Clin Pharmacokinet (2017) 56:977–985
DOI 10.1007/s40262-016-0494-0

ORIGINAL RESEARCH ARTICLE

CYP2C8 Genotype Significantly Alters Imatinib Metabolism in Chronic Myeloid Leukaemia Patients

Daniel T. Barratt[1,2] · Hannah K. Cox[1] · Andrew Menelaou[3] · David T. Yeung[3,4,5] · Deborah L. White[3,5] · Timothy P. Hughes[3,4,5] · Andrew A. Somogyi[1,2,6]

Published online: 19 December 2016

Abstract

Objective The aims of this study were to determine the effects of the *CYP2C8**3 and *4 polymorphisms on imatinib metabolism and plasma imatinib concentrations in chronic myeloid leukaemia (CML) patients.

Methods We genotyped 210 CML patients from the TIDELII trial receiving imatinib 400–800 mg/day for *CYP2C8**3 (rs11572080, rs10509681) and *4 (rs1058930). Steady-state trough total plasma N-desmethyl imatinib (major metabolite):imatinib concentration ratios (metabolic ratios) and trough total plasma imatinib concentrations were compared between genotypes (one-way ANOVA with Tukey post hoc).

Results *CYP2C8**3 ($n = 34$) and *4 ($n = 15$) carriers had significantly higher ($P < 0.01$) and lower ($P < 0.01$) metabolic ratios, respectively, than *CYP2C8**1/*1 ($n = 147$) patients (median ± standard deviation: 0.28 ± 0.08, 0.18 ± 0.06 and 0.22 ± 0.08, respectively). Plasma imatinib concentrations were consequently > 50% higher for *CYP2C8**1/*4 than for *CYP2C8**1/*1 and *CYP2C8**3 carriers (2.18 ± 0.66 vs. 1.45 ± 0.74 [$P < 0.05$] and 1.36 ± 0.98 µg/mL [$P < 0.05$], respectively).

Conclusions *CYP2C8* genotype significantly alters imatinib metabolism in patients through gain- and loss-of-function mechanisms.

Key Points

Cytochrome P450 (CYP) 2C8 metabolism plays a role in imatinib clinical pharmacokinetics.

The *CYP2C8* genotype of a chronic myeloid leukaemia patient can significantly affect their imatinib metabolism, and consequently imatinib exposure for a given dose.

1 Introduction

Imatinib is one of the most widely used first-line treatments for chronic myeloid leukaemia (CML), and is also indicated in Ph+ acute lymphoblastic leukaemia, c-KIT- and platelet-derived growth factor receptor (PDGFR)-positive gastrointestinal stromal tumours (GISTs), myelodysplastic/myeloproliferative diseases associated with *PDGFR* gene rearrangements, aggressive systemic mastocytosis (without D816V c-Kit mutation), hypereosinophilic syndrome and/or chronic eosinophilic leukaemia, and dermatofibrosarcoma

Electronic supplementary material The online version of this article (doi:10.1007/s40262-016-0494-0) contains supplementary material, which is available to authorized users.

✉ Daniel T. Barratt
daniel.barratt@adelaide.edu.au

1 Discipline of Pharmacology, Adelaide Medical School, University of Adelaide, Adelaide, SA 5005, Australia

2 Centre for Personalised Cancer Medicine, University of Adelaide, Adelaide, SA, Australia

3 South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA, Australia

4 Department of Haematology, SA Pathology, Adelaide, SA, Australia

5 Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

6 Department of Clinical Pharmacology, Royal Adelaide Hospital, Adelaide, SA, Australia

protuberans [1–4]. Currently up to 50% of CML patients discontinue imatinib due to lack of efficacy or adverse effects [5]; a significant problem requiring switching to other treatments that may be more costly or may have significant toxicities. Imatinib response is significantly associated with plasma imatinib concentrations, which can vary more than 25-fold between patients for a given dose [6–9]. Therefore, imatinib dose individualisation will likely be a key to achieving treatment goals in more patients.

