Original Article

Influence of Cytochrome P450, ABC and SLC Gene Polymorphisms on Imatinib Therapy Outcome of Patients with Gastrointestinal Stromal Tumours (GIST)

(gastrointestinal stromal tumours / *KIT* mutation / intestinal tumours / imatinib / pharmacogenetics / genetic polymorphisms)

K. WASIELEWSKI[1], B. WASAG[1,2], A. WOZNIAK[3], J. PIKIEL[4], A. KOWALIK[5], C. OSUCH[6], E. BYLINA[7], J. A. SIEDLECKI[8], P. RUTKOWSKI[7], J. LIMON[1,2]

[1]Department of Biology and Medical Genetics, Medical University of Gdansk, Gdansk, Poland
[2] Laboratory of Clinical Genetics, University Clinical Centre, Gdansk, Poland
[3]Laboratory of Experimental Oncology, Department of Oncology, KU Leuven and Department of General Medical Oncology, University Hospitals in Leuven, Leuven, Belgium.
[4]Regional Oncology Centre, Gdansk, Poland
[5]Department of Molecular Diagnostics, Holycross Cancer Centre, Kielce, Poland
[6]Department of General Surgery, Medical Faculty, Jagiellonian University, Krakow, Poland
[7]Department of Soft Tissue/Bone Sarcoma and Melanoma, [8]Department of Translational and Molecular Oncology, Maria Sklodowska-Curie Memorial Cancer Centre and Institute of Oncology, Warsaw, Poland

Abstract. The efficacy of imatinib-based therapy depends on the proteins involved in its metabolism and transportation. Therefore, the aim of our study was to investigate the possible correlation of selected P450, ABC and SLC polymorphic variants and the outcome of imatinib therapy. A total of 101 patients with advanced, *KIT*/*PDGFRA*(+) GIST treated with imatinib were enrolled to the study. DNA was extracted from peripheral blood samples and genotypes were determined by PCR-RFLP and direct sequencing. Deviation from the Hardy-Weinberg equilibrium was only observed for rs2740574. None of the studied SNPs was associated with GIST time to progression. No significant correlation between any specific variant and time to progression was found in the group with *KIT* exon 11 mutation. However, individuals of at least three potentially unfavourable genotypes presented significantly shorter time to progression in comparison to patients with two or less unfavourable genotypes.

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Corresponding author: Kacper Wasielewski, Department of Biology and Medical Genetics, Medical University of Gdansk, Dębinki 1, 80-211, Gdańsk, Poland. E-mail: k.wasielewski@gumed.edu.pl

Abbreviations: ABC – ATP-binding cassette, CML – chronic myeloid leukaemia, GIST – gastrointestinal stromal tumour, SLC – solute carries, SNP – single-nucleotide polymorphism, TKI – tyrosine-kinase inhibitor, TTP – time to progression.

Introduction

Gastrointestinal stromal tumours (GIST) are rare mesenchymal tumours with incidence of approximately 10 to 20 cases diagnosed per million per year (Goettsch et al., 2005; Nilsson et al., 2005; Tryggvason et al., 2005; Mucciarini et al., 2007; Chiang et al., 2014; Rubio-Casadevall et al., 2014). They may arise along the entire length of the gastrointestinal tract, but most of them are located in the stomach (60–70 %) and small intestine (25–30 %) (Miettinen and Lasota, 2001, 2003). It is accepted that GIST originate from precursors of interstitial cells of Cajal (ICC). The vast majority of GIST cells express the KIT receptor – tyrosine kinase protein that promotes cell proliferation. *KIT* or *PDGFRA* mutations are detected in approximately 90 % of GIST (Miettinen and Lasota, 2013). *KIT* mutations are found in 70–80 % of GIST and over 90 % of them are present in exon 11 (Corless et al., 2011; Miettinen and Lasota, 2013), which means that in over two thirds of GIST, *KIT* exon 11 mutation is found.

