Clinical Relevance of a Pharmacogenetic Approach Using Multiple Candidate Genes to Predict Response and Resistance to Imatinib Therapy in Chronic Myeloid Leukemia

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Abstract

Purpose: Imatinib resistance is major cause of imatinib mesylate (IM) treatment failure in chronic myeloid leukemia (CML) patients. Several cellular and genetic mechanisms of imatinib resistance have been proposed, including amplification and overexpression of the BCR/ABL gene, the tyrosine kinase domain point mutations, and MDRI gene overexpression.

Experimental Design: We investigated the impact of 16 single nucleotide polymorphisms (SNP) in five genes potentially associated with pharmacogenetics of IM, namely ABCB1, multidrug resistance 1; ABCG2, breast-cancer resistance protein; CYP3A5, cytochrome P450 3A5; SLC22A1, human organic cation transporter 1; and AGP, α1-acid glycoprotein. The DNAs from peripheral blood samples in 229 patients were genotyped.

Results: The GG genotype in ABCG2 (rs2231137), AA genotype in CYP3A45 (rs776746), and advanced stage were significantly associated with poor response to IM especially for major or complete cytogenetic response, whereas the GG genotype at SLC22A1 (rs883369) and advanced stage correlated with high rate of loss of response or treatment failure to IM therapy.

Conclusions: We showed that the treatment outcomes of imatinib therapy could be predicted using a novel, multiple candidate gene approach based on the pharmacogenetics of IM.

Imatinib mesylate (IM) is a selective tyrosine kinase inhibitor, especially against BCR/ABL fusion tyrosine kinase, that has achieved successful treatment outcomes and improved the life quality of chronic myeloid leukemia (CML) patients (1). The activity of IM is mediated by blocking the activity of BCR/ABL tyrosine kinase in CML cells. However, some of the patients fail to achieve optimal response, and a substantial proportion of patients develop resistance to IM (2). Several molecular mechanisms leading to IM resistance have been proposed: amplification and overexpression of the BCR/ABL gene, point mutations in the ATP-binding site with kinase reactivation (3), or overexpression of the MDRI gene (4).

It has been well established that the response to imatinib therapy is strongly associated with the disease factor such as disease stage, that is, chronic phase (CP), accelerated phase (AP), or blastic crisis (BC). Besides the disease factor, several determinants were known to be associated with the pharmacokinetics of imatinib with respect to absorption, distribution, and metabolism, influencing the systemic levels or intracellular concentration of imatinib which affects the response of imatinib therapy (Supplemental Table S1; ref. 5). IM is a substrate for the adenosine triphosphate binding cassette (ABC) transporters, ABCB1 and ABCG2, whereas the active uptake of IM into cells is mediated by the human organic cationic transporter-1 (OCT1; SLC22A1). Also, IM is metabolized through first-pass drug metabolism by the cytochrome P450 - CYP3A4 and CYP3A5. In addition, it is delivered in a bound form with a plasma protein referred to as α1-acid glycoprotein (AGP).

Accordingly, the intracellular or systemic level of imatinib should be influenced by these factors, such as the ABCB1, ABCG2, SLC22A1, CYP3A4, CYP3A5, or AGP genes (6). The interindividual variability of five candidate genes associated with drug transport/metabolism, namely, ABCB1 (4, 7–9), ABCG2 (10–12), SLC22A1 (13–16), CYP3A4/3A5 (6, 17, 18), and AGP (19–22), could affect the expression of corresponding proteins, thus influencing the treatment outcomes of imatinib therapy. We hypothesized that the SNPs of these five genes could predict the outcomes of imatinib therapy in CML patients. We evaluated its efficacy by three categories of parameters: (a) response to imatinib therapy [hematologic
**Translational Relevance**

The current study attempted to investigate the predictive role of multiple candidate gene single nucleotide polymorphisms (SNP) associated with the metabolism/transport of imatinib for the treatment of chronic myeloid leukemia. Several biomarkers were suggested to predict the response or resistance to imatinib therapy, such as IC50 cell assay, OCT1 activity assay, or blood trough level measurement. We hypothesized that pharmacogenomic information can be utilized to predict the response to imatinib therapy. Out of 16 SNPs in 5 genes (ABCB1, multidrug resistance 1; ABCG2, breast-cancer resistance protein; CYP3A45, cytochrome P450-3A5; SLC22A1, human organic cation transporter 1; AGP, α1-acid glycoprotein), SNPs in the ABCG2 and CYP3A45 genes correlated with cytogenetic response to imatinib therapy and SLC22A1 SNP correlated with loss of response or treatment failure to imatinib therapy. Based on the results from single marker analyses, we established the predictive model for the response or treatment failure to imatinib therapy. The present results can be used to identify high-risk group of patients with CML who potentially need dose escalation of imatinib or to switch to second-generation tyrosine kinase inhibitors.

