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ARTICLE

Genetic polymorphisms in *ABCG2* and *CYP1A2* are associated with imatinib dose reduction in patients treated for gastrointestinal stromal tumors

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Abstract

Imatinib has a mild toxicity profile, although severe adverse events may develop. In this pharmacogenetic pathway analysis the need for dose reduction and cessation of therapy was tested for an association with single nucleotide polymorphisms (SNPs) in genes related to imatinib pharmacology. Retrospective data from 315 patients with a gastrointestinal stromal tumor who received imatinib 400 mg o.d. was associated with 36 SNPs. SNPs that showed a trend in univariate testing were tested in a multivariate model with clinical factors and correction for multiple testing was performed. Dose reduction was associated with carriership of the A-allele in rs2231137 in *ABCG2* (OR 7.35, $p = 0.0002$) and two C-alleles in rs762551 in *CYP1A2* (OR 7.12, $p = 0.001$). Results remained significant after correction for multiple testing. Therapy cessation did not show an association with any of the tested SNPs. These results may help identifying patients at increased risk for toxicity who could benefit from intensified follow-up.

Introduction

Imatinib mesylate (Glivec®, Novartis, Switzerland) is a tyrosine kinase inhibitor (TKI) which primarily blocks the Bcr-Abl protein and the KIT receptor in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors (GIST), respectively [1, 2]. Imatinib offers a significant survival benefit in these malignancies and is considered first line therapy. Despite being a selective TKI, it confer a broad range of toxicities, albeit less than conventional cytostatic agents [3]. These adverse effects range from mild and amendable symptoms, to rare but fatal hepatitis [4].

The incidence of imatinib adverse events has been associated with a range of clinical factors. In a large collaborative effort, Van Glabbeke et al. found associations of sex, age and performance score with the incidence of several imatinib-induced, non-hematological adverse events such as fatigue, nausea, diarrhea and edema [5]. In addition to clinical factors, germline genetic polymorphisms have also been shown to be associated with TKI-induced toxicity [6–8]. Single nucleotide polymorphisms (SNPs) are the most prevalent genetic polymorphisms and known to potentially alter protein function. Therefore, SNPs in genes involved in the pharmacokinetic and pharmacodynamic pathways of imatinib may affect its toxicity [9]. Our group identified SNPs that may predict for worse progression free survival in GIST patients who received imatinib 400 mg once daily [10]. If SNPs are also associated with imatinib related toxicity, patients at risk for toxicity may be identified at the onset of treatment and serious adverse effects

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may possibly be avoided by starting with a reduced dose of imatinib.

This study aims to explore genetic variants in genes involved in the pharmacokinetics and pharmacodynamics of imatinib for an association with treatment-restricting toxicity.

Methods

Patients and DNA samples

Patients from four Dutch referral centers (Erasmus MC Cancer Institute, Leiden University Medical Center, Antoni van Leeuwenhoek - Netherlands Cancer Institute and University Medical Center Groningen) were recruited. Patients were included to the same standards, being the documented use of imatinib for GIST and the availability of a DNA sample, without further selection criteria. All patients were treated with imatinib with a standard starting dose of 400 mg once daily. Therapy was given for neoadjuvant, adjuvant, or palliative indications. The observation period lasted from January 2001 to July 2014. Clinical and toxicity data were collected from patient files. The decision for dose reduction or cessation of treatment was made upon the treating physician's discretion. The level of toxicities were scored according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

DNA was obtained from residual blood samples that were collected for routine patient care and stored at −20 °C. In the Antoni van Leeuwenhoek - Netherlands Cancer Institute only serum samples were stored, and in the Erasmus MC Cancer Institute informed consent was signed by patients. If a residual blood or serum sample was not available, DNA was obtained from residual pathology specimens. All samples were anonymized by a third party and the Code for Proper Secondary Use of Human Tissue was applicable and was adhered to (www.federa.org/codes-conduct) [11].