As a low hepatic extraction ratio drug, steady-state plasma imatinib concentrations are determined by variability in plasma protein binding and intrinsic clearance. Imatinib undergoes hepatic N-demethylation to the much less potent [10–13] major metabolite N-desmethyl imatinib (NDIM), with approximately 65% of systemic exposure corresponding to imatinib and 10–20% to NDIM [14, 15]. Both imatinib and NDIM undergo mostly hepatic excretion with very little renal contribution [16]. Therefore, imatinib biotransformation to NDIM is a clinically important inactivating process, with variable imatinib metabolism likely to be a major contributor to the large inter-patient variability in the dose–plasma concentration relationship; this may be partly genetically determined [17, 18].

Imatinib is metabolised to NDIM by cytochrome P450 (CYP) 2C8 and 3A4 in vitro, with evidence of dose- and time-dependent mechanism-based CYP3A4 inhibition [19–21]. Steady-state imatinib pharmacokinetics are unrelated to variable CYP3A activity (quinine 3-hydroxylation) in CML patients [22] (as in GIST patients [23, 24]), are not significantly influenced by CYP3A4 inducers or inhibitors [16, 25, 26], and there is no consistent evidence that *CYP3A4* or *CYP3A5* genetic polymorphisms alter imatinib metabolism [27–30]. We recently demonstrated that imatinib N-demethylation in human liver microsomes is mainly mediated by CYP2C8, for which the *CYP2C8**3 (rs11572080 [R139K] and rs10509681 [K399R]) is a gain-of-function haplotype [31]. The other major polymorphism in Caucasians, *CYP2C8**4 (rs1058930), has been associated with reduced paclitaxel, amodiaquine and fluoxetine metabolism [32–35]. Compared with other members of the CYP2C family, CYP2C8 metabolism and pharmacogenetics have not been the focus of much clinical pharmacology research in general, and *CYP2C8* genotype effects on imatinib metabolism in vivo have not been investigated.

Other enzymes (CYP1A2, CYP2D6, CYP2C9 and CYP2C19) play little or no role in imatinib N-methylation to NDIM [20, 36], and consequently their genetics have shown no significant effect on imatinib pharmacokinetics [28, 29].

We hypothesised that the *CYP2C8**3 polymorphisms would increase, and the *CYP2C8**4 polymorphism would decrease, imatinib metabolism to NDIM in CML patients.

This study aimed firstly to determine *CYP2C8* genotype differences in imatinib metabolism as indicated by steady-state metabolic ratio (trough plasma NDIM:imatinib concentration ratio) in CML patients treated with imatinib. It then aimed to identify any dose and time dependency of these genotype effects. Finally, the study aimed to investigate the potential clinically relevant consequences of any genotype effects on the metabolic ratio by determining genotype differences in steady-state plasma imatinib concentrations.

2 Methods

2.1 Patients, Data and Exclusions

The study was a retrospective analysis of the first 3 months of treatment for 210 predominantly Caucasian chronic-phase CML patients who participated in the TIDEL (Therapeutic Intensification in De Novo Leukaemia)-II study [37]. Briefly, all patients started treatment with imatinib 600 mg/day. If patient trough plasma imatinib concentrations at day 22 of treatment were <1000 ng/mL (based on previous studies indicating a correlation between the minimum plasma imatinib concentration achieved [>1000 ng/mL] and the likelihood of achieving complete cytogenetic remission and/or major molecular response) [7, 8], then the imatinib dose was increased to 800 mg/day. Imatinib dose reductions to 400 mg/day were allowed at any time for grade III/IV or persistent grade II toxicities. Patients were switched to nilotinib 400 mg twice daily if they were unable to dose escalate to imatinib 800 mg due to intolerance or had a loss of imatinib response at any time. Dose changes in response to failure to achieve pre-determined time-dependent treatment response targets [37] were made after the 90-day timepoint analysed in this retrospective analysis. The study was approved by the relevant Human Research Ethics Committees of participating study sites [37].

The following data from the TIDEL-II study were used in the main and supplementary analyses: imatinib dose and trough plasma imatinib and NDIM concentrations determined by HPLC with UV detection as described in Electronic Supplementary Material Online Resource 2 for days 8, 22 and 90 of treatment; and patient age and sex.