**Patients, Materials, and Methods**

**Study population.** A total of 229 Ph+ CML patients who received IM therapy at the Princess Margaret Hospital (Toronto, Ontario, Canada) from August 2000 to December 2006 were retrospectively enrolled in this study. The current study was approved by the Research Ethics Board of the University Health Network. Patient characteristics are summarized in Table 1. A total of 212 patients were started on IM at a dose of 400 mg/d and 17 patients were started at doses of 600 mg/d (n = 15) or 800 mg/d (n = 2). All CP patients were started on 400 mg/d (n = 203), whereas patients in AP or BC started at doses of 400 mg/d (n = 9), 600 mg/d (n = 15), or 800 mg/d (n = 2).

Before commencing IM therapy, in addition to routine history taking and physical examination, all patients had a complete blood cell count including WBC differential as well as standard biochemistry. Baseline tests also included bone marrow evaluation for morphology, conventional cytogenetic analysis, and BCR/ABL mRNA reverse transcription-PCR. Cytogenetic analysis was done by the G-banding technique. Patients were regularly monitored on an outpatient basis: biweekly physical examinations, blood counts, and biochemistry were obtained during the first month of IM therapy and then monthly until a cytogenetic response was achieved, and then every 3 mo thereafter. Until a complete cytogenetic response was confirmed, bone marrow evaluation and fluorescence in situ hybridization studies were performed every 3 mo. The quantification of peripheral blood BCR/ABL fusion gene transcripts was repeated every 3 mo regardless of cytogenetic response using quantitative BCR/ABL mRNA PCR.

**Sequenom MassARRAY genotyping system.** In the initial step, the candidate genotypes were selected by the literature review. If there was no available data in the literature review, we referred to the SNPs from the Entrez SNP site (http://www.ncbi.nlm.nih.gov/sites/entrez). The selection criteria for the SNP were the non-synonymous SNPs in exon region with minor allele frequency of >0.05 to 0.1. If we could not find any SNPs satisfying the criteria, we included synonymous SNPs in the exon region or SNPs with minor allele frequency of >0.05.

Genotyping was undertaken using the Sequenom iPLEX platform, according to the manufacturer’s instructions (www.sequenom.com; Sequenom Inc.). Whole blood samples were obtained according to the declaration of Helsinki. DNA was extracted using the Puregene DNA purification Kit (Genta Systems Inc.). The detection of SNPs was done by the analysis of primer extension products generated from previously amplified genomic DNA using a Sequenom chip-based matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer platform. Multiplex SNP assays were designed using SpectroDesigner software (Sequenom). Ninety-six-well plates containing 2.5 ng DNA in each well were amplified by PCR following the specifications of Sequenom. Unincorporated nucleotides in the PCR product were deactivated using shrimp alkaline phosphatase. Allele discrimination reactions were conducted by adding the extension primer(s), DNA polymerase, and a cocktail mixture of deoxynucleotide triphosphates and di-deoxynucleotide triphosphates to each well. MassExtend clean resin (Sequenom) was added to the mixture to remove extraneous salts that could interfere with MALDI-TOF analysis. The primer extension products were then cleaned and spotted onto a SpectroChip. Genotypes were determined by spotting an aliquot of each sample onto a 384 SpectroChip (Sequenom), which was subsequently read by the MALDI-TOF mass spectrometer. Duplicate samples and negative controls were included to check genotyping quality. Genotyping results were confirmed using direct sequencing in selected samples (n = 20). The sequences of primers are listed in Supplemental Table S2.

**BCR/ABL real-time quantitative PCR and tyrosine kinase domain mutation test.** Peripheral blood samples (5 mL) were also analyzed using quantitative PCR to determine the levels of BCR/ABL fusion gene transcripts according to the manufacturer’s instructions (ABI 9700 Thermal Cycler; Applied Biosystems) and following the recommendations established to standardize this procedure at the international level (23–25). GAPDH was used as the internal control gene in the BCR-ABL quantitation. The log reduction was calculated by using our molecular lab diagnostic baseline, which was established from ~50 untreated CML patients in chronic phase before commencement of IM. Nested PCR techniques were used to confirm the results in selected samples that had undetectable BCR/ABL transcript levels. Kinase domain mutations were screened in any patient in advanced-phase disease. For chronic-phase patients who start treatment with IM, mutation screening is indicated if there is inadequate initial response or any sign of loss of response. All blood samples were collected after informed consent had been obtained from patients in accordance with the Declaration of Helsinki.

