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Original article

Association of genotypes and haplotypes of multi-drug transporter genes ABCB1 and ABCG2 with clinical response to imatinib mesylate in chronic myeloid leukemia patients

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ABSTRACT

The introduction and success of imatinib mesylate (IM) has become a paradigm shift in chronic myeloid leukemia (CML) treatment. However, the high efficacy of IM has been hampered by the issue of clinical resistance that might due to pharmacogenetic variability. In the current study, the contribution of three common single nucleotide polymorphisms (SNPs) of ABCB1 (T1236C, G2677T/A and C3435T) and two SNPs of ABCG2 (G34A and C421A) genes in mediating resistance and/or good response among 215 CML patients on IM therapy were investigated. Among these patients, the frequency distribution of ABCG2 421 CC, CA and AA genotypes were significantly different between IM good response and resistant groups ($P = 0.01$). Resistance was significantly associated with patients who had homozygous ABCB1 1236 CC genotype with OR 2.79 (95%CI: 1.217–6.374, $P = 0.01$). For ABCB1 G2677T/A polymorphism, a better complete cytogenetic remission was observed for patients with variant TT/AT/AA genotype, compared to other genotype groups (OR = 0.48, 95%CI: 0.239–0.957, $P = 0.03$). Haplotype analysis revealed that ABCB1 haplotypes ($C_{1236}G_{2677}C_{3435}$) was statistically linked to higher risk to IM resistance (25.8% vs. 17.4%, $P = 0.04$), while ABCG2 diplotype $A_{34}A_{421}$ was significantly correlated with IM good response (9.1% vs. 3.9%, $P = 0.03$). In addition, genotypic variant in ABCG2 421C>A was associated with a major molecular response (MMR) (OR = 2.20, 95%CI: 1.273–3.811, $P = 0.004$), whereas ABCB1 2677G>T/A variant was associated with a significantly lower molecular response (OR = 0.49, 95%CI: 0.248–0.974, $P = 0.04$). However, there was no significant correlation of these SNPs with IM intolerance and IM induced hepatotoxicity. Our results suggest the usefulness of genotyping of these single nucleotide polymorphisms in predicting IM response among CML patients.

1. Introduction

Imatinib mesylate (IM), a competitive inhibitor of the BCR-ABL tyrosine kinase, has proven to be an extremely effective and generally well tolerated drug, producing durable responses in patients with chronic myeloid leukemia (CML). Despite the outstanding results obtained with IM for the treatment of CML, a significant proportion of patients show suboptimal response or develop resistance to IM. Mechanism of resistance to IM in CML patients involve BCR-ABL dependent and BCR-ABL independent pathways. BCR-ABL dependent mechanism which mainly involve point mutations in the tyrosine kinase domain (TKD) and amplification of BCR-ABL gene, account for approximately 50% of patients who develop resistance [1]. For the CML patients who do not fit into the BCR-ABL dependent mechanisms of resistance, several other BCR-ABL independent mechanisms have been postulated. Pharmacogenetic variability, which influences the pharmacokinetics of IM, could be a possible BCR-ABL independent mechanism mediating resistance.

IM is a substrate for the adenosine triphosphate binding cassette (ABC) transporters, ABCB1 and ABCG2. ATP Binding Cassette B1 (ABCB1) gene located at chromosome 7q21.1, consists

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of 28 introns and 28 exons and encodes for P-glycoprotein (P-gp), with 170 kDa or 1280 amino acids. ABCG2, also known as Breast Cancer Resistance Protein (BCRP) is the second member of the G family of ABC transporters. Located on chromosomal region 4q22, ABCG2 gene consists of 16 exons that span over 66 kb and encodes a 72-kDa membrane protein that is composed of 655 amino acids. Accordingly, ABCB1 and ABCG2 might be influencing the pharmacokinetics and intracellular or systemic level of IM. Both ABCB1 and ABCG2 display a high affinity for IM and have been demonstrated to confer resistance in vitro by extruding IM from haematopoietic cells [2]. Single nucleotide polymorphisms (SNPs) of these drug transporters could be potential determinants of variability in drug disposition and efficacy.

ABCB1 is highly polymorphic with existing 50 and more single nucleotide polymorphisms (SNPs) yet to be identified. SNPs within ABCB1 gene have been associated with PGp over-expression. Three polymorphisms (T1236C, G2677T/A and C3435T) of ABCB1 have significant effect on P-gp expression, functionality and substrate distribution [3]. The 3435C>T located on exon 26, is in linkage disequilibrium with the other two SNPs, 2677G>T/A (exon 21) and 1236C>T (exon 12), of which 2677G>T/A is responsible for a substitution in the amino acid sequence (Ala893Ser/Thr). Genetic polymorphisms in ABC transporters have been associated with altered transporter functions of various drugs [4]. Moreover, the ABCB1 SNPs are highly polymorphic within different ethnic groups. BCRP expression and function can be altered by SNPs in ABCG2 gene. Two most common ABCG2 polymorphisms are 34 G>A, which codes for Val12Met and 421 C>A which codes for Glu141Lys (Zamber et al., 2003).

