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ORIGINAL ARTICLE

Influence of single-nucleotide polymorphisms on deferasirox C_{trough} levels and effectiveness

J Cusato[1,4], S Allegra[1,4], D Massano[2], S De Francia[3], A Piga[2] and A D'Avolio[1]

Deferasirox (DFX) is the only once-daily oral chelator for iron overload and its pharmacokinetic has been related with response to therapy. Our aim was to evaluate DFX plasma concentrations according to single-nucleotide polymorphisms in genes involved in its metabolism (*UGT1A1*, *UGT1A3*, *CYP1A1*, *CYP1A2* and *CYP2D6*) and elimination (*MRP2* and *BCRP1*). Further aim was to define a plasma concentration cutoff value predicting an adequate response to therapy. Plasma concentrations were determined at the end of dosing interval (C_{trough}) using an high-performance liquid chromatography–ultraviolet method. Allelic discrimination was performed by real-time PCR. C_{trough} levels were influenced by *UGT1A1C>T* rs887829, *CYP1A1C>A* rs2606345, *CYP1A2A>C* rs762551, *CYP1A2C>T* rs2470890 and *MRP2G>A* rs2273697 polymorphisms. A DFX plasma efficacy cutoff value of 20 000 ng ml^{-1} was identified; *CYP1A1C>A* rs2606345 AA and *CYP1A2C>T* rs2470890 TT genotypes may predict this value, suggesting a negative predictive role in therapy efficacy. Our data suggest the feasibility of a pharmacogenetic-based DFX dose personalization.

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INTRODUCTION

Deferasirox (DFX, ICL670, Exjade; Novartis Pharma AG, Basel, Switzerland) is a once-daily oral iron chelator for therapy of blood transfusion-related iron overload in patients above 2 years old.[1–4] DFX dose between 20 and 30 mg kg^{-1} per day generally produces a net negative iron balance;[1] however, a recent retrospective study demonstrated that doses of DFX > 30 mg kg^{-1} per day are safe and more effective in reducing the iron burden.[5] The current maximum Food and Drug Administration-approved dose of DFX has been recently increased to 40 mg kg^{-1} per day in the United States.[6]

Major side effects that may occur after continuous DFX treatment include renal and hepatic failure, gastrointestinal hemorrhage and bone marrow suppression.[7–9]

DFX is rapidly absorbed with a bioavailability of ~70% and its peak plasma concentrations and radioactivity (metabolites) are reached between 1.5 to 4 h.[8] Serum drug concentration is proportional to administered dose[10,11] and mean half-life is ~11–19 h, supporting a once-daily oral dosing regimen.[2,11] DFX is present in blood circulation, mainly in the uncharged and iron complex form. This drug highly binds to plasma proteins (>99%) and its volume of distribution is moderate.[4,12,13]

DFX is mainly metabolized in the liver (glucuronidation) and eliminated through hepatobiliary excretion in feces.[1,3,4,12,13] UDP-glucuronyltransferase 1A1 (UGT1A1) is the main UGT isoform responsible for DFX glucuronidation;[14–18] *in vitro* studies showed that the enzyme system involved in DFX metabolism is the cytochrome-P450, CYP1A1, CYP1A2 and, to a lesser extent, CYP2D6 enzymes.[4] DFX and metabolites are mostly excreted in bile through multidrug resistance protein 2 (MRP2, also known as ABCC2).[4] Breast cancer resistance protein (BCRP1, also known as ABCG2) may influence drug toxicity;[14] many studies confirmed that BCRP expression in different cell types confers resistance to a variety of anticancer agents and reduces drug accumulation in cells. Thus, BCRP could be the cause of drug resistance for certain types of chemotherapeutic agents, including mitoxantrone and topotecan. As BCRP is expressed in different organs involved in absorption, distribution and elimination of drugs, it has a role in drug disposition.