**Definition of response criteria and end points.** Response criteria were the same as previously defined in studies using IM (2, 26, 27). Briefly, a hematologic response was defined as normalized peripheral blood cell counts (WBC <10 × 10^9/L, platelet count <450 × 10^9/L) without evidence of peripheral blasts, promyelocytes, or myelocytes, and without evidence of extramedullary disease including disappearance of palpable splenomegaly lasting for at least 4 wk. Cytogenetic responses were categorized as complete (CCyR; 0% Ph+ cells in marrow by conventional cytogenetics or fluorescence in situ hybridization), partial (1–34% Ph+ cells in marrow, or minor (35–90% Ph+ cells in marrow). A major cytogenetic response (MCyR) was defined as the sum of CCyR and partial cytogenetic response (0–33% Ph+ cells in marrow). Major molecular response (MMoR) was defined as ≥3 log reduction
of BCR/ABL fusion gene transcripts by quantitative PCR compared with the base line, and complete molecular response (CMoR) was defined as disappearance of detectable BCR/ABL fusion gene transcripts, equivalent to a 5 log reduction. For major and complete cytogenetic response, bone marrow evaluation and fluorescence in situ hybridization studies were done every 3 mo until a complete cytogenetic response was confirmed. For molecular response, the peripheral blood BCR/ABL real-time quantitative-PCR was repeated every 3 mo regardless of cytogenetic response. Time to treatment failure was defined as the interval between the initiation of IM therapy and the occurrence of events that indicated that patients had failed IM, including primary hematologic resistance, cytogenetic resistance, or loss of response (LOR). Time to LOR was defined as the interval between the date of any confirmed response and the date at which the criteria for response were no longer met, such as (a) transformation from CR to AP or BC, (b) loss of CCyR/ MCyR, and (c) confirmed increase of ≥1 log of BCR/ABL mRNA transcript for patients in CCyR or more.

Time to transformation-free survival was defined as the interval between the initiation of IM therapy and confirmation of progression to AP or BC or death from any cause, whereas overall survival (OS) was calculated from the initiation of IM therapy until the date of death from any cause or the date of last follow-up.

**Statistical analysis.** The 16 SNPs were primarily evaluated for adequacy of Hardy-Weinberg Equilibrium using χ² test. The frequency of each genotype was calculated using Haploview software (available at [http://www.broad.mit.edu/mpg/haploview](http://www.broad.mit.edu/mpg/haploview)). Additive, dominant, and recessive models were tested in each SNP for the association with probabilities of OS and transformation-free survival (TFS) were also estimated using the Kaplan-Meier method. Demographic and disease characteristics were compared according to the genotypes of 16 SNPs in additive, dominant, and recessive models using Fisher’s exact test or Mann-Whitney’s U-test. In univariate analyses, the treatment outcomes such as MCyR, CCyR, MMoR, CMoR, LOR, treatment failure, TFS, and OS were also compared according to the genotypes of 16 SNPs in additive, dominant, and recessive models using the log-rank test. In multivariate analyses using Cox proportional hazard models, the disease stage (CP versus AP or BC), the presence of additional cytogenetical abnormality, race (caucasian versus non-caucasian), age, and significant genotypes were considered as covariates for each event: ABCG2 (rs2231137 and CYP3A5 (rs776746) for MCyR and for CCyR; ABCG2 (rs2131142) for MMoR and for CMoR, SLC22A1 (rs683369) for LOR and treatment failure; ABCB1 (rs1045642) for OS, and ABCG2 (rs2131142) and SLC22A1 (rs683369) for dose escalation of IM. The multivariate analyses using Cox proportional hazard models were conducted using backward stepwise modeling and a P value for the likelihood ratio test of >0.05. The hazard ratios (HR) and 95% confidence intervals (95% CI) were also estimated. Based on the results from multivariate models, we generated two scoring models (a) using the disease stage and genotype data of ABCG2 (rs2231137 and CYP3A5 (rs776746) for the prediction of MCyR and CCyR, and (b) using the disease stage and genotype data of SLC22A1 (rs683369) for LOR and treatment failure. In each predictive model, a score of 1 was assigned to adverse genotype (i.e. GG genotype for ABCG2, rs2231137; AA genotype for CYP3A5, rs776746; or GG genotype for SLC22A1, rs683369) or advanced-disease stage, and a score 0 was assigned to referent genotype (i.e. AA or AG genotype for ABCG2, rs2231137; GG or GA genotype for CYP3A5, rs776746; CC or CG genotype for SLC22A1, rs683369) or chronic-phase disease. After summing up the scores for the predictive model of MCyR or CCyR, three risk groups were obtained: low (composite score 0; n = 27), intermediate (composite score 1; n = 173), and high risk (composite score 2 or 3; n = 28). And for the predictive model of LOR or treatment failure two risk groups were generated: low (composite score 0; n = 198) and high risk (composite score 1; n = 30).