Three patients were excluded for all timepoints due to unclear dosing histories. Additional timepoint-specific exclusions ($n = 5$) were made due to documented deviations in time of blood sampling. Demographics of the 207 patients included in this study, and a summary of dose and timepoint data used, are shown in Table 1.

Table 1 Demographics and summary of dose and time point data for TIDELII chronic myeloid leukaemia patients analysed in this study

Demographics, dose and timepoint	Value
Age at start of treatment (years) [median (range)]	54 (22–86)
Sex (male:female) (*n*)	118:89
Dose and timepoints with trough plasma imatinib concentration data (*n*)	
Treatment day 8	
Dose	
400 mg/day	0
600 mg/day	199
800 mg/day	0
Timepoint exclusion	1
Missing concentration data	7[a]
Treatment day 22	
Dose	
400 mg/day	1
600 mg/day	198
800 mg/day	0
Timepoint exclusion	3
Missing concentration data	5[b]
Treatment day 90	
Dose	
400 mg/day	13
600 mg/day	150
800 mg/day	23
Timepoint exclusion	1
Missing concentration data	20[b]

NDIM N-desmethyl imatinib

[a] An additional 3 patients had a plasma NDIM concentration below the lower limit of quantification

[b] An additional 1 patient had a plasma NDIM concentration below the lower limit of quantification

2.2 Genotyping

CYP2C8 rs11572080, rs10509681 and rs1058930 were determined by probe-based allelic discrimination assays as detailed in Online Resource 1.

2.3 Statistical Analysis

Chi squared analysis (GraphPad PRISM 5, GraphPad Software, San Diego, CA, USA) was used to test for genotype deviations from Hardy–Weinberg equilibrium. All other data analyses were conducted in the R statistical package (version 3.3.1) [38].

The metabolic ratio was calculated as plasma NDIM concentration (μmol/L) divided by plasma imatinib concentration (μmol/L). Histograms and quantile–quantile (Q–Q) plots indicated that plasma imatinib and NDIM concentrations (ng/mL) and the metabolic ratio were not normally distributed. Square-root-transformed plasma imatinib concentration, and $\log_e$-transformed plasma NDIM concentration and metabolic ratio, data were normally distributed. Transformed data were used in all statistical comparisons.

Day 8 metabolic ratios and plasma imatinib concentrations were compared between genotypes by one-way ANOVA with Tukey post hoc test (lm function in stats package [38], Anova function in car package [39], and glht function of the multcomp package [40]). Tests for linear trend between genotypes (gene–dose effect) were performed in GraphPad PRISM 5. The proportion of patients achieving the day 22 plasma imatinib 1000 ng/mL concentration target was also compared (Chi squared test) between genotypes.

Forward stepwise (inclusion criteria: type II ANOVA $P < 0.05$) linear mixed-effects modelling was used to investigate the main (fixed) effects of dose (factor: 400, 600 and 800 mg/day), time (factor: day 8, 22 and 90), age, sex and genotype on the metabolic ratio and plasma imatinib or NDIM concentrations, with random effect (intercept) for patient ID (lmer function of lme4 package [41]). To determine dose and time dependency of any significant genotype effects on metabolic ratios or plasma imatinib concentrations, genotype was held as a fixed effect and each patient held as a random intercept, whilst testing (type II ANOVA $P < 0.05$) random slopes and intercepts for dose and/or day. The effects of individual predictors from multiple regression analyses (averaging over other terms in the model) were determined using the allEffects function of the effects package [42]. Simultaneous Tukey post hoc tests on significant main effects were conducted using the glht function of the multcomp package [40].

The dose proportionality of trough plasma imatinib concentrations within patients decreasing to 400 mg/day, and increasing to 800 mg/day, from 600 mg/day before day 90 of treatment was determined as described in Online Resource 3.

Point-wise *P*-values (no correction for multiple testing) are presented unless specified otherwise.