Surgical resection of the primary tumour is the main and most effective treatment method. However, in case of inoperable/metastatic tumours, a selective TKI (tyrosine-kinase inhibitor) such as imatinib is administered (Casali and Blay, 2010). Its mechanism of action is based on structural similarity between imatinib and ATP. Imatinib binds to the ATP-binding site in the enzymatic

domain of the KIT or PDGFRA receptor, competitively inhibiting substrate phosphorylation (Rubin et al., 2007). This inhibition disrupts signal transduction and in consequence decreases cell proliferation (Buchdunger et al., 2000). Imatinib is administered orally, usually at the initial dose of 400 mg once a day (Liegl-Atzwanger et al., 2010). Over 50 % of patients undergoing imatinib therapy achieve at least partial response, while complete responses are rare (Debiec-Rychter et al., 2006; Blanke et al., 2008). Unfortunately, most of patients finally develop resistance to imatinib due to secondary *KIT* mutations (Demetri et al., 2010; Miettinen and Lasota, 2013) which are thought to arise as an effect of long exposure of tumour cells to imatinib (Wardelmann et al., 2006). As a consequence, disease progression is observed in previously well-responding patients.

However, the time that a tumour requires to develop resistance differs among patients undergoing imatinib therapy. Such differences are observed even within the same molecular subgroup of patients, such as for instance patients with *KIT* exon 11 mutation. One of the possible explanations of this phenomenon is individual difference in imatinib pharmacokinetics. For example, if during imatinib therapy the metabolism and clearance of the drug are increased, its level may be lower than expected, and thus the tumour exposure to imatinib is insufficient (Wardelmann et al., 2006), which may promote faster development of secondary mutations and tumour progression. The metabolism, transport and clearance of imatinib depend on the efficiency of the proteins engaged in these processes. These proteins belong mainly to the cytochrome P450, ATP-binding cassette (ABC) and solute carries (SLC) families. They are encoded by genes in which many polymorphic variants have been detected, and some of them may substantially affect protein efficiency and thus change the substrate metabolism. It was demonstrated that polymorphic variants in *ABCB1* and *SLCO1B3* genes may affect imatinib clearance in patients with chronic myeloid leukaemia (CML) (Yamakawa et al., 2011). Therefore, the aim of our study was to investigate the influence of selected polymorphisms of cytochrome P450, ABC and SLC genes on the outcome of imatinib therapy in patients with GIST. The following polymorphisms *CYP2C9* (rs28371674), *CYP2C19* (rs4244285), *CYP2C19* (rs4986893), *CYP2D6* (rs1800716), *CYP3A4* (rs2740574), *CYP3A5* (rs776746), *ABCB1* (rs1045642), *ABCG2* (rs2231142), *SLC22A4* (rs1050152) and *SLC22A5* (rs2631372) were selected for the study.

Material and Methods

Study population

A group of 101 patients with unresectable/metastatic GIST, undergoing imatinib monotherapy, were enrolled to the study. Patients were recruited in four Polish medical centres: Department of Soft Tissue/Bone Sarcoma and Melanoma, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology at Warsaw; Department of General Surgery, Jagiellonian University in Krakow; Department of Clinical Oncology, HolyCross Centre of Oncology, Kielce; and Regional Oncology Centre at Gdansk. A written informed consent was obtained from each patient and the study was approved by the local bioethics committee at the Medical University of Gdansk. Peripheral blood samples were collected from each patient. The patients were divided into five subgroups according to mutation type in primary tumour: a) *KIT* exon 9 mutation (11 patients); b) *KIT* exon 11 mutation (71 patients); c) *PDGFRA* exon 14 mutation (1 patient); d) *PDGFRA* D842V exon 18 mutation (5 patients); e) no *KIT/PDGFRA* mutation (13 patients). Only the group of *KIT* exon 11 mutation patients was large enough to perform statistical analysis. Therefore, further analysis was performed only in this group of patients. Detailed information is presented in Table 1. To avoid the possible influence of imatinib dose on the treatment outcome, three individuals receiving higher or unknown dose of TKI were excluded from the analysis.

Genotyping analysis

DNA was extracted using the standard phenol-chloroform extraction method. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the following single-nucleotide polymorphisms (SNPs): rs28371674, rs4244285, rs4986893, rs1800716, rs776746, rs1045642, rs2231142 and rs2631372. For amplification, DreamTaq polymerase (ThermoFisher Scientific, Waltham, MA) and standard three-temperature protocol was applied. PCR products were digested according to the manufacturer's protocol (ThermoFisher Scientific).