### Table 1. Patient and disease characteristics (N = 229)

<table>
<thead>
<tr>
<th>Initial diagnosis of Ph+ CML</th>
<th>No. of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female/male (ratio)</td>
</tr>
<tr>
<td>Age, y</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Race</td>
<td>White/non-white (ratio)</td>
</tr>
<tr>
<td>Disease stage</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>Treatment prior to IM</td>
<td>INF</td>
</tr>
<tr>
<td>At the time of imatinib therapy</td>
<td>Age, y</td>
</tr>
<tr>
<td>Disease duration from diagnosis, mo</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Disease stage</td>
<td>Chronic phase</td>
</tr>
<tr>
<td>Cyto genetics</td>
<td>Additional abnormalities*</td>
</tr>
<tr>
<td>Maximum dose of IM</td>
<td>400 mg/d</td>
</tr>
<tr>
<td></td>
<td>600 mg/d</td>
</tr>
<tr>
<td></td>
<td>800 mg/d</td>
</tr>
</tbody>
</table>

Abbreviations: INF, interferon; BMT, bone marrow transplantation.

*Additional cytogenetic findings were detected: -Y (n = 5); double Ph+ chromosome (n = 5); t(7;8) (n = 2); t(9;22;22) (n = 2); t(9;22;17) (n = 1); t(1;22;18) with inv (5) (n = 1); t(7;8) with +8 and +der(22) (n = 1); t(8;17) (n = 1); t(8;16) (n = 1); inv (9q) (n = 1); -18q (n = 1); t(12;16) (n = 1); t(3;19) (n = 1); t(4;6), 47-52, +X, +6, +8, +18, +19, +der(22) (n = 1); t(17;20), +der(17), +der(20) (n = 1); +8 (n = 1); -Y (n = 1).

Table 1. Patient and disease characteristics (N = 229)
To validate the genetic effect, we carried out bootstrap algorithm to construct the bootstrap confidence interval. The bootstrap, like cross-validation, is a resampling technique; however, it differs in the manner of resampling. Bootstrap data sets are created by sampling with replacement, whereas cross-validation methods sample without replacement. The bootstrap method has been shown to give a nonparametric maximum likelihood estimate of the prediction error and can correct for the bias of the estimate. We applied bootstrap based on 500 replications. The results were generated using PROC SURVEYSELECT procedure of SAS version 9.1, and presented as the bootstrap HR confidence intervals of the genetic effects and important clinic factors.

The rates of MCyR, CCyR, MMoR and CMoR were evaluated at the time of 1, 1.5, 2, 3, 4, and 5 years after IM therapy. The MCyR or CCyR was compared in each time point according to the predictive model for MCyR or CCyR using $\chi^2$ test. The cumulative incidences of MCyR and CCyR were also compared according to the predictive model for MCyR or CCyR in overall patients and in a group confined to the patients in CP.

The serial mRNA levels of the BCR/ABL fusion transcript were also analyzed every 3 mo up to 24 mo as a logarithmic-transformed format. The differences of the BCR/ABL fusion transcript mRNA levels were compared according to the risk score model based on the disease stage and genotype data of ABCG2 (rs2231137) and CYP3A5 (rs776746) using repeated measures ANOVA test.

Multiple comparisons were implicated in this study. That considered, this study is exploratory and hypothesis-generating. All statistical tests were two-sided with the significance level set at 0.05 unless otherwise stated. The statistical data were obtained using an SPSS software package (SPSS 13.0 Inc.) and SAS version 9.1 (SAS Institute). The cumulative incidence curves were obtained using R package, version 2.4.1 (available at http://CRAN.R-project.org).

Results

Demographic and disease characteristics prior to IM therapy. The demographic and disease characteristics before the commencement of IM therapy are summarized in Table 1. A total of 203 patients (89%) were in CP, 23 (10%) were in AP, and 3 (1%) were in BC before commencement of IM.

The genotype frequencies of the candidate genes. The genotype frequencies for 16 candidate SNPs are summarized in the Supplemental Table S3. We failed to detect polymorphisms for three loci (rs28383469, rs1126724, and rs3182034). The LDs of five gene polymorphisms are shown in the Supplemental Fig. S1. There were strong LDs within SNPs of the SLC22A1 gene, especially among rs1867351, rs683369, and rs628031: $D’ = 1.00$ between rs1867351 and rs683369, between rs683369 and rs628031, and between rs1867351 and rs628031.

The comparison of genotype frequency according to the patients’ demographics did not show any differences except for SLC22A1 (rs1867351, $P = 0.03$) and AGP (rs3182041, $P = 0.08$) between the patients in CP versus those in AP/BC.

Overall treatment outcomes of imatinib therapy. The treatment outcomes in overall patients are summarized in Fig. 1. With a median duration of IM administration of 40.8 months, the incidence of hematologic response was 96% (95% CI, 92-99%) at 3 months. The incidence of MCyR and CCyR was 86% (95% CI, 81-91%) and 62% (95% CI, 56-69%) at 12 months after initiation of IM therapy, respectively. The incidence of MMoR was 33% (95% CI, 27-40%), 52% (95% CI, 45-59%), and 64% (95% CI, 57-72%) at 1, 2, and 3 years, and the incidence of CMoR was 29% (95% CI, 23-37%) and 32% (95% CI, 26-40%) at 2 and 3 years.