Patients treated with DFX, especially those heavily iron loaded, do not achieve adequate iron chelation and a negative iron balance, even when receiving DFX doses exceeding 30 mg kg^{-1} per day (poor responders). Others may experience DFX-related adverse events at the dose required to maintain the iron burden balance (intolerant patients). If adverse events are managed by decreasing the dose of DFX or interrupting treatment, these patients will not be able to achieve adequate iron chelation and maintain a negative iron balance during their regular blood transfusions. Therefore, a high interindividual variability of DFX exposure may occur, leading to inadequate chelation treatment or to a toxicity increase. Even if some DFX adverse events may be dose dependent,[1] related to levels of circulating drug achieved, in order to discriminate better among the potential causes about treatment failure, pharmacogenetic analyses are absolutely required.

To date, there are only two DFX pharmacogenetic studies; Chirnomas *et al.*[6] evaluated the association of single-nucleotide polymorphisms (SNPs), as *UGT1A1*, *UGT1A3*, *BCRP* and *MRP2*, with clinical outcome in 15 patients, reporting no statistically significant results. Lee *et al.*[17] analyzed some SNPs of *UGT1A1*, *UGT1A3*, *UGT1A7*, *UGT1A9*, *MRP2* and *BCRP*, reporting an increase of

[1]Laboratory of Clinical, Pharmacology and Pharmacogenetics, Unit of Infectious Diseases, Department of Medical Sciences, Amedeo di Savoia Hospital, University of Turin, Turin, Italy; [2]Centre for Microcitemie, Department of Paediatrics, S. Luigi Gonzaga Hospital, University of Turin, Turin, Italy and [3]Department of Biological and Clinical Sciences, S. Luigi Gonzaga Hospital, University of Turin, Turin, Italy. Correspondence: Dr A D'Avolio, Laboratory of Clinical, Pharmacology and Pharmacogenetics, Unit of Infectious Diseases, Department of Medical Sciences, Amedeo di Savoia Hospital, University of Torino, Corso Svizzera, Turin 164-10149, Italy.
E-mail: antonio.davolio@unito.it
[4]These authors contributed equally to this work.
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hepatotoxicity and nephrotoxicity in association with *MRP2* haplotype and *UGT1A1*6* genotype.

In this work, our first aim was to retrospectively evaluate the influence of some SNPs of genes involved in DFX metabolism and transport, and their correlation with drug plasma exposure, in a cohort of β-thalassemic adult patients.

A high interindividual variability of DFX exposure[16] has been reported in literature; further aim of our work was to define a plasma concentration cutoff value predicting an adequate response to therapy, in order to be able to discriminate clearly poor and good responders.

SUBJECTS AND METHODS

Patients and inclusion criteria

We performed a monocentric cohort study in β-thalassemic adult patients treated at the Microcitemia Center of San Luigi Gonzaga Hospital in Orbassano (Turin, Italy) between September 2011 and September 2012. Inclusion criteria were as follows: β-thalassemia disease with transfusional iron overload, age above 18 years and treated with DFX for at least 6 months with a self-reported adherence of 90%. Patients enrolled were monitored in the period between two consecutive determinations (about 9 months) of liver iron concentration (LIC) by biomagnetical susceptometry (SQUID).[19,20] Study protocol ('Studio dei determinanti farmacogenetici nella farmacocinetica e nella risposta clinica del deferasirox', registration number 79/2012) was approved by the local ethics committee. A written informed consent for the study was obtained from each enrolled subject. Patient cohort has been sorted into responders and non-responders after enrollment.

For all patients, the following data were available: demographic data as gender, age, weight and height; HIV/HCV infections, LIC at baseline and at the end of follow-up period, transaminases, γ-glutamyl transpeptidase, serum creatinine, serum ferritin, direct and indirect bilirubin levels, and proteinuria/creatinuria ratio.