3 Results

3.1 Genetic Variability

One hundred and sixty patients were *CYP2C8**1/*1, 31 were *1/*3, 3 were *3/*3 and 16 were *1/*4. *CYP2C8* rs11572080 and rs10509681 were in complete linkage disequilibrium. Given the low frequency of the *CYP2C8**3/*3 genotype, these patients were grouped with *CYP2C8**1/

*3 for statistical analyses, and collectively referred to as *CYP2C8**3 carriers. All *CYP2C8**1/*4 patients were wildtype at both *CYP2C8**3 loci.

CYP2C8 genotype distribution did not deviate significantly from Hardy–Weinberg Equilibrium ($P = 1.0$).

3.2 Metabolic Ratio

Day 8 plasma NDIM:imatinib concentration ratios ranged from 0.07 to 0.63 (median = 0.23). The metabolic ratio was significantly higher for *CYP2C8**3 carriers compared to *CYP2C8**1/*1 and *CYP2C8**1/*4, and significantly lower for *CYP2C8**1/*4 compared to *CYP2C8**1/*1 (Table 2; Fig. 1a). *CYP2C8* genotype accounted for 10% of variability in the metabolic ratio. There was no significant linear trend if *CYP2C8**1/*3 and *CYP2C8**3/*3 were separated ($P = 0.4$).

Linear mixed-effects analysis of day 8, 22 and 90 data identified significant dose ($P = 0.0004$), day ($P = 5 \times 10^{-6}$) and *CYP2C8* genotype ($P = 4 \times 10^{-5}$) main effects (Fig. 2), but no significant effects of age or sex ($P > 0.1$).

Accounting for other significant main effects, post hoc analyses showed that the imatinib 800 mg/day dose was associated with a significantly lower metabolic ratio than 600 (17% lower, $P < 0.01$) and 400 (25% lower, $P < 0.01$) mg/day, with no significant difference between 400 and 600 mg/day. Day 22 metabolic ratios were significantly higher than at day 8 (11% higher, $P < 0.0001$) and day 90 (7% higher, $P < 0.05$), with no significant difference between days 8 and 90.

The *CYP2C8**3 carrier genotype was associated with a significantly higher metabolic ratio than *CYP2C8**1/*1 (18% higher, $P < 0.01$) and *CYP2C8**1/*4 (39% higher, $P < 0.001$); *CYP2C8**1/*4 genotypes were 15% lower than *CYP2C8**1/*1 ($P = 0.09$). Random slopes for genotype effect within dose and day did not significantly improve the model ($P > 0.9$).

3.3 Plasma Imatinib Concentration

Day 8 trough total plasma imatinib concentrations ranged from 280 to 5000 ng/mL (median = 1480 ng/mL) and were significantly (>50%) higher in *CYP2C8**1/*4 patients than in *CYP2C8**1/*1 and *CYP2C8**3 carrier genotypes (Table 2; Figs. 1b and 3a).

Dose was the only significant ($P = 1 \times 10^{-11}$) main effect in linear mixed-effects analysis of day 8, 22 and 90 data; plasma imatinib concentrations were a median 52% higher at 600 mg/day than at 400 mg/day, and 149% higher (non-dose-proportional) at 800 mg/day than at 600 mg/day (see Online Resource 3).

Accounting for dose, the *CYP2C8* genotype was not a significant predictor of plasma imatinib concentrations

Table 2 *CYP2C8* genotype differences in plasma N-desmethyl imatinib:imatinib metabolic ratio and plasma imatinib concentrations in chronic myeloid leukaemia patients