With median follow-up of 47.3 months, 46 cases (20%) of IM treatment failure were documented including due to resistance ($n = 38$) or intolerance ($n = 8$). The probability of freedom from LOR was 71% (95% CI, 63-78%) at 2 years, 60% (95% CI, 51-69%) at 3 years, and 53% (95% CI, 44-62%) at 4 years after any response to IM therapy had been attained. The probability of freedom from treatment failure was 69% (95% CI, 62-76%), 58% (95% CI, 51-65%), and 49% (95% CI, 40-58%) at 2, 3, and 4 years after initiation of IM therapy. The 5-year probability of TFS and OS was 90% (95% CI, 87-94%) and 95% (95% CI, 92-99%), respectively.

Treatment outcomes of IM therapy according to the candidate genotypes. Treatment outcomes were compared according to each candidate genotype for the nine statistical end points using additive, dominant, and recessive models. All the significant results in the univariate analyses are presented in Table 2. In summary, the GG genotype in ABCG2 SNP (rs2231137) and AA genotype in CYP3A5 SNP (rs776746) had adverse impacts on achievement of MCyR and CCyR, whereas AC or CC genotypes in ABCG2 SNP (rs2231142) showed adverse effect in terms of MMoR or CMoR. With respect to the parameters indicating treatment failure, the GG genotype in SLC22A1 SNP (rs683369) was associated with higher risk of LOR or treatment failure. As regard to OS, the TT genotype in ABCG2 SNP (rs1045642) correlated with higher risk of death. In the subgroup analysis confined to chronic-phase patients, similar outcomes have been noted.

For CYP3A5 SNP (rs776746), the minor allele frequency was 0.118 in the overall population. However, it differs between Caucasian (0.044) and non-Caucasian (0.331). The Hardy-Weinberg Equilibrium $P$ value in each group was appropriate both in Caucasian ($P = 0.28$) and non-Caucasian ($P = 0.38$). Accordingly, we did subpopulation stratification according to the CYP3A5 (rs776746). In subpopulation analysis, the MCyR was significant in both Caucasian ($P = 0.002$) and non-Caucasian ($P = 0.008$). The CCyR was significant in both Caucasian ($P = 0.03$) and non-Caucasian ($P = 0.003$). The stratified analysis adjusted for race also provided significant results ($P = 0.01$ for MCyR and $P = 0.02$ for CCyR).

The results of multivariate analyses are summarized in Table 3. When considering the confounding effect of the disease phase to the treatment outcomes of imatinib therapy, the GG genotype in ABCG2 (rs2231137), AA genotype in CYP3A5 (rs776746), and advanced stage were significantly associated with lower response rate to IM therapy, especially for MCyR or CCyR, whereas the GG genotype at SLC22A1 (rs683369) and advanced stage correlated with higher rate of LOR or treatment failure. The CC genotype in ABCG2 (rs2231142) was also identified as an independent predictor of more frequent need for IM dose escalation.

We also carried out the haplotype analysis for the SLC22A1 genes. We generated the haplotype of SLC22A1 gene polymorphisms based on three genotypes (rs1867351, rs683369, and rs628031). The frequencies of SLC22A1 haplotypes were 37.4% (ACG), 25.3% (CCG), 18.8% (ACA), and 18.4% (AGA). The SLC22A1 gene haplotype was not correlated with MCyR, CCyR, LOR, or treatment failure (data not shown).

Internal validation using bootstrap method. The results showing the bootstrap HR confidence intervals of the genetic effects and important clinic factors are shown in Supplemental Table S3. The bootstrap results support our previous result.
Because the bootstrap algorithm provides a nonmodel based confidence interval of the hazard ratio, it is more robust than the model-based estimates.

**Predictive models for the response or treatment failure to imatinib therapy.** As the above results showing that the ABCG2 genotype (rs2231137) and CYP3A5 genotype (rs776746) were significantly associated with response to imatinib therapy together with disease stage, we generated the predictive model incorporating information of two genotypes and disease stages. Three risk groups were obtained: low (composite score 0; \( n = 27 \)), intermediate (composite score 1; \( n = 173 \)), and high risk (composite score 2 or 3; \( n = 28 \)). The MCyR was significantly different according to the three risk groups at 1 year (\( P < 0.001 \)), 1.5 years (\( P < 0.001 \)), 2 years (\( P < 0.001 \)), 3 years (\( P = 0.01 \); Fig. 2A), whereas the CCyR was also different according to the three risk groups at 1 year (\( P < 0.001 \)), 1.5 years (\( P < 0.001 \)), 2 years (\( P < 0.001 \)), and 3 years (\( P = 0.01 \); Fig. 2B).

