

## Influence of ABCB1 C3435T and ABCG2 C421A Gene Polymorphisms in Response to Imatinib Mesylate in Chronic Myeloid Leukemia Patients

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**Abstract.** Despite the excellent efficacy results of IM treatment in CML patients, resistance to IM has emerged as a significant problem. Genetic variations in genes involved in drug transportation might influence the pharmacokinetic and metabolism of IM. The genotype of a patient is increasingly recognized in influencing the response to the treatment. In this study, associations between IM response and 2 SNPs in genes involved in IM pharmacokinetics (ABCB1 C3435T, ABCG2 C421A) were investigated among 45 Malaysian CML patients undergoing IM treatment. The results showed the wild type CC genotypes were higher in CML patients showing IM resistant compared to IM good response CML patients with  $p = 0.004$ . Resistance was lower for patients homozygous for the A allele of C421A compared to patients with C allele (33% vs. 64%,  $p=0.0001$ ). These preliminary results prompt us to suggest the possibility of exploring these SNPs as biomarkers to predict the response to IM in CML patients prior to IM treatment.

**Keywords:** ABCB1, ABCG2, Chronic Myeloid Leukemia, Imatinib Mesylate, Single-Nucleotide Polymorphism

### 1. Introduction

Chronic Myeloid Leukemia (CML), a myeloproliferative disorder, comprises 14% of all leukemias. The synthetic tyrosine kinase inhibitor Imatinib mesylate (IM), also known as Glivec or Gleevec, has been well documented as first line treatment for CML. Despite the excellent results encountered with IM therapy, many CML patients in advanced phases do not respond to IM, others who initially respond to IM may eventually develop resistant disease. Development of resistance to IM is a multifactorial phenomenon in patients with CML and may be mediated by a diversity of mechanisms. However, there are 2 broad mechanisms of resistance. (1) BCR-ABL dependent and (2) BCR-ABL independent [1]. BCR-ABL dependent pathways include ABL kinase domain mutations and BCR-ABL gene amplifications. But several CML patients showing IM resistance were identified who do not fit into the BCR-ABL dependent mechanisms. It is presumed that the mechanisms of IM resistance in such patients might be mediated through BCR-ABL independent pathways. BCR-ABL independent pathways of IM resistance may include several mechanisms. Not much data are available on this issue as very few studies have been carried out on this aspect worldwide [2] and nil from Malaysia. Membranebound efflux transporters play important roles in mediating chemosensitivity and resistance of tumour cells. There are seven subfamilies (ABCA through to ABCG). Members of the ABC family are important in the mechanism of multidrug resistance [3]. Important genes

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encoding ABC transporters associated with multidrug resistance (MDR1) include ABCB1 encoding P-Glycoprotein (PGP) also known as ABCB1 with protein product MRP1 and ABCG2 also named MXR (mitoxanthrone resistance protein) or BCRP (Breast Cancer Resistance Protein). Of these transporters, ABCB1 and ABCG2 are involved in IM transport. P-glycoprotein, a member of the adenosine triphosphate (ATP) binding cassette family of membrane transporters, is well known to be expressed on the surface of cancer cells and to be associated with multidrug resistance of tumour cells and affect the pharmacokinetics of many drugs and xenobiotics [4; 5]. In humans, this multidrug transporter plays a key role in determining drug bioavailability and difference in drug response has been reported to exist amongst different ethnic groups [6; 7; 8]. ABCG2 is another transporter protein mediating drug resistance. The expression of ABCG2 has not been well worked out in cancer. With the recent advances in pharmacogenomics, genetic polymorphisms have been revealed as one of the most important factors influencing the pharmacokinetics and pharmacodynamics of many drugs. Polymorphisms or nucleotide diversity occurring in the genes encoding drug metabolizing enzymes, transporters and target molecules may affect the pharmacokinetic and pharmacodynamics of respective drugs. Single nucleotide polymorphisms (SNPs) in ABCB1 and ABCG2 genes have the potential to alter protein function and could also influence the efficacy of absorption or elimination [9; 10]. This study aimed to investigate the allele and genotype frequencies of SNPs ABCB1 C3435T and ABCG2 C421A in CML patients undergoing IM treatment and to determine whether different genotype pattern of these SNPs have any influence in mediating good response and resistance to IM.