CYP2C8 genotype	Day 8 metabolic ratio		Day 8 plasma imatinib concentration		Day 22 plasma imatinib target[a] [yes:no (%)]
	Median ± SD (*n*)	Mean difference (95% CI) ($\log_e$)	Median ± SD (*n*) (μg/mL)	Mean difference (95% CI) ($\sqrt{}$ng/mL)	
*1/*1	0.22 ± 0.08 (147)	(Intercept = −1.49)	1.45 ± 0.74 (150)	(Intercept = 38.9)	122:28 (81)
*3 carrier	0.28 ± 0.08 (34)	vs. *1/*1: 0.19 (0.06 to 0.33)[##]	1.36 ± 0.98 (34)	vs. *1/*1: −1.4 (−5.6 to 2.8)	25:7 (78)
(*1/*3)	(0.27 ± 0.08 (31))		(1.35 ± 0.93 (31))		(22:7 (76))
(*3/*3)	(0.33 ± 0.11 (3))		(1.61 ± 1.28 (3))		(3:0 (100))
*1/*4	0.18 ± 0.06 (15)	vs. *1/*1: −0.24 (−0.43 to −0.04)[#]	2.18 ± 0.66 (15)	vs. *1/*1: 7.1 (1.1 to 13.1)[#]	15:0 (100)
		vs. *3: −0.43 (−0.66 to −0.21)[†††]		vs. *3: 8.5 (1.6 to 15.3)[†]	
P-value[b]		3×10^{-5}		0.01	0.04

Metabolic ratio and plasma imatinib concentration medians and SD are untransformed; mean differences are for transformed data

CI confidence interval, *SD* standard deviation

Mean difference and Tukey's post hoc [#] $P < 0.05$ and [##] $P < 0.01$ vs. *CYP2C8**1/*1 and [†] $P < 0.05$ and [†††] $P < 0.001$ vs. *CYP2C8**3 carrier

[a] Trough plasma imatinib concentration target ≥1000 ng/mL

[b] One-way ANOVA of transformed data for metabolic ratio and plasma imatinib concentration. Chi squared test for day 22 plasma imatinib concentration target

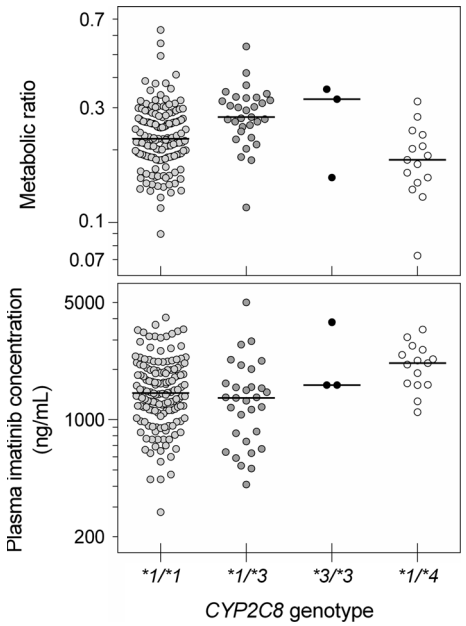

Fig. 1 *CYP2C8* genotype differences in steady-state trough total plasma N-desmethyl imatinib:imatinib concentration ratios and plasma imatinib concentrations in chronic myeloid leukaemia patients on day 8 of treatment

($P = 0.08$), and nor were day ($P = 0.6$), age ($P = 0.5$) or sex ($P = 0.2$), when analysing day 8, 22 and 90 data together. Given the disparity in results for the *CYP2C8* genotype between day 8 and multiple timepoint analyses (combined days 8, 22 and 90), an interaction between *CYP2C8* genotype and day was examined. The genotype × day interaction was not significant ($P = 0.18$); however, Fig. 3a illustrates a decrease in *CYP2C8* genotype differences over time in treatment and a significant decrease in trough plasma imatinib concentrations on day 90 among *CYP2C8**1/*4 genotype patients ($P = 0.002$ for day main effect [post hoc $P < 0.01$ day 90 vs. day 8 and day 22]), none of whom had a prescribed dose reduction. There were no significant ($P > 0.1$) day effects on plasma imatinib concentrations within *CYP2C8**1/*1 or *CYP2C8**3 carrier patients.

Of 198 patients without a dose change prior to day 22, 162 (82%) reached the day 22 plasma imatinib concentration target of >1000 ng/mL. The proportion of patients achieving this target was significantly different between *CYP2C8* genotypes (Table 2), with all *CYP2C8**1/*4 patients above 1000 ng/mL on day 22 compared with 81% for *CYP2C8**1/*1 and 78% for *CYP2C8**3 carriers.