In order to confirm the above result in the group restricting to chronic-phase disease, we generated another predictive model incorporating information of the two genotypes of ABCG2 (rs2231137) and CYP3A5 (rs776746), and assigned score 1 to adverse genotype and score 0 to referent genotype. Three risk groups were generated after summing up the scores: low (composite score 0; \( n = 30 \)), intermediate (composite score 1; \( n = 191 \)), and high risk (composite score 2; \( n = 7 \)). Similar results were noted for M CyR and CCyR as shown in Fig. 2C and D.

Then, we compared the cumulative incidence of M CyR and CCyR according to the predictive model using two genotypes and disease stages. The median time to M CyR was significantly different (\( P = 0.003 \)): 106 days (95% CI, 66-146 days) for the low-risk group, 146 days (95% CI, 110-181 days) for the intermediate-risk group, and 279 days (95% CI, 224-334 days) for the high-risk group. The median time to CCyR was also different in favor of the low-risk group (\( P < 0.001 \); Fig. 3A): 168 days (95% CI, 148-188 days) for the low-risk group, 239 days (95% CI, 186-292 days) for the intermediate-risk group, and 962 days (95% CI, 354-1,569 days) for the high-risk group. Also, a similar result was noted when analysis was confined to the patients in chronic phase with respect to M CyR or CCyR (Fig. 3B). The cumulative incidence curves of MMoR according to this predictive model are available in Fig. 3C.

Based on the multivariate result showing the GG genotype for SLC22A1 (rs683369) and advanced stage as adverse risk factors for LOR or treatment failure, we generated the predictive model for LOR and treatment failure incorporating the SLC22A1 genotype and disease stage. As mentioned in the statistical section, two risk groups were generated: low (composite score 0; \( n = 198 \)) and high risk (composite score 1; \( n = 30 \)). The 3-year probabilities of freedom from LOR were 72\% and 54\% for the low- and high-risk groups, respectively (\( P < 0.001 \); Fig. 3D). A similar result was noted when analysis was confined to patients in chronic phase for LOR (\( P < 0.001 \); Fig. 3E). The 3-year probabilities of freedom

### Table 2. Results of the univariate analyses for the treatment outcomes according to the candidate genotypes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Gene, rs number</th>
<th>Referent genotype</th>
<th>Adverse genotype</th>
<th>( P )</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCyR</td>
<td>ABCG2, rs2231137</td>
<td>AA or AG</td>
<td>GG</td>
<td>0.05</td>
<td>0.68 (0.46-1.00)</td>
</tr>
<tr>
<td>MCyR</td>
<td>CYP3A5, rs776746</td>
<td>GG or GA</td>
<td>AA</td>
<td>0.01</td>
<td>0.34 (0.14-0.83)</td>
</tr>
<tr>
<td>CCyR</td>
<td>ABCG2, rs2231137</td>
<td>AA or AG</td>
<td>GG</td>
<td>0.02</td>
<td>0.63 (0.42-0.94)</td>
</tr>
<tr>
<td>CCyR</td>
<td>CYP3A5, rs776746</td>
<td>GG or GA</td>
<td>AA</td>
<td>0.03</td>
<td>0.39 (0.16-0.96)</td>
</tr>
<tr>
<td>MMoR</td>
<td>ABCG2, rs2231142</td>
<td>AA</td>
<td>AC or CC</td>
<td>0.004</td>
<td>0.40 (0.20-0.81)</td>
</tr>
<tr>
<td>CMoR</td>
<td>ABCG2, rs2231142</td>
<td>AA</td>
<td>AC or CC</td>
<td>0.006</td>
<td>0.42 (0.21-0.85)</td>
</tr>
<tr>
<td>LOR</td>
<td>SLC22A1, rs683369</td>
<td>CC or CG</td>
<td>GG</td>
<td>0.0008</td>
<td>4.86 (1.74-13.52)</td>
</tr>
<tr>
<td>Treatment failure</td>
<td>SLC22A1, rs683369</td>
<td>CC or CG</td>
<td>GG</td>
<td>0.02</td>
<td>3.24 (1.18-8.89)</td>
</tr>
<tr>
<td>OS</td>
<td>ABCB1, rs1045642</td>
<td>CC or CT</td>
<td>TT</td>
<td>0.04</td>
<td>3.70 (0.96-14.7)</td>
</tr>
</tbody>
</table>
from treatment failure were 68 ± 4% and 42 ± 10% for the low- and high-risk groups, respectively (P < 0.001). The result of analysis confined to chronic phase was also similar for treatment failure (P = 0.006).

**Serial changes of the BCR/ABL fusion transcript levels according to the predictive model.** We also applied repeated measures ANOVA model on the BCR/ABL fusion gene transcript mRNA levels according to the ABCG2 genotype (rs2231137), CYP3A5 genotype (rs776746), and disease stage. The P value for the overall comparison of repeated measures was <0.0001 (Fig. 3F).

