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ORIGINAL PAPER

Multidrug resistance gene (MDR1) polymorphisms correlate with imatinib response in chronic myeloid leukemia

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Abstract The human multidrug resistance gene (*MDR*1, *ABCB*1) codes for P-glycoprotein (P-gp) that affects the pharmacokinetics of many drugs. MDR1 single nucleotide polymorphisms (SNPs) are associated with drug clearance. Imatinib is a substrate of P-gp-mediated efflux. We investigated the MDR1 T1236C, G 2677T/A, and C3435T polymorphism in 52 patients with chronic myeloid leukemia treated with imatinib. The distribution of MDR1 1236, 2677, or 3435 genotypes was significantly different between the resistance patients and sensitivity patients. The resistance incidence correlated with the number of T alleles at locus 1236 and 3435. Resistance was higher for patients homozygous for the 1236T allele when compared to patients with CT/CC genotype groups (75% vs. 31.3%, $P = 0.004$). For the G2677T/A polymorphism, a better complete cytogenetic remission was observed for patients with genotype AG/AT/AA, when compared to other genotype groups (TT/GT/GG, $P = 0.02$). Patients with 3435 TT/CT genotypes showed a higher resistance when compared with patients with CC genotype (59.4% vs. 25%, $P = 0.023$). In conclusion, determination of 1236T, C3435T, and G2677T MDR1 polymorphisms might be useful in response prediction to therapy with imatinib in patients with CML.

Keywords Chronic myeloid leukemia · P-glycoprotein; MDR1 · Polymorphism · Imatinib

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Introduction

Imatinib-mysylate (imatinib) was a small molecular inhibitor of tyrosine kinase activity of the Bcr-Abl fusion protein, which is now frontline therapy for chronic myeloid leukemia (CML). The treatment of patients with CML with imatinib has resulted in complete cytogenetic response (CCR) rates of 65–85% [1]. Despite of its striking efficacy, however, resistance develops over time in many patients. The multidrug resistance gene (*MDR*1, *ABCB*1) product is an ATP-driven efflux pump contributing to the pharmacokinetics of drugs that are P-glycoprotein (P-gp) substrates and to the multidrug resistance of cancer cells. Imatinib is a substrate of P-gp-mediated efflux [2]. MDR1 gene is polymorphic, and more than 50 single nucleotide polymorphisms (SNP) have been identified so far. SNP polymorphism affects the expression and function of the P-gp in many ways [3, 4]. The SNP C3435T was found to be associated with altered P-gp activity, and when it appears in a haplotype, with reduced functionality.

The SNPs T1236C, G 2677T/A, and C3435T are the most common variants in the coding region of *ABCB*1 [3]. Identifying influential SNPs may allow to predict the drug disposition in individual patients and respond optimally to imatinib in patients with CML [2, 5]. Moreover, linkage disequilibrium of an indicator SNP used in association studies with the functional polymorphism may vary among ethnic populations [6]. Regulatory polymorphisms can have different effects in different tissues. Therefore, it is important that we analyze the T1236C, G2677T/A, and C3435T most relevant SNPs to identify genetic variants underlying susceptibility to imatinib efficacy in Chinese.

The aim of this study was to examine the association between MDR1 1236T, C3435T, and G2677T polymorphisms

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in patients with CML as well as the association between MDR1 polymorphism and response to imatinib.

Patients and methods

Patients

A total of 52 patients, aged 18 years or older, were eligible if they had Ph-positive chronic-phase CML. Those patients consisted of 33 men and 19 women with a median age of 44 years (ranging from 18 to 76 years).

Chronic-phase (CP) CML was defined by the presence of less than 15% blasts, less than 20% basophils, and less than 30% blasts plus promyelocytes in peripheral blood (PB) or bone marrow (BM) and a platelet count of at least 100,000 per cubic millimeter, with no extramedullary involvement [1]. Cytogenetic failure was defined as either cytogenetic resistance (at least 5% of metaphase cells being Ph^+ after at least 1 year on imatinib) or relapse after achievement of major cytogenetic response (MCyR).