Both Chirnomas and Galanello effectiveness definitions were used. Chirnomas *et al.*[6] define as inadequate responders patients with a rising ferritin trend over 3 consecutive months, at least one higher than 1500 ng ml^{-1}, or a rising liver iron documented by biopsy or change in T2* or Ferriscan magnetic resonance imaging and on a dose of >30 mg kg^{-1} per day of DFX; instead, adequate responders have a ferritin trend below 1000 ng ml^{-1} (evaluating the interval between the LIC at the beginning and at the end of the study) documented declining liver iron burden by magnetic resonance imaging or biopsy and an administration of 30 mg kg^{-1} per day or less of DFX.[6] Galanello *et al.*[21] qualify good responders as patients with a negative net iron balance and non-responders as a positive net iron balance ones. Net iron balance (total body iron excretion) was calculated based on the amount of red blood cell transfused (iron intake in mg = Kin) and on the changes in total body iron from baseline to study end: net iron balance = (Kin+[Us (t0)–Us (t)])/($t - t$0). Iron intake (mg of iron) was calculated as Kin = (total amount of RBCs transfused) × 1.08. The total amount of RBCs transfused was calculated as the total amount of blood (ml) multiplied by the hematocrit of each unit (%) divided by 100. Complete data sets (volume and hematocrit) were available for all transfusions in three quarters of the patients. If an individual hematocrit was missing, the average hematocrit of the blood given as transfusions at the respective center was used, and if this was not available the value was assumed to be 65%. If the amount of blood transfused was given only as units, instead of in ml with the hematocrit, the volume of RBC was assumed to be 185 ml, and thus 200 mg of iron was assumed to be given per unit. Us(t) is the total body iron extrapolated from the LIC (in mg Fe/g dw) at time t (t0 = 0, for baseline measurement): Us(t) = 10.6 × LIC × (body weight in kg). Both the iron intake Kin and the changes in total body iron Us(to) − Us(t) are expressed in mg of iron; therefore, the net body iron balance is expressed in mg iron per day.[21]

Pharmacokinetic analysis

Plasma DFX concentrations were determined from samples obtained at the end of dosing interval (C_{trough}, 24 ± 3 h) at steady state. Patient samples (collected in the lithium-heparin tube, 5 ml) were centrifuged at 1500 r.p.m. for 10 min at 4 °C within 30 min from blood sampling and plasma was stored in criovials at − 20 °C before the analysis. DFX concentrations were determined using a high-performance liquid chromatography system coupled with an ultraviolet determination, according to the method described and fully validated in a previous study.[16]

Pharmacogenetic analysis

A venous blood sample was obtained from each patient (3 ml EDTA), together with the sampling for pharmacokinetic analyses. Whole blood was stored at − 80 °C and DNA extracted using the Qlamp DNA Mini Kit (Qiagen, Valencia, CA, USA). The purified and elute DNA was directly used for the PCR real-time (Biorad, Milano, Italia) reaction. The allelic discrimination analysis was performed using the TaqMan assays (Applied Biosystems, Foster City, CA, USA).

The analyzed polymorphisms were *UGT1A1 G>A* (rs4148323, UGT1A1*6), *UGT1A1 A>G* (rs3806596), *UGT1A1 C>T* (rs887829), *UGT1A3 C>T* (rs1983023), *CYP1A1 C>A* (rs2606345), *CYP1A1 T>C* (rs4646903), *CYP1A2 A>C* (rs762551), *CYP1A2 C>T* (rs2470890), *CYP2D6 C>G* (rs1135840), *MRP2 G>A* (rs2273697), *BCRP1 G>A* (rs2231142) and *BCRP1 T>C* (rs13120400).

Statistical analysis

All the variables were tested for normality with the Shapiro–Wilk test. The correspondence of each parameter was evaluated with a normal or non-normal distribution, through the Kolmogorov–Smirnov test. Non-normal variables were described by median values (plasma concentration).

All the polymorphisms were tested for Hardy–Weinberg equilibrium by the χ^2-test, in order to determine the observed genotype frequencies. Linkage disequilibrium was evaluated with Haploview 4.2 software (Broad Institute of MIT and Harvard, Cambridge, MA, USA). Correlation between