## 2. Material and Methods

### 2.1. Sample Collection

In this multi-centric study, Philadelphia (Ph) chromosome positive CML patients undergoing IM treatment in various centres in Malaysia were recruited. Ph positive CML patients (Chronic phase or Accelerated phase), treated with standard dose IM (400mg) on frontline treatment and who demonstrated the following clinical features were classified as IM resistant CML patients [11]:

- No major or complete cytogenetic response by 12 months after initiation of therapy, loss of complete cytogenetic response or development of IM resistance
- Failure to achieve a complete hematologic response after 3 months
- No major molecular response for at least 6 months

Those CML patients showing very good response to IM, at the cytogenetic and molecular level (good responders) were also included for comparison of results. After obtaining written-informed consent, 3ml peripheral blood was collected from 45 CML patients (22 IM good responders, 23 IM resistant) and 91 normal healthy individuals in EDTA vacutainer tubes. DNA from the peripheral blood of the study subjects were extracted using QIAGEN amp DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

### 2.2. Genotyping

The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method was used for the genotyping of 3435 C>T and C421A SNPs. For the 3435 C>T SNP, the sequences of oligonucleotide primers used were forward primer (5'-TGCTGGTCCT GAAGTTGATC TGTGAAC-3') and reverse primer 5'-ACATTAGGCA GTGACTCGAT GAAGGCA-3' [12]. The reagents and conditions for PCR amplification consisted of 10 X PCR buffer, 200µmol deoxynucleoside-5'-triphosphate (dNTPs), 0.2µmol of each specific primer, 1.5 mmol MgCl<sub>2</sub>, 1 unit AmpliTaq Gold DNA Polymerase and 50 ng genomic DNA templates in a 25 µl reaction. PCR conditions were 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 seconds, 61°C for 30 seconds and 72°C for 30 seconds with a final 5 minutes cycle at 72°C. The PCR products were digested with restriction enzyme Fermentas FastDigest Mbo I and DNA fragments generated after restriction enzyme digestion were separated on 3% of Bioline Agarose HiRes gel (Bioline, London, UK) with 0.1% of GelRed DNA stain. For the genotyping of 421 C>A SNP, the primer sequences used were forward primer (5'-GTTGTGATGGGCACTCTGATGGT-3') and reverse primer (5'-CAAGCCACTTTTCTCATTGTT-3') [13]. The reaction mixture for PCR amplification consisted of 10 X PCR buffer, 200µmol dNTPs, 0.2µmol of each specific primer, 1.5 mM MgCl<sub>2</sub>, 1 unit AmpliTaq Gold DNA

Polymerase and 50 ng genomic DNA templates and PCR grade water was added to a final volume of 25 $\mu$ L. PCR amplification consisted of 3 steps: denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds after denaturation for 95°C for 3 minutes, followed by 35 cycles. PCR products digested with Fermentas FastDigest restriction enzyme Taa I and the DNA fragments generated were run on 5% of Bioline Agarose HiRes gel (Bioline, London, UK) with 0.1% of GelRed DNA stain. Allele and genotype frequencies for each SNP in all normal subjects were determined.

### 2.3. Statistical Analysis

The difference in allele or genotype frequencies in patients undergoing imatinib treatment were determined by using the chi-square test or Fisher's exact test (if  $n < 5$ ). All tests were two-sided, and the  $P$  value when  $< 0.05$  was considered as significant.

## 3. Results and Discussion

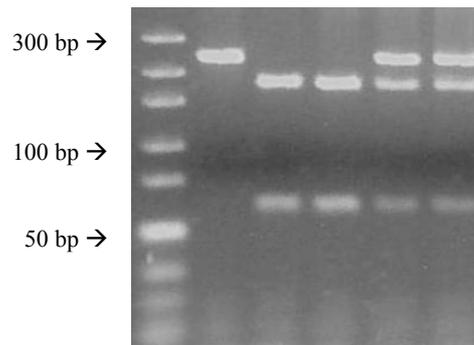


Fig. 1: Electrophoresis patterns for MDR1 alleles analyzed by PCR-RFLP. L: O'GeneRuler Ultra Low Range DNA Ladder, 10-300 bp ; lane 1: homozygous T allele (248bp); lane 2 & 3: homozygous C allele (172bp, 60bp, 16bp); lane 3 & 4: heterozygous (248bp, 172bp, 60bp, 16bp)

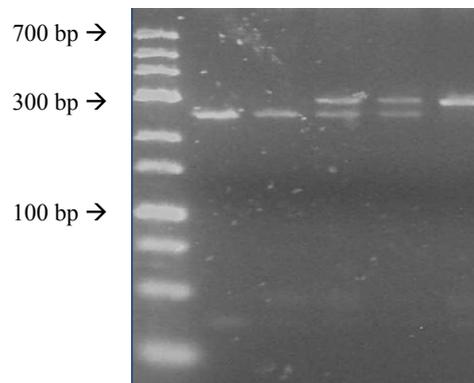


Fig. 2: Electrophoresis patterns for MDR1 alleles analyzed by PCR-RFLP. L: O'GeneRuler Low Range DNA Ladder, 25-700 bp; lane 1: homozygous C allele (251bp, 21bp, 17bp); lane 2 & 3: heterozygous (268bp, 251bp, 21bp, 17bp); lane 3 & 4: homozygous A allele (268bp, 21bp)

Table 1: ABCB1 C3435T and ABCG2 C421A polymorphisms in healthy controls and CML patients with sensitive or resistance to IM treatment