The etiologic (KIT) mutation was not determined, because in contrast to the evident effect of these mutations in GIST on survival, such an effect is not probable for toxicity. Etiologic mutations will not affect imatinib pharmacokinetics, and toxicity is a result of imatinib interaction with healthy cells, which do not carry these mutations.

SNP selection

Using the candidate gene pathway approach, a review of literature was performed to identify SNPs in genes encoding for imatinib metabolizing enzymes and targets [12]. For selection, Haploview and HapMap data (release 28) was used to find SNPs in linkage disequilibrium (LD, >95%), and only one SNP was selected if multiple were in high LD. The National Institute of Environmental Health Sciences database was used to select the SNPs with an expected functional change, and SNPs were required to have a minor allele frequency of at least 0.1 for inclusion [10]. A total of 36 SNPs in 18 genes were selected, see Supplementary Table S1.

Genotyping

DNA was isolated from blood (270 patients), serum (32 patients) or FFPE samples (13 patients) using the MagnaPure Compact (Roche Diagnostics, Almere, the Netherlands). To enhance genotyping results, DNA isolated from serum and FFPE samples was pre-amplified using real-time PCR genotyping assays [13]. Using the QuantStudio™ 12 K Flex Real-time PCR system (Life Technologies, Bleiswijk, the Netherlands) with a custom-made array, DNA was genotyped according to the manufacturer's protocol. A number of SNPs were additionally genotyped with real-time PCR genotyping assays (Life Technologies, Bleiswijk, the Netherlands) according to the manufacturer's protocol or in house developed Pyrosequencing assays (Qiagen, Venlo, the Netherlands) in order to achieve a satisfactory call rate for all SNPs (>90%).

All 36 SNPs had a call rate of >90%, 33 of which had a call rate of >95%. Out of the 36 SNPs, 32 were in Hardy-Weinberg Equilibrium (HWE). For the remaining 4 SNPs, the relative low minor allele frequency was deemed to be the cause for the deviation. The minor allele frequencies were in accordance to those reported in the NCBI database. Haploview 4.2 [14] and Plink 1.7 [15] were used to explore haplotypes in the study population. SNPs in the same gene were considered to be in a haplotype in case D' was at least 95%. Only patients with a ≥95% probability of the assigned allele were included in the analyses.

Statistics

Two co-primary endpoints were deemed clinically relevant: the need for dose reduction and the need for therapy cessation due to toxicity. Toxicity was *a-priori* considered to be related to the clinical variables age, sex, and WHO performance score, based on clinical experience and literature [5]. The endpoints were first tested for associations with clinical variables using the Students's *t*-test or chi-square test, depending on the variable. To univariately test for associations with SNPs and haplotypes the chi-square test was also used. The general genetic model was used for the genetic variables, unless the paucity of the homozygote variant necessitated otherwise. If this test showed a trend for an association, with $p < 0.1$, it was selected for inclusion as covariate in the multivariate analysis. The multivariate analysis used logistic regression and included the clinical

Table 1 baseline characteristics of study population

	Median	range
Age at start imatinib		
in years	62.2 number	17.9–92.6 %
Sex		
male	200	63.5
female	115	36.5
WHO performance score		
0–1	279	88.6
2–3	10	3.2
unknown	26	8.3
Metastases at diagnosis		
no	225	71.4
yes	89	28.3
unknown	1	0.3
Previous surgery for GIST		
no	123	39.0
yes	192	61.0
Indication for imatinib		
neo-adjuvant	63	20.0
adjuvant	38	12.1
palliative	213	67.6
unknown	1	0.3

Baseline characteristics of study population, in which all patients received imatinib in a starting dose of 400 mg

Table 2 Incidence of imatinib toxicity

	Number	%
Dose reduction	32	10.2
Cessation of therapy due to toxicity	28	8.9
Final dose of imatinib		
200 mg	12	37.5
300 mg	14	43.8
400 mg	4	12.5
800 mg	2	6.3
Highest toxicity, any	298	94.6
grade 1	206	65.4
grade 2	68	21.6
grade 3	20	6.3
grade 4	4	1.3
Toxicity absent	17	5.4

variables and the selected genotypes, the latter as a categorical variable. A single SNP or haplotype was added to these variables. To account the 36 SNPSs tested, a correction for multiple testing was performed. A p-value in the multivariate analysis was considered significant when it was lower than 0.00139 (that is 0.05 divided by 36, the number of tested SNPs). SPSS version 20 (IBM Corp., Armonk, NY, USA) was used.