In addition, the frequency of bone marrow assessments was reduced every 6–12 month, with a further reduction in the frequency of assessments in patients in complete cytogenetic response to every 12 months.

Genotyping

DNA was extracted from PB or BM samples by standard phenol/chloroform extraction method. Polymerase chain reaction (PCR)-restriction fragment length polymorphism was used for the detection of C1236T, G2677T/A, and C3435T SNP. The primer design was based on published sequences for genotyping procedure of MDR1 polymorphism using genomic DNA. The primers are shown in Table 1. The accuracy of genotyping was confirmed by conventional polymerase chain reaction/restriction fragment length polymorphism assay as described previously [2].

Statistical analysis

The difference in allele or genotype frequencies in all patients taking imatinib treatment was determined by using the chi-square test. All tests were two-sided, and differences were considered not significant when P values were >0.05. All analyses were performed with the SPSS for Windows (version 13.0).

Results

All patients were considered for our study and were treated with 400 mg imatinib per day for at least 12 months. Of 52 patients with CML, 27(51.9%) were sensitivity to imatinib, and 25(48.1%) were resistance to imatinib.

The overall frequencies of the MDR-1 1236CC, CT, and TT genotypes were 25, 36.5, and 38.5%, respectively, and 2677 GG, GT, TT, and others (GA, AA) genotypes were 25, 40.3, 11.5, 23.11%, respectively. The overall frequency of the MDR-1 3435 CC, CT, and TT genotypes was 38.5, 50, and 11.5%, respectively. There was a significant difference between the two groups that were resistance or sensitivity to imatinib (Table 2). We observed a significant difference in genotype frequencies at loci between patients with resistance and sensitivity to imatinib when the analysis of individual polymorphisms was performed ($P < 0.05$).

The distribution of MDR1 1236, 2677, or 3435 genotypes was significantly different between the resistance group and sensitivity group when the analysis of individual polymorphisms was performed. The resistance incidence correlated with the number of T alleles at locus 1236 and 3435. Resistance was higher for patients homozygous for the 1236T allele when compared to patients with CT/CC genotype patients (75% vs. 31.3%, $P = 0.004$, Fig. 1a). For the G2677T/A polymorphism, a better complete cytogenetic remission (CCR) was observed for patients with genotype AG/AT/AA, when compared to other genotype groups (TT/GT/GG, $P = 0.02$, Fig. 1b), and genotype GT compared to

Table 1 Primers and length of products amplified by PCR-RFLP

Primer	Sequence	Product length
1236C reverse	5′- CTGCACCTTCAGGTTCTGG -3′	236 bp
1236T reverse	5′- CTGCACCTTCAGGTTCGGA -3′	
1236 forward	5′- GTCACTTCAGTTACCCATCTCG -3′	
2677G forward	5′- TGAAAGATAAGAAAGAACTAGAATGTG -3′	222 bp
2677T forward	5′- TGAAAGATAAGAAAGAACTAGAATGTT -3′	
2677A forward	5′- TGAAAGATAAGAAAGAACTAGAATGTA -3′	
2677 reverse	5′- AGTCCAAGAACTGGCTTTGC -3′	
3435C reverse	5′- CTTTGCTGCCCTCACG -3′	197 bp
3435T reverse	5′- CTTTGCTGCCCTCACA -3′	
3435 forward	5′- TGTTTTCAGCTGCTTGATGG -3′	

Table 2 Patients' characteristics according to MDR1 polymorphisms

	Men	Women	*P*	Resistant to imatinib	Sensitivity to imatinib	*P*
C1236T			0.368			0.007
CC	10 (30.3)	3 (15.8)		3 (12)	10 (37)	
CT	10 (30.3)	9 (47.4)		7 (28)	12 (44.4)	
TT	13 (39.4)	7 (36.8)		15 (60)	5 (18.5)	
G2677T/A			0.416			0.03
GG	8 (24.2)	5 (26.3)		5 (20)	8 (29.6)	
GT	11 (33.3)	10 (52.6)		14 (56)	7 (25.9)	
TT	5 (15.2)	1 (5.3)		4 (16)	2 (7.4)	
AG/AT/AA	9 (27.3)	3 (15.8)		2 (8)	10 (37)	
C3435T			0.297			0.023
CC	14 (42.4)	6 (31.6)		5 (20.8)	15 (53.6)	
CT	14 (42.4)	12 (63.2)		14 (58.3)	12 (42.9)	
TT	5 (15.2)	1 (5.3)		5 (20.8)	1 (3.6)	

