequent in CML patients [12].

Haplotype analysis rather than separate analysis of variants can predict the therapeutic response to Imatinib and may explain the contradictory results of the previous studies [20, 23, 29]. In the present study, no significant association was found between the *ABCB1* C1236T, G2677T/A and C3435T genetic variants and treatment response to Imatinib in CML patients ($P > 0.05$). In consistent with our findings, some studies in Japan, Czech Republic, Indonesia, Morocco and United Kingdom [14, 24, 25, 28, 30] reported no association between *ABCB1* variants and clinical response to Imatinib in CML patients although several studies found some associations. In 2014, Ali *et al* [23] reported that the optimal response to Imatinib was significantly associated with CGC, TTT, TGC, CGT, TGT, CTC, CTT and TTC haplotypes and in patients with TGT haplotype it was associated with lower efficacy. Au *et al* [9] reported a significant association between C1236T and G2677T/A variants with optimal response to

Imatinib. They also showed that patients with CGC haplotype were more resistant to Imatinib than the other patients. Likewise, in 2008, Dulucq *et al* [11] indicated that the TT genotype at C1236T loci and TT/TA genotypes at G2677T/A loci correlated with the higher MMR in patients. They found that patients with CGC haplotype had lower MMR and higher MMR was due to TTC and TTT haplotypes. In the subsequent study in 2010, Dulucq *et al* [31] could not approve the results of the previous study in 2008. Furthermore, Ni *et al* [12] concluded that 3435TT and 3435CT genotypes were associated with a higher risk of Imatinib resistance and it was observed in patients with 1236T allele, particularly in homozygous types. Also, a better complete cytogenetic remission was observed in patients with 2677AG/AT/AA genotypes. Another study done by Maffioli *et al* [13] showed that the less resistance rate was observed in patients with 2677T allele. They found an association between 3435CC genotype and TGC haplotype and failure to achieve optimal treatment response to Imatinib. The results of the Vivona *et al* [32] revealed the higher frequency of 1236CT/2677GT/3435CT haplotype in patients with MMR. It was seen a significant correlation between 1236CT/3435CT/2677GT and 1236TT/3435TT/2677TT haplotypes and reduction of P-gp activity and MMR in Imatinib treated CML patients of chronic phase (CP) [20]. A meta-analysis failed to identify an association between G2677T or C3435T loci and Imatinib efficacy in Asian and Caucasian CML patients. They found that the C1236T variant was statistically correlated with increased risk of Imatinib resistance in Asian CML patients but not Caucasian CML patients [33]. In addition, Elghannam *et al* [34] demonstrated that a better molecular response, CHR and CCyR was observed in CP-CML patients with 2677TT genotype. Glady *et al* [21] reported that the homozygous T allele at G2677T/A loci was observed in the patients with poor treatment responses to Imatinib. Moreover, Angelini *et al* [18] suggested that the 3435CT/TT genotypes were associated with less frequency of complete molecular response. A subsequent meta-analysis indicated that the 1236CC genotype, 2677T/A allele or 3435C allele were protective factors against resistance to Imatinib [17]. Furthermore, Deenik *et al* [22] showed that molecular resistance was significantly correlated with TT-genotype of each *ABCB1* variants. The results of Salimizand *et al* [35] study indicated that 3435T allele was significantly higher in patients than the healthy groups. They explained that poor cytogenetic response was observed in patients with 3435C allele. They also found that patients with *ABCG2* 421CC-*ABCB1* 3435TT diplotype had poor Imatinib response.

Many factors such as small sample size, patient's ethnicity, heterogeneity of patients, treatment protocol, before treatments approaches, disease phase, Imatinib dosage and response criteria lead to inconsistent results. Furthermore, the effect of other genes involved in Imatinib metabolisms, *BCR-ABL1* gene mutations and differences in the frequency of alleles in different populations can also explain the contradictions.

Conclusion

Our results indicated that there is no association between optimal response to Imatinib and C1236T, G2677T/A and C3435T variants of *ABCB1*. Therefore, genotyping of *ABCB1* variants is not useful to predict the therapeutic response and make the best treatment decision. Further pharmacogenetic studies with the larger samples of homogenous patients in relation to genetic variants of other pharmacokinetic markers involved in Imatinib metabolisms such as *ABCG2*, *SLC22A1*, *CYP3A4* and *CYP3A5* are needed to determine the variable responses to Imatinib in CML patients.

Acknowledgement

The authors thank all the patients who participated in this study. This work was financially supported by Isfahan University of Medical Sciences, Isfahan, Iran.

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