The accuracy of PCR-RFLP was confirmed by direct sequencing. For this purpose, samples were amplified in independent PCR reactions and sequenced. Finally, rs2740574 and rs1050152 were determined by bidirec-

Table 1. Characteristics of the studied population (KIT exon 11 mutation)

Number of patients	71 (%)
Gender, N (%)	
Female	28 (39.4)
Male	43 (60.6)
Age at diagnosis in years	
Mean (range)	57 (30–78)
Imatinib dose, N	
400 mg	68
800 mg	1
NA	2
Primary tumour site, N	
Stomach	24
Duodenum	2
Small intestine	34
Rectum	7
NA	4

NA – data not available

tional sequencing (3130 Genetic Analyzer, Applied Biosystems, Foster City, CA).

Statistical analysis

To compare the observed and expected genotype frequencies, the Hardy-Weinberg equilibrium of alleles was tested with one-dimensional χ^2 test. To determine the relationship between the studied SNPs and time to progression (TTP), defined as the number of days between the beginning of treatment and progression occurrence, survival curves were estimated and plotted with the Kaplan-Meier method. In the studied population, the average TTP was 1743 days (range: 178–4284). Log-rank test of equality was used to compare the curves. Statistical significance for all the tests was defined as $P < 0.05$. Statistical analysis was performed using Statistica, version 10 (StatSoft Poland).

Results

A final group of 71 Caucasian GIST patients were analysed in this study. There were 43 men and 28 women with inoperable/metastatic tumours harbouring *KIT* exon 11 mutation (Table 1). Each patient was treated initially with 400 mg of imatinib daily. Genotype distribution of the studied SNPs is presented in Table 2.

Deviation from the Hardy-Weinberg equilibrium was only observed for rs2740574. Probably it was due to accidental occurrence of a single GG homozygote. In case of rs4986893, all patients were GG homozygotes, which is in agreement with the previously published studies of Caucasian population (Harmsze et al., 2010). This SNP was excluded from statistical analysis.

In the present study, TTP was significantly longer in patients with stomach tumours than in individuals with intestinal/duodenal tumours ($P = 0.02$). Therefore, patients were divided into subgroups based on the location of primary tumour: stomach – 24 cases; intestinal/duodenal – 35 cases; rectum – 7 cases. The entire statistical analysis further described was only possible in the group of patients with intestinal/duodenal tumours.

The outcome of the imatinib therapy was represented by TTP. The Kaplan-Meier method was applied to correlate the studied SNPs with TTP. No statistically significant correlation between the presence of a specific allele and TTP was found. The results are summarized in Table 3. Although neither of the individual studied SNPs had an impact on TTP, it was assumed that at least some of them might have a cumulative effect on the treatment outcome. Among the studied SNPs, five were chosen to perform a possible cumulative effect analysis. The following SNPs were selected: rs2740574 in CYP3A4 – the main imatinib metabolizer – and four transporters whose polymorphic variants were found to play a role in imatinib therapy (rs1045642, rs2231142, rs1050152, rs2631372). Based on previously published data, unfavourable genotypes were selected: rs2740574 – AG/GG (Hesselink et al., 2003); rs1045642 – TT (Deenik et al., 2010; Kim et al., 2009); rs2231142 – AC/CC (Kim et al., 2009; Koo et al., 2015); rs1050152 – TT (Angelini et al., 2013a, b); rs2631372 – CC (Angelini et al., 2013a). Patients were divided into two groups, one consisting of individuals with two or less unfavourable genotypes (30 cases), and one including patients with three or more unfavourable genotypes (5 cases). Comparison of the survival curves with log-rank test for these two groups (Fig. 1) revealed shorter TTP in the