Pharmacogenetic studies have been helpful in the evaluation of sensitivity profile of drugs. SNPs of ABCB1 and ABCG2 genes can cause inter-individual variations in the pharmacokinetics for IM. Therefore, we aimed to determine whether different genotype and haplotype pattern of SNPs ABCB1 (1236T>C, 2677G>T/A, and 3435C>T) and ABCG2 (34G>A and 421C>A) have any influence in mediating clinical response in CML patients undergoing IM treatment. IM pharmacogenetics may have an obvious correlation with the cytogenetic and molecular response of IM. Apart from IM response, the effects of pharmacogenetic covariates on hematological, non-hematological adverse side effects and hepatotoxicity among CML patients undergoing IM treatment were also examined.

2. Materials and methods

2.1. Sample collection

This multi-centric study protocol was approved by Universiti Sains Malaysia Human Ethics Committee and registered under National Medical Research Register (NMRR), Ministry of Health Malaysia. A total of 215 Philadelphia (Ph) chromosome positive CML participants (age range between 11 to 78 years) undergoing 400 mg IM daily for at least 6 months in several local hospitals and medical centers in Malaysia were enrolled after obtaining written informed consent. Out of the 215 CML patients, 106 were males and 109 were females with a mean age of 41.5 years. When these CML patients were categorized based on IM treatment response, 107 were IM good responders and 108 IM were resistant Ph+ CML patients belonging to chronic and accelerated phase. Blood samples of these patients were collected after getting written informed consent and stored in EDTA vacutainer tubes until analysis.

2.2. Evaluating imatinib response and tolerance

Hematologic, cytogenetic and molecular criteria were accessed in order to justify clinical response to IM by referring "European Leukemia Net: guideline for managing CML patients" [5]. Molecular response was classified based on BCR-ABL control gene transcript ratios, expressed on the International Scale, where major molecular response (MMR) and complete molecular response (CMR) were defined as ratios $\leq 0.1\%$ and $\leq 0.0032\%$ respectively. Cytogenetic response was classified as complete (0% Ph+ metaphases), partial (>0 to 35% Ph+ cells), minor (>35 to 65% Ph+ metaphases), minimal (>65–95% Ph+ metaphases), and none (>95–100% Ph+ metaphases) based on GTG banded analysis of a minimum 20 bone marrow metaphases. CML patients were categorized as in complete hematological response (CHR) when they achieved $< 450 \times 10^9$/L platelet count, $< 10 \times 10^9$/L WBC count and $< 5\%$ basophils. Those patients with CHR in 3 months, MCgR in 12 months and MMR in 18 months were considered as IM good responders. Those patients who did not achieve the above response criteria within the specified time frame were categorized under non responders/resistant group. In this study, common IM adverse side effects were non-hematological toxicity (nausea and/or vomit, skin hypo-pigmentation and/or rash, join pain and/or muscle cramp, headache and/or fatigue) and hematological toxicity (anemia, thrombocytopenia and neutropenia) in all grades, after administration with 400 mg IM. Parameters of hepatic injury were assessed by biochemical parameters like levels of bilirubin, alkaline phosphatase (ALP) and alanine transaminase (ALT).

2.3. Genotyping

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. DNA yield was standardized into 50 ng/μl after measurement with Infinite® 200 PRO NanoQuant. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was designed for amplification of the SNPs ABCB1 1236 T>C, 2677 G>T/A, 3435 C>T, and also the ABCG2 G34A and C421A SNPs. Forward and reverse primers were self-designed for 1236 T>C (F: 5′-CGAAGAGTGGGCACAAACCAG-3′ and R: 5′-GCATGGGTCATCTCACCATC-3′) and 2677 G>T/A (F: CCT TCA TCT ATG GTT GGC-3′ and R: 5′- GCA TAG TAA GCA GTA GGG AG-3′). Primers for 3435C>T was obtained from Ameyaw et al. [6] while 34G>A and 421C>A were referred to Kobayashi et al. [7]. Each PCR mixture consisted of 1× MyTaq Reaction Buffer (Bioline Ltd, London, UK), 1 unit MyTaq DNA Polymerase (Bioline Ltd, London, UK), 50 ng genomic DNA templates and ddH_2O in a total volume of 20 μl. PCR conditions involved denaturation at 95° C for 1 min, and repeated 35 cycles consisting of 3 steps: denaturation at 95° C for 15 seconds; annealing at 63° C/56° C/61° C/61° C/60° C for 15 seconds and; extension at 72° C for 10 seconds, followed by 3 mins final extension at 72° C. PCR products were subsequently digested with Fermentas FastDigest (Fermentas, Lithuania) Eco0109 I for 30 mins, 37° C (1236T>C), Ban I 37° C for 30 mins (2677G>T), Rsa I for 30 mins, 37° C (2677G>A), Mbo I for 30 mins, 37° C (3435C>T), BseM I for 55° C, 20 mins (34G>A) and Taa I for 10 mins at 65° C (421C>A) respectively. Digested DNA fragments were electrophoresed on 3.5% Bioline Agarose HiRes gel (Bioline Ltd, London, UK) and stained with SyBr Green. As part of quality control checking, genotyping results were directly sequenced (First BASE Laboratories Sdn Bhd, Malaysia) in 10% of samples after the purification steps (GeneJET PCR Purification Kit, Fermentas).

2.4. Statistical analysis

Hardy–Weinberg equilibrium was verified for all examined SNPs. Difference in genotype frequencies among the two groups of CML patients and the associations of the various genotypes with good response and resistance to IM were determined using Pearson X^2 test. Odds ratios (OR) along with 95% confidence intervals (CI) and two-sided *P*-values were calculated by Epi Info