3.4 Plasma N-Desmethyl Imatinib Concentration

Dose ($P = 7 \times 10^{-12}$) and day ($P = 2 \times 10^{-5}$), but not genotype ($P = 0.5$), showed significant main effects in linear mixed-effects analysis of day 8, 22 and 90 plasma NDIM concentrations. Day 22 plasma NDIM concentrations were significantly higher than at day 8 ($P < 1 \times 10^{-4}$) and day 90 ($P < 0.01$), with no significant difference between days 8 and 90 (see Online Resource 3, Fig. S1).

As with plasma imatinib concentrations, Fig. 3b shows a significant decrease in trough plasma NDIM concentrations on day 90 among *CYP2C8**1/*4 genotype patients ($P = 0.002$ for day main effect [post hoc $P < 0.01$ day 90 vs. day 22]).

4 Discussion

CYP2C8 makes up as little as 7% of total hepatic CYPs, and drug metabolism driven solely by the CYP2C8 enzyme is relatively uncommon [43]. Consequently, CYP2C8 metabolism and pharmacogenetics have not been focal points for much previous clinical pharmacology research. Where substrates are also metabolised by more abundant CYPs such as CYP3A4, the contribution of CYP2C8 is often discounted, and until recently this was the case for imatinib. However, recent in vitro studies [20, 31] combined with these clinical findings demonstrate that CYP2C8 and its genetic variability can still play a significant role in this context, or at least where the CYP3A4 pathway may be suppressed by mechanism-based inhibition.

Here we show that CML patients who carry the *CYP2C8**3 haplotype have significantly higher trough total plasma metabolic ratios, reflecting *CYP2C8**3 gain-of-function for imatinib N-demethylation reported in human liver microsomes [31]. The 27% increase in metabolic ratio on day 8 is an order of magnitude less than the in silico prediction (>200%) of Filppula and colleagues [20] for a genotype that doubles CYP2C8 activity, but is consistent with human liver microsome results where *CYP2C8**3 increases intrinsic clearance by only approximately 20% [31]. Our data indicated a possible *CYP2C8**3 allele–dose effect on the metabolic ratio (Table 2); however, this was not statistically significant, likely because of too few ($n = 3$) *CYP2C8**3/*3 genotype patients.

Conversely, the *CYP2C8**1/*4 genotype was associated with lower metabolic ratios, indicating reduced imatinib N-demethylation activity. Similar reduced function has been reported for other substrates in vitro and in vivo [32–35]. There are currently no corresponding published data on the in vitro metabolism of imatinib in *CYP2C8**1/*4 human liver microsomes, although the 18% reduction in metabolic ratio on day 8 was less than the in silico prediction (43% reduction) of Filppula and colleagues [20] for a genotype that halved CYP2C8 activity.

Metabolic ratios were also influenced by dose, being significantly lower at imatinib 800 mg/day than at 600 or