Table 1. Demographic and clinical characteristics of patients

Characteristics	
Number of patients, *n*	55
Male n,%	28, 50.9
Median age, years (IQR)	34.21 (25.11–37.24)
Median BMI, kg m^{-2} (IQR)	20.93 (19.78–22.83)
Caucasian *n* %	54, 98.20
HCV infection n %	
Absent	17, 30.90
Past	20, 36.40
Present	18, 32.70
Metavir score n %	
F0	19, 34.50
F1	23, 41.80
F2	3, 5.50
F3	10, 18.20
F4	0, 0
Median serum ferritin, ng ml^{-1} (IQR)	1303.50 (625.50–2456)
Median ALT, mU ml^{-1} (IQR)	18 (15–58)
Median AST, mU ml^{-1} (IQR)	19.50 (15–39.50)
Median GGT, mU ml^{-1} (IQR)	14 (11–21.50)
Median creatinine, mg dL^{-1} (IQR)	0.69 (0.63–0.82)
Median GFR, ml min^{-1} (IQR)	99.48 (82.51–130.95)
Median dose DFX mg per day (IQR)	1500 (1250–2000)
Median dose DFX mg kg^{-1} day (IQR)	29.62 (21.93–30.53)
Median ΔLic, mg (IQR)	2.08 (0.65–5.10)
Outcome according to Chirnomas et al.[6] n%	
Responders	16, 29.6
Non-responders	38, 70.4
Outcome according to Lee et al.[17] *n %*	
Responders	26, 48.1
Non-responders	28, 51.9
Median DFX plasma levels, ng ml^{-1} kg^{-1} (IQR)	420 (171–989)

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DFX, deferasirox; IQR, interquartile range; GFR, glomerular filtration rate; GGT, Gamma-glutamyl transferase.

Table 2. Genotype frequencies of different SNPs and their effect on DFX C_{trough} levels

Genotypes	*rs*	N	*DFX/Kg (ng ml^{-1} kg^{-1})*	P-*value*	*Genotypes combined*	N	*DFX/Kg (ng ml^{-1} kg^{-1})*	P-*value*
UGT1A1								
G > A	rs4148323							
GG		54	434.7872		ne	ne	ne	ne
A > G	rs3806596							
AA		12	534.8726	0.481	ne	ne	ne	ne
AG		30	411.4781					
GG		12	651.2071					
C > T	rs887829							
CC		23	331.492	0.132	CC/CT	48	441.4781	**0.045**
CT		25	449.5248		TT	6	1306.1392	
TT		6	1306.1392					
UGT1A3								
C > T	rs1983023							
CC		10	1251.8893	0.181	CC	10	1251.8893	0.091
CT		28	434.4605		CT/TT	44	441.4781	
TT		16	341.849					
CYP1A1								
C > A	rs2606345							
CC		5	1016.5163	0.051	CC/CA	29	644.67	**0.017**
CA		24	608.5771		AA	25	317.06	
AA		25	317.06					
T > C	rs4646903							
TT		44	411.4781	0.086	ne	ne	ne	ne
TC		10	10898.12					
CYP1A2								
A > C	rs762551							
AA		27	317.06	**0.040**	AA	27	317.06	**0.014**
AC		25	648.6768		AC/CC	27	648.6768	
CC		2	577.8036					
C > T	rs2470890							
CC		4	718.2829	**0.015**	CC/CT	31	648.6768	**0.004**
CT		27	648.6768		TT	23	265.6828	
TT		23	265.6828					
CYP2D6								
C > G	rs1135840							
CC		8	498.6291	0.664	ne	ne	ne	ne
CG		23	331.492					
GG		23	420.0909					
MRP2								
G > A	rs2273697							
GG		34	318.487	**0.032**	ne	ne	ne	ne
GA		20	632.9945					
BCRP1								
G > A	rs2231142							
GG		44	533.2513	0.338	ne	ne	ne	ne
GA		10	322.3368					
T > C	rs13120400							
TT		26	341.849	0.406	ne	ne	ne	ne
TC		21	449.5248					
CC		7	648.6768					

Abbreviations: DFX, deferasirox; ne, not evaluated because not significant or without trend; SNP, single nucleotide polymorphism. Significant values are in bold.

continuous variables was performed by parametric and non-parametric test (Pearson and Spearman), considering the level of statistical significance (*P*-value) < 0,05. Moreover, Kruskal–Wallis and Mann–Whitney tests have been used to compare plasma concentration and SNPs.