Table 2. Genotype distribution in the studied population

Polymorphism	Number of patients	Genotypes (frequency)	HWE P value
rs2740574	66	AA – 63 (95.5 %) AG – 2 (3.0 %) GG – 1 (1.5 %)	0.0004
rs776746	68	GG – 62 (91.2 %) AG – 6 (8.8 %)	0.93
rs28371674	68	CC – 53 (77.9 %) CT – 15 (22.1 %)	0.6
rs4244285	67	GG – 48 (71.6 %) AG – 19 (28.4 %)	0.39
rs4986893	66	GG – 66 (100 %)	-
rs1800716	68	GG – 45 (66.2 %) AG – 18 (26.5 %) AA – 5 (7.3 %)	0.3
rs1045642	68	TT – 21 (30.9 %) CT – 30 (44.1 %) CC – 17 (25.0 %)	0.64
rs2231142	65	CC – 46 (70.8 %) AC – 18 (27.7 %) AA – 1 (1.5 %)	0.89
rs1050152	66	CC – 25 (37.9 %) CT – 33 (50.0 %) TT – 8 (12.1 %)	0.84
rs2631372	66	GG – 31 (47.0 %) CG – 28 (42.4 %) CC – 7 (10.6 %)	0.99

HWE – Hardy-Weinberg equilibrium

Table 3. Log-rank test results of statistical analysis performed in the group of patients with duodenal and intestinal tumours harbouring KIT exon 11 mutation

SNP	P value
CYP3A4 (rs2740574)	*
CYP3A5 (rs776746)	*
CYP2C9 (rs28371674)	0.38
CYP2C19 (rs4244285)	0.87
CYP2C19 (rs4986893)	*
CYP2D6 (rs1800716)	0.71
ABCB1 (rs1045642)	0.98
ABCG2 (rs2231142)	*
SLC22A4 (rs1050152)	0.98
SLC22A5 (rs2631372)	*
Cumulative effect analysis	**0.057**

* Statistical analysis was not performed due to insufficient frequency of minor alleles