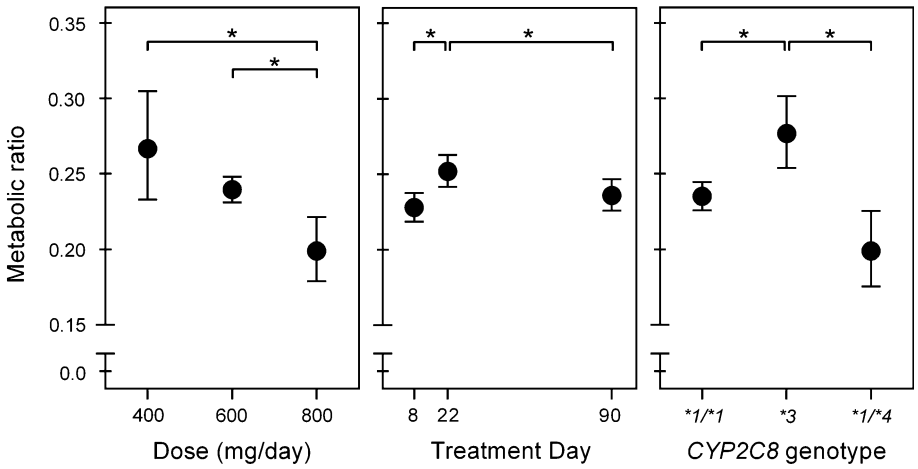

Fig. 2 Dose, day and *CYP2C8* genotype effects on trough total plasma N-desmethyl imatinib:imatinib metabolic ratio in chronic myeloid leukaemia patients. *Circles* and *bars* are geometric means and 95% confidence intervals, respectively, holding other main effects to typical values (proportional distribution). *CYP2C8**3 genotype group combines *CYP2C8**1/*3 and *3/*3 genotypes. Tukey post hoc * $P < 0.05$

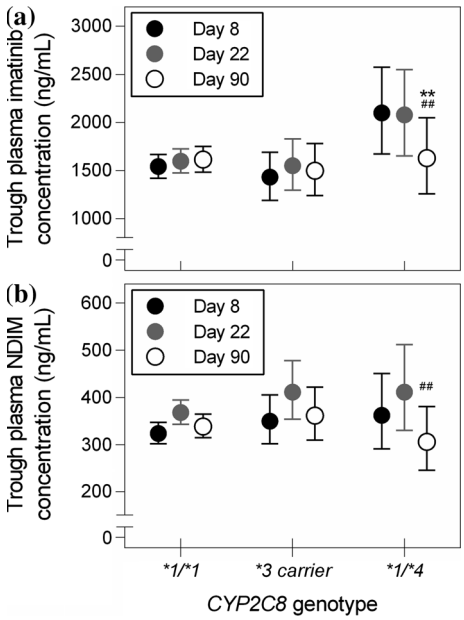

Fig. 3 *CYP2C8* genotype effects on trough total plasma imatinib (**a**) and N-desmethyl imatinib (**b**) concentrations in chronic myeloid leukaemia patients over time in treatment. *Circles* and *bars* are back-transformed means and 95% confidence intervals, respectively, holding imatinib dose to typical values (proportional distribution) [linear mixed-effects model of $\sqrt{}$(plasma imatinib concentration) or $\log_e$(plasma N-desmethyl imatinib concentration) with dose and genotype × day interaction main effects, and patient ID random effect (intercept)]. *NDIM* N-desmethyl imatinib. Tukey post hoc ** $P < 0.01$ vs. day 8 and $^{\#\#}$ $P < 0.01$ vs. day 22 for one-way ANOVA in *CYP2C8**1/*4 patients

400 mg/day, which was coupled with greater than dose-proportional increases in plasma imatinib concentrations. Whilst an interesting finding, this was not a focus of this study, and further discussion of the apparent dose effect on the metabolic ratio and consequent non-dose-proportionality is provided in Online Resource 3.

The clinical relevance of the *CYP2C8* genetic effects on imatinib metabolism is less obvious. The magnitude of *CYP2C8**1/*4 genotype effect on the day 8 plasma imatinib concentration (+50%) was similar to the within-patient difference between 400 and 600 mg/day (+52%) in this study, and the 50% increase predicted in silico for a genotype with low (half) CYP2C8 activity [20]. The *CYP2C8**1/*4 genotype effect was also of similar or greater magnitude than other gene polymorphisms previously associated with changes in plasma imatinib concentrations clinically (25–50% reported for *ABCB1*, *SLC22A1* and *ABCG2* [27, 30, 44]). In addition, there was a significant *CYP2C8* genotype effect on the likelihood of achieving day 22 target plasma imatinib concentrations of 1000 ng/mL, with all *CYP2C8**1/*4 patients reaching this threshold that has been associated with significantly improved long-term treatment outcomes [6, 7].