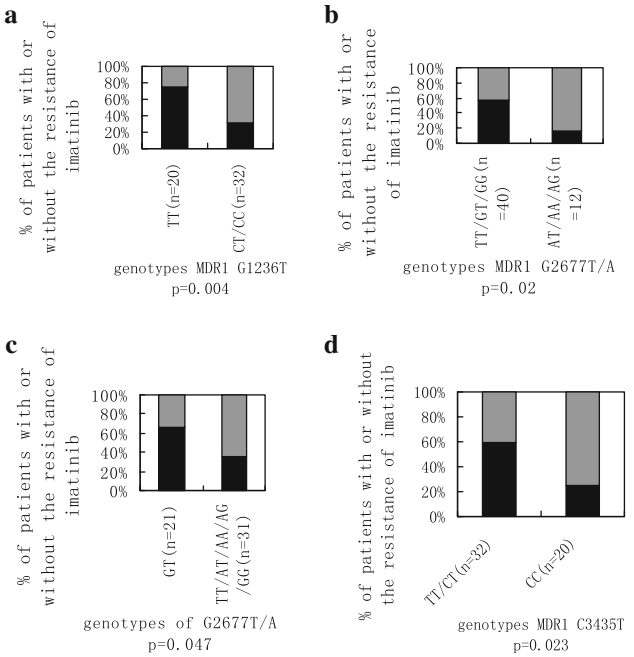

Fig. 1 Genotypes of MDR1 (*Black* indicates the resistance to imatinib, and *gray* indicates sensitivity to imatinib)

others (TT/AT/AA/AG/GG $P = 0.047$, Fig 1c). Patients with 3435 TT/CT genotypes showed a higher resistance when compared with patients with CC genotype (59.4% vs. 25%, $P = 0.023$, Fig. 1d). There were no significant differences according to gender (Table 3). Genotype frequencies were in accordance with Hardy–Weinberg equation.

Table 3 MDR1 polymorphisms in patients with resistance or sensitivity to imatinib

	Resistant to imatinib	Sensitivity to imatinib	*P*
C1236T			
CC	3 (23.1)	10 (76.9)	0.055
CT/TT	22 (56.4)	17 (43.6)	
CT	7 (36.8)	12 (63.2)	0.259
CC/TT	18 (54.5)	15 (45.5)	
TT	15 (75)	5 (25)	0.004
CC/CT	10 (31.3)	22 (68.8)	
C3435T			
CC	5 (25)	15 (75)	0.023
CT/TT	19 (59.4)	13 (40.6)	
CT	14 (53.8)	12 (46.2)	0.404
CC/TT	10 (38.5)	16 (61.5)	
TT	5 (83.8)	1 (16.7)	0.084
CC/CT	19 (41.3)	27 (58.7)	
G2677T/A			
GG	5 (38.5)	8 (61.5)	0.528
Others	20 (51.3)	19 (48.7)	
GT	14 (66.7)	7 (33.3)	0.047
Others	11 (35.5)	20 (64.6)	
TT	4 (66.7)	2 (33.3)	0.411
Others	21 (45.7)	25 (54.3)	
AG/AT/AA	2 (16.7)	10 (83.3)	0.02
GG/GT/TT	23 (57.5)	17 (42.5)	

Discussion

SNPs in CYP3A have the potential to affect the expression and function of the P-gp in many ways [2, 4]. It is well known that different patients may respond differently to the same drug. The MDR1 polymorphisms might alter P-gp expression and activity toward specific anticancer agents, thereby influencing their therapeutic efficacy; the functional consequences of the changes at positions 2677 and