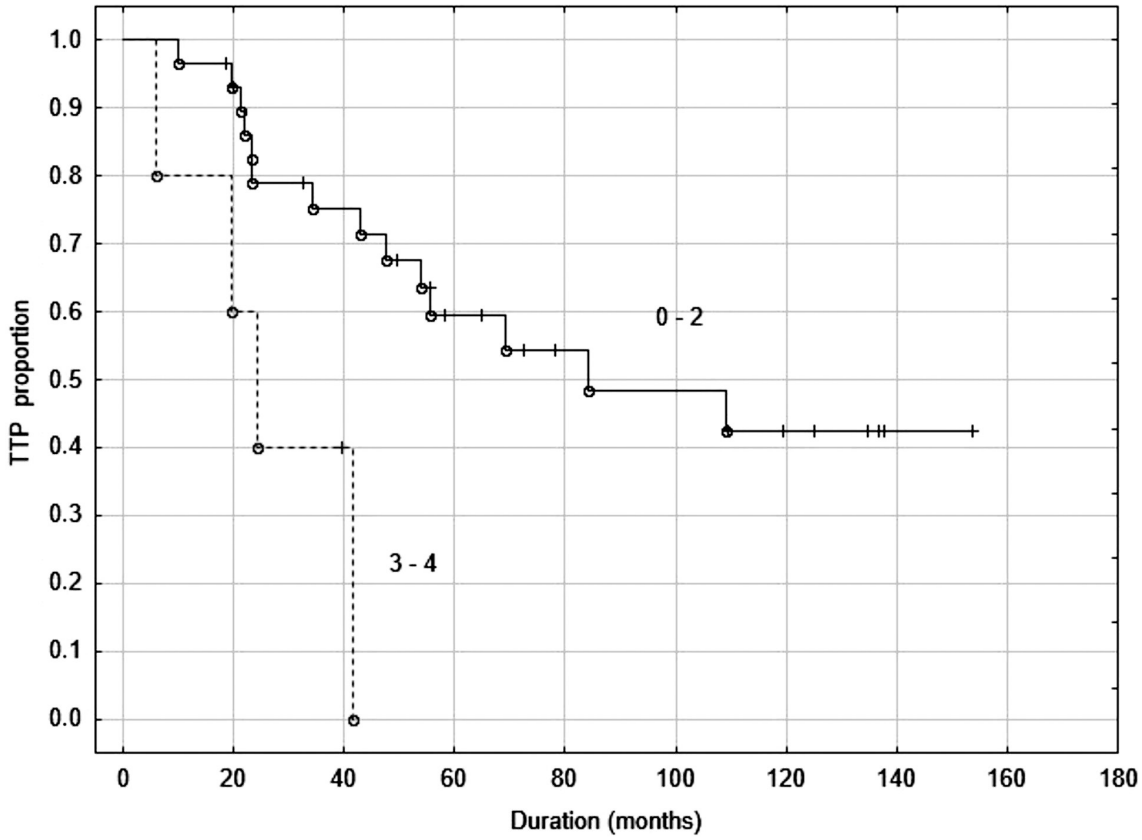

Fig. 1. Kaplan-Meier TTP estimates for cumulative effect analysis. Numbers above curves indicate counts of unfavourable genotypes.

group of patients with three or more unfavourable genotypes (median = 1669 days in the first group and 729 days in the second; 1483 days in the entire studied population); however, this result was not statistically significant (P = 0.057).

Discussion

Imatinib proved to be a milestone in GIST treatment. It is particularly effective against tumours harbouring *KIT* exon 11 mutations, which are detected in the majority of GIST (Miettinen and Lasota, 2013). Less frequent *KIT* exon 9 mutants require higher imatinib dose (800 mg) to achieve the response (Gastrointestinal Stromal Tumour Meta-Analysis Group, 2010). This fact indicates that genetic factors may substantially contribute to the TKI treatment outcome. Therefore, patients in this study were divided into five subgroups according to the mutation type. Group sizes reflected the frequency of mutation in the GIST population as previously published (Heinrich et al., 2003; Corless et al., 2004; Liegl-Atzwanger et al., 2010). It was also demonstrated that imatinib plasma levels affect progression-free survival of GIST patients (Widmer et al., 2008; Demetri et al., 2009; Marrari et al., 2010). This means that the mutational status, dosage and pharmacokinetic profile of imatinib are associated with the response to treatment. The pharmacokinetic profile of imatinib depends on the proteins responsible for both metabolism and transport of the compound. The activity of a specific protein can be related to polymorphic variants. It was previously shown that many of these variants may affect drug pharmacokinetics and anticancer therapy outcomes (van Schaik, 2005; Kim et al., 2009; Deenik et al., 2010; Angelini et al., 2013a, b; Koo et al*.,* 2015).

In this study, 10 SNPs were selected in order to evaluate their potential impact on the GIST treatment outcome. Six of them were located within the sequences of genes encoding enzymes of the cytochrome P450 family (rs28371674, rs4244285*,* rs4986893, rs1800716, rs2740574, rs776746), two ABC transporters (rs1045642, rs2231142) and two SLC transporters (rs1050152, rs2631372). Surprisingly, none of the studied SNPs was associated with GIST TTP. Such a result could be expected for SNPs of CYP2 and CYP3A5 enzymes, which are not strictly involved in imatinib metabolism (CYP3A4 is the main imatinib metabolizer). Also the results of rs2740574 in CYP3A4 can be explained due to insufficient frequency of the minor allele. More unexpected results concern ABC and SLC transporter polymorphic variants, which were previously demonstrated to affect imatinib treatment outcomes of both GIST and CML (Kim et al., 2009; Deenik et al., 2010; Angelini et al., 2013a, b; Koo et al., 2015). This might be an effect of the heterogeneity of the studied population.

Although all of the patients had primary tumours with *KIT* exon 11 mutation, no other important risk factors such as mitotic index or tumour size were available for the study. However, we assumed that a cumulative effect of possibly important polymorphic variants may be stronger and thus easier to reveal. Indeed, estimation of survival curves indicates that the presence of at least three unfavourable genotypes (among rs2740574, rs1045642, rs2231142, rs1050152, rs2631372) substantially reduced TTP in comparison with two or less unfavourable genotype carriers (Fig. 1). Of note, these results are of borderline significance (P = 0.057), and the studied group of patients with at least three unfavourable genotypes was relatively small. Thus, a similar study on a larger population should be performed. In addition, imatinib blood levels were not evaluated in the analysed group of patients, which is another limitation of the present study. Confirmation of the presented and similar findings could substantially contribute to establishment of a more individualized GIST treatment procedure, providing the patients with better chances for successful therapy.

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