Receiver operating characteristic curve has been used to calculate cutoff values of DFX.

Any predictive power of variables considered was finally evaluated through univariate and multivariate logistic regression analyses. Factors with *P*-value < 0.2 in univariate analysis were considered in multivariate analysis. Furthermore, for the univariate analyses, *P*-value were corrected according to Benjamini–Hochberg rules when appropriate. All tests were performed with IBM SPSS Statistics 20.0 per Windows (Chicago, IL, USA).

RESULTS

Study population

Fifty-four patients were included: 27 (50%) were male and 53 (98.15%) Caucasians. Median age was 34.27 years (interquartile range, IQR, 26.36–37.39 years) and median body mass index was

22.25 kg m^{-2} (IQR, 20.24–23.75 kg m^{-2}). Median DFX dose was 29.57 mg kg^{-1} per day (IQR, 22.58–31.40 mg kg^{-1} per day). Median plasma DFX concentration was 12.37 ng ml^{-1} (IQR, 5.74–26.99 ng ml^{-1}) and this was normalized per kilogram, to allow the correlation with pharmacogenetic data. Patients characteristics were resumed in Table 1.

Effect of *UGT1A1*, *UGT1A3*, *CYP1A1*, *CYP1A2*, *CYP2D6*, *MRP2* and *BCRP1* on plasma DFX concentration (C_{trough})

All the studied polymorphisms were in Hardy–Weinberg equilibrium and in accordance to the expected frequencies; their associations with DFX C_{trough} were resumed in Table 2. Linkage disequilibrium among the 12 SNPs in the studied population was shown in Figure 1.

UGT1A1 C>T rs887829 SNP was not associated with DFX C_{trough} if we considered the three genotypes: patients with CC, CT and TT genotypes showed, respectively, 331.49, 449.53 and 1306.14 ng ml^{-1} kg^{-1} concentrations ($P=0.132$); instead, considering patients carrier of C allele, median levels for CC/CT and TT patients were 441.48 and 1306.14 ng ml^{-1} kg^{-1}, respectively, with a statistically significant *P*-value of 0.045 (Figure 2).

CYP1A1 C>A rs2606345 polymorphism presented borderline association with DFX: patients with CC, CA and AA showed, respectively, 1016.52, 608.58 and 317.06 ng ml^{-1} kg^{-1} ($P=0.051$). Considering patients carrier of C allele, CC/CA genotypes had higher DFX levels than the AA one: median concentration of CC/CA group and AA genotype were 644.67 and 317.06 ng ml^{-1} kg^{-1}, respectively, showing statistical significance ($P=0.017$).

CYP1A2 SNPs were associated with DFX C_{trough}: patients with AA, AC and CC for rs762551 A>C one showed, respectively, 317.06, 648.68 and 577.80 ng ml^{-1} kg^{-1} ($P=0.040$), and patients with CC, CT and TT for rs2470890 C>T one had 718.28, 648.68 and 265.68 ng ml^{-1} kg^{-1}, respectively, ($P=0.015$). Considering patients carrier of C allele, AC/CC for rs762551 and CC/CT genotypes for rs2470890 showed median levels of 317.06 and 648.68 ng ml^{-1} kg^{-1} ($P=0.014$) for rs762551 AA and AC/CC, respectively, and 648.68 and 265.68 ng ml^{-1} kg^{-1} ($P=0.004$) for rs2470890 CC/CT and TT, respectively (Figure 3).

Patients with GG and GA genotypes for *MRP2 G>A* rs2273697 had 318.49 and 632.99 ng ml^{-1} kg^{-1} drug concentrations, respectively, ($P=0.032$) (Figure 4).

Other SNPs analyzed were not significantly associated with DFX exposure.

Receiver operating characteristic curve analysis

Using receiver operating characteristic curve analysis, a DFX plasma concentration cutoff value of ~20 000 ng ml^{-1} (sensitivity = 50.0% and specificity = 79.0%, $P=0.008$) was found to be associated with therapy effectiveness on the basis of Chirnomas definition. This cutoff value was confirmed as the same for Galanello efficacy definition as well (sensitivity = 50.0% and specificity = 88.5%, $P=0.038$).