**Discussion**

In the current study, we have shown that (a) the treatment outcomes of imatinib therapy can be predicted using the SNP approach in CML patients; (b) the response to imatinib was strongly associated with SNPs for ABCG2 (rs2231137) and CYP3A5 (rs776746), and disease stage; and (c) the treatment failure including LOR was associated with SNP for CYP3A5 (rs776746), and disease stage. The present study suggested that the pharmacogenetic variability of individuals might predict the treatment outcomes of imatinib therapy in CML patients.

Picard et al. reported that trough plasma levels of imatinib are associated with cytogenetic and molecular response to IM therapy in CML patients, suggesting an importance of individual pharmacokinetic differences on the response to IM therapy (28). Hence, interindividuation variations in uptake and clearance by transporter protein, metabolic inactivation, and/or plasma binding inhibition could lead to changes in systemic imatinib levels. The current study included 16 SNPs in 5 genes associated with imatinib metabolism and transport, and this multiple candidate genes approach may gain a benefit of excluding potential effects of gene-to-gene interaction between genes.

A previous study suggested that not only ABCB1 but also ABCG2 is overexpressed in hematopoietic stem cells and that imatinib can be a substrate for ABCG2 (29). The ABCG2 gene, located on chromosome 4q22, encodes for a 655 amino-acid polypeptide [breast cancer resistance protein (BCRP)] that is capable of pumping imatinib out of cells (30). ABCG2 is expressed not only in the hematopoietic stem cells but also in the hepatic cells or intestinal epithelial cells, affecting the bioavailability of imatinib (30, 31). Whether ABCG2 is involved in the resistance mechanism of imatinib is a matter of debate (32–36). Gardner et al. reported that the ABCG2 SNP Q141K (rs2231142 in the current article) is associated with the intracellular level of imatinib in vitro model (6). Several SNPs were known to modify the transporter activity of ABCG2 (11, 12). In the present study, the ABCG2 genotype V12M (rs2231137) was found to be significantly associated with cytogenetic response to imatinib therapy. Similar to a previous result using an in vitro model (6), the ABCG2 genotype Q141K (rs2231142) was associated with molecular response to imatinib therapy in univariate analyses although it was not statistically significant in multivariate analyses.

Of interest, two SNPs in the ABCG2 gene showed good correlation with different parameters of treatment response. The GG genotype for ABCG2, rs2231137 was associated with a lower CCyR, whereas the non-AA genotype for ABCG2, rs2231142, was also associated with a lower MMR. The linkage disequilibrium between these two SNPs was significant.

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**Table 3.** Multivariate regression model based on Cox’s proportional hazard model for each end point

<table>
<thead>
<tr>
<th>P, HR (95% CI)</th>
<th>Candidate genes, SNP ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prognostic factors</strong></td>
<td></td>
</tr>
<tr>
<td>Adverse group</td>
<td><strong>ABCG2</strong>; rs2231137</td>
</tr>
<tr>
<td>MCR</td>
<td>0.04, 0.67</td>
</tr>
<tr>
<td>CCyR</td>
<td>0.03, 0.64</td>
</tr>
<tr>
<td>MMR</td>
<td>0.08, 0.36</td>
</tr>
<tr>
<td>CMR</td>
<td>0.005, 0.48</td>
</tr>
</tbody>
</table>

**Response to imatinib mesylate therapy, P, HR (95% CI)**

<table>
<thead>
<tr>
<th>LOR</th>
<th>Treatment failure</th>
<th>Transformation-free survival</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001, 5.47</td>
<td>(1.95-15.36)</td>
<td>(1.39-10.69)</td>
<td>0.003, 9.76</td>
</tr>
<tr>
<td>0.002, 2.67</td>
<td>(1.24-5.32)</td>
<td>(1.45-4.91)</td>
<td>(2.18-43.65)</td>
</tr>
<tr>
<td>0.011, 2.57</td>
<td>(2.18-43.65)</td>
<td>(9.6-1.01)</td>
<td>(2.18-43.65)</td>
</tr>
</tbody>
</table>

**Resistance to imatinib mesylate therapy, P, HR (95% CI)**

<table>
<thead>
<tr>
<th>LOR</th>
<th>Treatment failure</th>
<th>Transformation-free survival</th>
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</tr>
</tbody>
</table>

Abbreviations: MCR, major cytogenetic response; CCyR, complete cytogenetic response; MMR, major molecular response; CMR, complete molecular response; AP, accelerated phase; BC, blastic crisis.
This potential linkage disequilibrium between rs2231137 and rs2231142 may explain why both these SNPs associate with response to IM, either CCyR or MMoR. The reason one was associated with CCyR and another with MMoR may be due to different frequencies of minor alleles in each SNP.