Alternatively, whilst the *CYP2C8**3 carriers had significantly higher metabolic ratios, they did not have significantly lower plasma imatinib concentrations. In addition, whilst *CYP2C8**3 and *4 genotype effects on the metabolic ratio appeared relatively consistent across time and the doses investigated, genotype (in particular *CYP2C8**4) differences in plasma imatinib concentrations were less apparent at later timepoints (Fig. 3) and non-significant when all timepoints were combined in linear mixed-effects analysis. Plasma imatinib concentrations are more confounded than the metabolic ratio by variability in patient adherence to the treatment regimen, dose-sample interval (e.g. due to variability in sampling time or error), and plasma protein binding (over 5-fold variability in plasma α1 acid glycoprotein concentrations within and between CML patients [45]). For example, plasma imatinib concentration results may have been confounded by increased variability in patient adherence as the amount of time in treatment progresses [46], particularly amongst *CYP2C8**1/*4 patients (Fig. 3). Whilst every effort was made to identify and limit the effects of non-adherence by excluding data based on documented non-adherence, it is difficult to

exclude occult non-adherence entirely. Based on the data presented in Fig. 3, it is speculated that significantly higher plasma imatinib concentrations in *CYP2C8**1/*4 patients could have resulted in adverse effects (not captured under the intolerance criteria of the TIDELII study [37]) leading to patient non-adherence after the major plasma imatinib concentration checkpoint on day 22 of the study. Significant decreases in both plasma imatinib and NDIM concentrations on day 90 in *CYP2C8**1/*4 patients, with no significant change in metabolic ratio (data not shown), suggest non-adherence rather than increased metabolism. However, there were no adverse effect data or other adherence markers (e.g. pill counts) available to test such a hypothesis.

Genotyping for the *CYP2C8**4 polymorphism could foreseeably aid in decision making regarding whether to start patients on the standard imatinib 400 (*CYP2C8**4) or higher 600 (wildtype *CYP2C8*) mg/day dose, if the hypothesis regarding adverse effects and non-adherence among *CYP2C8**1/*4 patients at 600 mg/day can be proven. No *CYP2C8**4/*4 patients were identified in this study, and so it is unknown whether this genotype would lead to more significant differences in metabolism and plasma imatinib exposure (and possibly adverse events). However, such patients would be rare in Caucasian (<1 in 150 patients), and rarer still in non-Caucasian ($\leq$1 in 2500 patients), patient populations [47].

This study intentionally focused on the novel investigation of the *CYP2C8* genotype; however, other potential contributors to variability in imatinib metabolism and pharmacokinetics are acknowledged. In addition to the aforementioned transporter genetics, variability in plasma protein binding, and thus the imatinib and NDIM unbound fraction, will influence total plasma concentrations and the metabolic ratio (unpublished unbound plasma imatinib and NDIM concentration data from a subset of TIDELII patients indicate *CYP2C8* genotype effects on metabolic ratio and total plasma imatinib concentrations are similar after adjusting for unbound fraction). The relative and combined contributions, and thus importance, of *CYP2C8* genotype (or CYP2C8 function more generally), transporter genetics and plasma protein binding to variability in imatinib pharmacokinetics in CML patients remain to be determined. Whether a pharmacogenetic approach to individualised imatinib dosing might be complementary or redundant in the context of potential therapeutic drug monitoring/target concentration intervention should also be considered [6].

5 Conclusion

This study shows that *CYP2C8**3 and *4 genotypes significantly alter imatinib metabolism clinically and confirms a role for CYP2C8 in imatinib pharmacokinetics, in addition to increasing our limited knowledge of *CYP2C8* clinical pharmacogenetics per se. Future prospective studies would need to be specifically designed and sufficiently powered [48, 49] to test whether personalising imatinib treatment based on transporter and metabolism genetics and plasma protein binding can improve imatinib treatment outcomes.

Compliance with Ethical Standards

Funding This study was funded by the School of Medical Sciences (now Adelaide Medical School), University of Adelaide and a Royal Adelaide Hospital Research Foundation Clinical Project Grant.

Conflicts of Interest DLW received research funding and honoraria from Novartis and BMS. TPH acted on the advisory board of, and received research funding and honoraria from, Novartis, BMS and Ariad. DTY received research funding and honoraria from, Novartis, BMS and Ariad. DTB, HKC, AM and AAS have no conflicts of interest to declare.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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