Results

Study population

A total of 315 patients were included in the study, see Table 1 for the baseline characteristics. In 32 patients (10.2%) a dose reduction due to toxicity was performed, and 28 patients (8.9%) ceased imatinib treatment due toxicity, see Table 2. Only 5 patients had dose reductions prior to ceasing imatinib entirely due to toxicity. The final imatinib dose was 200 mg in 12 patients, and 300 mg in 14, whereas in 6 patients the dose was later escalated to 400 and 800 mg (in between dosing not recorded). The time between start of imatinib and dose reduction was a median 3.1 months (range 0.7–68.8 months), and the majority of dose reductions occurred early in treatment (see Supplementary Figure S2). In case of subsequent dose escalation, it followed in median 2.9 months (range 1.2–22.4 months).

Almost all patients ($N = 298$, 94.6%) suffered from at least one adverse event, 92 patients (29.2%) had at least a grade 2 toxicity, and 24 patients had a grade 3 toxicity or higher (7.6%). Just 20 patients (6.3%) had a grade 3 toxicity. Only 4 patients (1.3%) had a grade 4 adverse event. None of the patients died as a direct result of imatinib toxicity. The toxicities to cause cessation of treatment were diverse in nature and in some patients it was a combination of several adverse events (see Supplementary Table S3).

Dose reduction needed

Increased age was associated with the need for dose reduction in the multivariate analysis (OR 1.05 per year, $p = 0.015$), and sex and WHO performance score were not, as shown in Table 3. Carriers of the A allele in rs2231137 in *ABCG2* had higher chance of dose reduction (34.8%) compared to wildtype patients (8.4%) and this showed a significant difference in both univariate and multivariate analysis (OR 7.35, $p = 0.0002$). Two C alleles in rs762551 in *CYP1A2* conferred a higher chance of dose reduction compared to CA and AA genotypes (28.6% *vs* 10.3% and 6.3%, respectively). This was also found to be a statistically significant difference in the multivariate tests (OR 7.12, $p = 0.0010$). For homozygous carriers of the T allele in rs28656907 in *ABCB1* an association was found (OR 0.19, $p = 0.040$) and patients with this genotype had a lower change of needing a dose reduction. However, this result failed to match the significance level set by the correction for multiple testing. For all (non-significant) results, a Supplementary Table S4 is available.