In the present study, the SLC22A1 genotype was well correlated with treatment outcomes, especially for LOR or treatment failure. The human organic cation transporter, OCT1, was known to mediate active influx of imatinib into cells, thus a critical determinant of intracellular imatinib concentration (37). White et al. suggested that the interpatient variability of OCT1 activity was an important determinant of molecular response to IM (38, 39). They reported that patients with low OCT1 activity showed higher failure rates to achieve MMoR by 18 months (82% versus 17% of failure rate) and to achieve a 2-log reduction of BCR/ABL mRNA transcript by 12 months (45% versus 8% of failure rate; ref. 38). Other studies also suggested a significant association of SLC22A1 mRNA transcription levels with imatinib response (40, 41). Wang et al. reported that the group with high baseline OCT1 expression showed better progression-free ($P = 0.01$) or overall survival ($P = 0.004$) compared with those with low baseline, and that the pre-imatinib OCT1 expression level was much higher in those achieving CCyR at 6 months than noncytogenetic responders (27). Our result is quite consistent with the result of White et al. (42) The group with the GG genotype of SLC22A1 (rs683369) showed a 5.5-fold and 3.9-fold higher risk of treatment failure or loss of response to IM therapy, respectively (Table 3). The interindividual variability of the SLC22A1 genotype or gene expression could affect the variability of OCT1 protein activity (38). Controversy still remains on whether the SLC22A1 genotype could determine the levels of SLC22A1 mRNA transcription or OCT1 activity (13–15), thus facilitating further study to investigate the correlation of the SLC22A1 genotype and OCT1 activity. Although it was not statistically significant in the multivariate analysis, the SLC22A1 genotype (rs683369) was associated with different MMoR, suggesting that the SLC22A1 genotype could affect the response to imatinib therapy (Supplemental Fig. S2). Maybe because of the relatively low frequency of the SLC22A1 GG genotype (18%), it could not be identified in a multivariate analysis.

The cytochrome P450 (CYP) 3A (CYP3A) family is the predominant drug metabolizing enzyme and accounts for approximately 30% of hepatic CYP and >70% of intestinal CYP expression. Imatinib is predominantly metabolized by CYP3A4 and CYP3A5 in the liver (17, 18, 43, 44). This study showed that the AA genotype in the CYP3A5 genotype (rs776746) had a 66% and 61% lower rate of MCyR and CCyR compared with the referent genotype group, suggesting an...
important role of the CYP3A5 genotype (rs776746) in predicting response to IM therapy.

One of the interesting findings was that the ABCB1 genotype (MDR1) overexpression may confer the resistance of imatinib in cell line model (4, 37). The ABCB1 gene, which belongs to the ABC superfamily, encodes for a transmembrane glycoprotein (P-glycoprotein) capable of pumping imatinib out of the tumor cell (45). Accordingly, P-glycoprotein was suggested as a potential determinant of intracellular concentration of imatinib through ATP-dependent efflux pathway (46). C3435T (rs1045642), C1236T (rs1128503), and G2677T or A (rs2032582) have been the most frequently investigated polymorphisms for the ABCB1 gene (46–48). However, unlike other study results, we could not identify any association of ABCB1 gene polymorphisms with clinical outcomes of imatinib therapy for CML. The approach targeting other genotypes of the ABCB1 gene besides C3435T, C1236T, and G2677T or A genotypes will be necessary.

Several end points were included in the present study to evaluate which end point could discriminate the patients by their risk based on individual differences of pharmacogenetic markers. At least three events (i.e. CCyR, LOR, and treatment failure) could be reasonably accepted as statistical end points for pharmacogenetic markers to predict the outcomes to imatinib therapy in CML patients. These end points could be adopted in a future studies evaluating the power of other candidate SNP markers to predict the treatment outcomes of imatinib therapy.

Because of the limitation of the retrospective study, the information of the Sokal score or percentage of Ph+ metaphase was not available, which were suggested as potential predictive factors for the response to imatinib therapy in CML. Further studies should consider these variables as important covariates. The other issue is that the disease stage is linked to the dose administered. This may confound any analysis where nonlinearity of pharmacokinetics may be important. However, subgroup analysis confined to the chronic-phase patients reproduced a similar result to those from overall patients (Fig. 2C and D and Fig. 3B and E).

A potential debate is the use of GAPDH as a control gene for determining BCR/ABL transcript level. GAPDH was used as internal control gene to monitor BCR-ABL transcript levels for the past 7 years in our institute. And now our institute has switched to BCR as internal control gene. Our validation study showed that the log reduction difference by using GAPDH and BCR from 95% of patients is within 0.5 log (data not shown).
Accordingly, the internal and external reproducibility of measurement of BCR/ABL transcript level was maintained in the current study. In conclusion, with a novel, multiple candidate gene approach based on the pharmacogenomics of IM, we identified several SNP candidates in patients with CML that are potential predictors of clinical response to IM, the ABCG2 (rs2231137) or CYP3A5 genotype (rs776746); and of resistance to IM, SLC22A1 (rs683369). Further studies are warranted to evaluate the role of these SNPs in the early identification of individuals with CML who may not respond optimally to standard IM therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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