Gene	<i>n</i> , frequency (%)			<i>p</i> -value	<i>n</i> , frequency (%)			
	Controls	CML			IM responsive	IM resistance	<i>p</i> -value	
<i>ABCB1</i> C3435T	Genotype	CC	33 (36.3)	16 (35.6)	0.999	6 (27.3)	10 (43.5)	0.353
		CT	45 (49.4)	22 (48.8)	0.720	13 (59.1)	9 (39.1)	0.366
		TT	13 (14.3)	7 (15.6)	0.8037	3 (13.6)	4 (17.4)	0.999
	Allele	C	0.61	0.60	0.999	0.57	0.63	0.312
		T	0.39	0.40		0.43	0.37	

<i>ABCG2</i>	Genotype	CC	38 (41.8)	11 (24.4)	0.058	1 (4.5)	10 (43.5)	0.004
		CA	41 (45.0)	25 (55.6)	0.278	14 (63.6)	11 (47.8)	0.373
C421A	Allele	AA	12 (13.2)	9 (20)	0.321	7 (31.9)	2 (8.7)	0.071
		C	0.64	0.52	0.115	0.36	0.67	0.0001
		A	0.36	0.48		0.64	0.33	

The introduction of IM had a marked impact on treatment outcomes in CML patients. Despite the excellent response obtained, resistance to IM has emerged as a daunting problem in the management of CML patients. The molecular basis for inter-individual variation in IM response is still unclear. While a number of factors may contribute to inter-individual variability. The genotype of a patient is increasingly implicated in influencing drug disposition and activity. SNPs in the human genome contribute to wide variations in how individuals response to medications either by changing the pharmacokinetics of drugs or by altering the cellular response to therapeutic agents [14]. Both *ABCB1* (MDR1) and *ABCG2* (BCRP) have been demonstrated to display high affinity for IM and confer IM resistance in vitro by extruding IM from hematopoietic cells [15; 16]. Moreover, both transporters have shown elevated expression profile in CML stem cells [17]. Several known SNPs have been reported to modify their activity [18; 19]. The silent C3435T polymorphism (rs1045642) in exon 26 of *ABCB1* was demonstrated to be a functional polymorphism altering mRNA stability, modifying the P-gp expression and consequently reducing its' drugs substrate specificity. The SNPs C421A (rs2231142) which substitutes a glutamine for a lysine, is the most frequent non-synonymous polymorphism in *ABCG2*. We undertook this study to investigate whether the SNPs C3435T and C421A could play any contributing role in mediating response to IM in Malaysian CML patients.

In our study, there was no significant difference in the genotype and allele frequencies of *ABCB1* C3435T between the CML patients and controls as well as between CML patients showing good response and resistant to IM. However in the case of *ABCG2* C421A, the heterozygous and homozygous variant genotypes were higher in CML patients compared to controls but statistically insignificant ( $p=0.278$ ,  $0.321$  respectively). Comparison of the allele frequencies between CML patients and controls showed the variant allele A to be higher in CML patients but statistically insignificant ( $p=0.115$ ). Similarly, the heterozygous (CA) and homozygous wild type (CC) of C421A genotypes were comparatively higher in CML patients showing IM good response compared to IM resistant CML patients but statistically insignificant with ( $p=0.373, 0.071$ ). Meanwhile, CC genotype was shown significantly to be higher among IM resistant group with  $p=0.004$ . When the frequency of variant allele A was compared between CML patients showing good response and resistance to IM, the frequency was higher in CML patients with good response with  $p=0.0001$  indicating that BCRP efflux transporter encoded by *ABCG2* might have an important role in transporting IM.

Recently Dulucq *et al.* [20] has reported that *ABCB1*/MDR1 polymorphisms are associated with major molecular responses to standard dose IM and the SNP 3435 C>T has been repeatedly shown to predict changes in the function of P-Gp. Deenik [21] and colleagues also reported that patients with homozygous for 3435T showed lower probabilities to obtain a Major Molecular Response (MMR) and Complete Molecular Response. Similarly, higher resistance rate in homozygous *ABCB1* 3435T allele was reported by Ni *et al.* [22]. Another study conducted by Kim *et al.* [23] on CML patients, did not find an association between *ABCB1* polymorphisms and MMR. The frequencies with which these polymorphisms exist in a population have also been shown to be ethnically related [24]. Likewise, *ABCG2* C421A genotyping done in Japanese population had shown correlation with respect to MMR and IM trough concentration, in the study by Takahashi *et al.* [25]. Further prospective studies in larger populations and Meta Analysis by combination of several studies are needed to determine the effects of these *ABCB1* and *ABCG2* genes haplotypes on IM treatment which may provide more power to detect small but clinically significant effects and also to overcome the bias.

#### 4. Conclusion

The results of our study demonstrated a significant correlation of the SNPs *ABCG2* C421A with imatinib efficacy and a lack of association of SNPs *ABCB1* C3435T with IM response in Malaysian CML patients.

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