3435 are still controversial. P-gp is encoded by the MDR1 gene, which is highly polymorphic, and interethnic differences in the frequencies of several SNPs have been reported. In particular, the two most common SNPs, C3435T and G2677T(A), have been found to differ significantly among different ethnic groups [6]. C3435T, one of the most important MDR1 gene polymorphism, is demonstrated to be the main functional polymorphism affecting mRNA levels by altering mRNA stability. Gurney et al. [7] reported that ABCB1 SNPs are associated with imatinib clearance, but its mechanism is still uncertain. Three SNPs (C3435T, G2677T, and 1236C) are in strong linkage disequilibrium. The MDR1 SNPs were associated with efficacy of patients with CML treated with imatinib [2, 5].

In the current study, we studied the C1236T, G2677T/A, and C3435T polymorphisms on patients who had resistance or sensitivity to imatinib, and then the alleles and genotype frequencies of resistance patients were compared with the results of sensitivity patients. We observed a significant difference between sensitivity and resistance patients for C1236T polymorphism ($P = 0.007$), G2677T/A ($P = 0.03$) and C3435T ($P = 0.023$). According to our data, we may predict the response to imatinib in patients with CML.

Dulucq et al. [2] recently reported that homozygous for allele 1236T had higher molecular response (MMR) and had higher imatinib concentrations, and for G2677T/A, the presence of G allele of had a worse response. Moreover, one of the haplotypes (1236C-2677G -3435C) was statistically associated with less frequent MMR. Likewise, the study of Gurney et al. [5] showed that patients with each of 1236T, 2677T, and 3435T homozygotes had higher estimated imatinib clearance compared to patients with CC or GG genotypes. In consistent with above, our data showed that there was significantly higher resistance to imatinib in carriers of the 3435T allele (3435TT/CT genotype) when compared to the non-carriers (3435CC genotype), and among the patients homozygous for allele 1236T, 75% occurred the resistance to imatinib versus 31.3% for the other genotypes (1236CT/CC) ($P = 0.004$), suggesting that increased risk of resistance of patients with CML with imatinib treatment may be associated with the T allele, particularly if homozygous. Studies on the 3435C > T SNP show a correlation between allele frequency and risk of cancer development, as well as various responses to drug treatment [6, 7]. Carriers of the TT genotype are more at risk of developing acute lymphoblastic leukemia than other individuals, whereas the CC genotype carriers exhibit a different prognosis [8]. Homozygotes for the T nucleotide at each of the loci C1236T, G2677T/A, and C3435T had higher estimated imatinib clearance compared to patients with CC or GG genotypes [5].

The silent C3435T mutation is suggested to be linked in with 2677T functional mutation in exon 21, resulting in substitution of alanine in position 893 by serine (2677T) or threonine (2677A), respectively [3, 4]. The effect of C3435T mutation may reflect that of G2677T, and there may be racial differences in the relation between C3435T and G2677T [9]. Our results showed that a better response in carriers of at least one 2677A allele (2677AG/AT/AA genotype) when compared to non-carriers (2677TT/GT/GG genotype). Studies on 2677G > T/A/C polymorphism have yielded contradictory results, possibly due to small sample sizes and the different ethnic groups [6, 9]. Further studies are needed to determine the effects of these *ABCB*1 gene haplotypes on imatinib treatment in prospective studies in large populations.

In conclusion, the results of our study demonstrated a significant correlation of the SNPs polymorphisms with imatinib efficacy. One of the halpotypes (2677A-1236C genotype) or 3435C homozygote was statistically linked with less resistance to imatinib. So, the haplotype and genotype analysis of three SNPs polymorphism in patients with CML treated with imatinib may be used as a basis for studies on the relationship between *ABCB*1 genotypes and drug efficacy and may provide some insight into who is likely to respond optimally to imatinib.

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Conflict of interest We do not have any conflicts of interest regarding our manuscript.

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