Table 3 Multivariate logistic regression analyses of toxicity

Dose reduction needed	N event (%)	*p* value	OR	95% CI	*p* value
Age		0.004	1.05	1.01–1.08	0.015
Sex					
male	16 (8.0)	0.094	1		
female	16 (13.9)		1.87	0.86–4.04	0.114
WHO score					
0–1	28 (10.0)	0.278	1		
2-3	2 (20.0)		1.57	0.30–8.28	0.595
rs2231137 *(ABCG2)*					
GG *vs*	23 (8.4)	<0.001	1		
GA + AA	8 (34.8)		7.35	2.55–21.2	*0.0002
rs762551 *(CYP1A2)*					
AA *vs*	16 (10.3)	0.002	1		
AC	8 (6.3)		0.81	0.32–2.07	0.657
vs CC	8 (28.6)		7.12	2.21–22.9	*0.001
rs28656907 (ABCB1)					
CC *vs*	11 (13.9)	0.079	1		
CT	14 (9.7)		0.67	0.27–1.66	0.385
vs TT	2 (3.0)		0.19	0.04–0.93	0.040
Cessation of therapy due to toxicity	N event (%)	*p* value	OR	95% CI	*p* value
Age		0.079	1.03	0.99–1.06	0.163
Sex					
male	14 (7.0)	0.120	1		
female	14 (12.2)		1.88	0.81–4.37	0.143
WHO score					
0-1	23 (8.2)	0.843	1		
2-3	1 (10.0)		0.95	0.11–8.10	0.961
rs2631370 *(SLC22A5)*					
TT *vs*	6 (4.9)	0.068	1		
TC	19 (12.3)		2.07	0.76–5.62	0.153
vs CC	2 (5.3)		1.01	0.19–5.36	0.988
rs1045642 *(ABCB1)*					
CC *vs*	9 (10.5)	0.003	1		
CT	6 (3.9)		0.40	0.12–1.33	0.136
vs TT	13 (17.3)		1.98	0.72–5.41	0.184
rs1050152 *(SLC22A4)*					
CC *vs*	11 (10.2)	0.095	1		
CT	16 (10.9)		0.95	0.39–2.32	0.919
vs TT	1 (1.7)		0.15	0.02–1.25	0.080

Multivariate logistic regression analyses of toxicity during imatinib treatment, OR= odds ratio, 95% CI = 95% confidence interval, * these results remained significant after statistical correction for the number of tested SNPs. Multivariate results are reported for the base model with clinical variables characteristics as covariates without inclusion of SNPs. Genetic variables results are presented for the singular SNP or haplotype added to the base model.

Cessation of therapy due to toxicity

Ceasing imatinib therapy due to toxicity was not associated with age, sex or WHO performance score. SNPs in *SLC22A5, ABCB1* and *SLC22A4* only showed an association in the univariate analysis, but these associations did not remain significant in the multivariate analysis. For all non-significant results, a Supplementary Table S5 is available.

Discussion

This exploratory pharmacogenetic study on the toxicity of imatinib 400 mg once daily has found an association of rs2231137 in *ABCG2* and rs762551 in *CYP1A2* with the need for a dose reduction. To the best of our knowledge, this is the largest pharmacogenetic study to explore imatinib toxicity, and the results remained statistically significant after correction for the number of tested SNPs.

This study used the need for dose reduction and cessation of therapy due to toxicity as primary endpoints as they were deemed to be clinically relevant endpoints for a drug that has a relatively mild toxicity profile. Although toxicity can be debilitating, non-hematological toxicity can be treated with other drugs and hematological toxicity is often asymptomatic and acceptable considering the need for anti-tumor therapy. A combination of several mild ailments, however, may lead to a decision to reduce the dosage, or

stop treatment altogether. The treatment setting, being neo-adjuvant, adjuvant or palliative, was not associated with one of the clinical endpoints, nor with the prevalence of polymorphisms in the selected SNPs (data not shown).

A dose reduction was needed more often in older patients, in line with results of a large cohort which showed age to be associated with toxicity [5]. In large clinical trials, the need for a dose reduction has consistently been reported to be around 15% in patients receiving a dose of 400 mg daily for GIST [16–18]. The percentage of 10% found in this study is comparable and the slightly lower percentage may better reflect clinical practice, as most trial protocols dictate a mandatory dose reduction in case of certain grade of (non-)hematological toxicities. Furthermore, the percentage of patients with a poor performance score was lower than in most trials.

An association for dose reduction was found with the A-allele in rs2231137 in *ABCG2*, with 34.8% needing dose reduction *vs* 8.4% in wild type patients. The ATP-binding cassette sub-family G member 2 is encoded by the *ABCG2* gene and it functions as a cellular transmembrane transporter able to excrete xenobiotic molecules [19]. Imatinib is known to be transported through this molecule in the intestinal epithelium [20]. Associations with selected (non-) hematological adverse events and this SNP were neither found in a study with Malayan patients, nor in Chinese patients who had a GIST [7, 21]. The A-allele has been associated with better response to imatinib in Korean patients, but a mechanism in which this SNP may lead to higher imatinib plasma levels is uncertain, as an association with imatinib steady state trough levels was not found in two cohorts of GIST patients from China and Korea [22–24]. Possibly, the minor allele frequency is too low in Asian patients to provide enough statistical power to detect a difference in serum levels. An alternative possibility may that this SNP influences the intracellular imatinib level, instead of the serum level.

Several other studies have reported an association with rs2231142, another frequently investigated SNP in *ABCG2*, and imatinib efficacy, but it was not associated with the toxicity endpoints in that study, nor were SNPs in the genes encoding for organic cation (influx) transport proteins [21–23]. SNPs previously reported to be associated with imatinib efficacy in advanced GIST were not associated to one of the clinical endpoints [10].

Patients with the less prevalent CC genotype in rs762551 in *CYP1A2* had a significantly higher chance of the need for a dose reduction; 28.6% had a dose reduction *vs* 10.3 and 8.3%. The CC genotype is considered to yield a slow metabolizers CYP1A2 phenotype compared to the AA genotype, and if patients with a CC genotype have a higher plasma level, it may explain for the increased need for dose reduction. Obviously, in vivo other enzymes in the cytochrome P450 system could compensate in case of slow acting CYP1A2, but the effect of the CC genotype is strong enough to show in the multivariate analysis. This effect has not yet been reported previously.

An association with dose reduction was found for rs28656907 in *ABCB1*, but this did not remain significant when corrected for multiple testing. This SNP has been shown to increase *ABCB1* expression [25]. This gene (also known as MDR1) encodes for the drug transporter P-glycoprotein. SNPs in this gene, such as with rs1045642, rs1128503 and rs2032582, have been studied extensively in CML patients receiving imatinib. The T-allele in rs1128503 has been shown to confer a better response in Asian patients [26]. However, association studies with imatinib toxicity have yielded mixed results [21]. One study found rs1045642 to associated with periorbital edema in a co-dominant model, but this was not tested in a multivariate analysis [7].

Cessation of treatment due to toxicity was not associated with any of the tested SNPs. Possibly, this is due to the low frequency of events. Imatinib is a drug with a relatively mild toxicity profile. Phase I studies showed dose limiting toxicity to occur in patients taking imatinib 500 mg twice daily, whereas currently the standard dose for imatinib is much lower at 400 mg once daily, and all patients in this cohort received that dose [27].

Specific grades of toxicity were not explored because of the retrospective character of data collection. Instead, the clinically relevant endpoints were used, that are accurately noted in patient files. By choosing these particular endpoints, any result this study would find, was prone to be challenging in determining a molecular explanation. Therapy restricting toxicities may well be due a combination of adverse events, with each having a different molecular pathway. Although the etiologic mutations for GIST do not have a direct effect on toxicity, there could be an indirect time-related effect. Etiologic mutations influence patient survival and longer survival allows for more time for an endpoint to occur. This potential effect can be considered to be negligible as the median duration until the event was 3 months and the sheer majority of events was early in treatment.

For this study, DNA was obtained from blood samples, serum samples and FFPE samples. FFPE material contained tumor specimen and genotyping could potentially have been affected by loss of heterozygosity, as seen in GIST. However, almost all of the tested SNPs were in HWE, and deviations from HWE did not point towards loss-of-heterozygosity. DNA was obtained from FFPE samples in only 13 out of 315 patients (4%).The multivariate analysis did not yield different conclusions if performed without these patients (data not shown), which have therefore been retained in the analysis.

In conclusion, this pharmacogenetic study found SNPs in *ABCG2* and in *CYP1A2* in association with the need for a dose reduction of imatinib 400 mg in patients being treated for GIST. In 10% of patients dose reduction is needed and, these SNPs, if validated could potentially identify those patients in advance of the adverse events occurring. This may help in identifying which patients will suffer more from imatinib toxicity and could benefit intensified from follow-up. This would be a step towards personalizing and optimizing imatinib therapy in GIST patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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