Univariate and multivariate regression analyses of DFX concentration

Univariate logistic regression analysis was performed to evaluate the effect of age, gender, body mass index and genetic factors on DFX C_{trough} efficacy cutoff value of 20 000 ng ml^{-1} (Table 3). Stepwise forward regression analysis was used to identify the minimum set of independent predictive variables of the cutoff effectiveness and estimate the contribution of each factor to pharmacokinetic variability; only *CYP1A1* AA (rs2606345) and *CYP1A2* TT (rs2470890) genotypes remained in the final model with *P*-value of $P=0.017$ and $P=0.037$, respectively.

We replicated Mann–Whitney test without correction per kilogram of plasma concentrations to confirm the efficacy of these genotypes to predict values above 20 000 ng ml^{-1}: *P*-values were $P=0.035$ for AA genotype of *CYP1A1 C>A* rs2606345 and $P=0.002$ for TT one of *CYP1A2 C>T* rs2470890 (Figure 5).

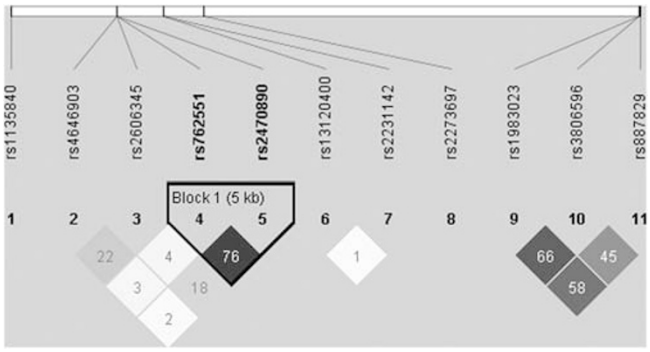

Figure 1. Linkage disequilibrium among *UGT1A1*, *UGT1A3*, *CYP1A1*, *CYP1A2*, *CYP2D6*, *MRP2* and *BCRP1* gene single-nucleotide polymorphisms (SNPs). The panel shows linkage disequilibrium of the studied population among the 12 SNPs. Each value in the box represents r^2 calculated by the Haploview 4.2 software. Dark grey, regions with high r^2 values; light grey, regions with low r^2 values.

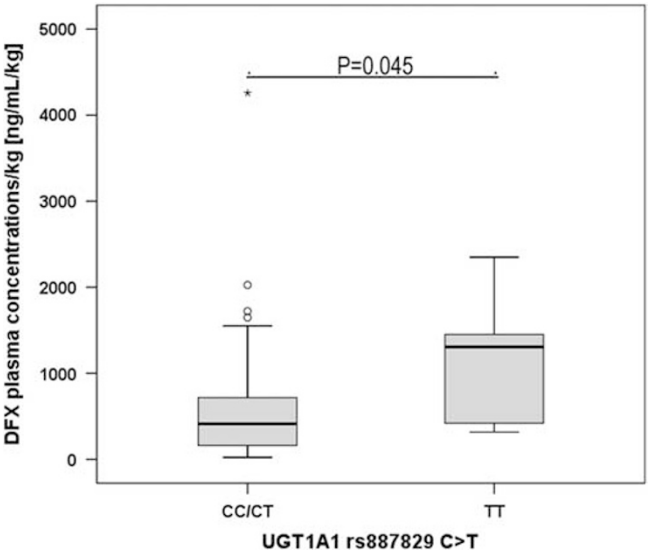

Figure 2. Influence of *UGT1A1* rs887829 C>T polymorphism on deferasirox C_{trough} levels. Box plot of *UGT1A1C>T* rs887829 SNP influence on plasma deferasirox concentration at 24 h after dosing; boxes and black lines in boxes represent, respectively, interquartile ranges (IQR) and median values; open dots and stars represent outlier values. Median values (horizontal line), IQR (bars), patient values (black square), highest and lowest value (whiskers), and *P*-value are shown. CC/CT ($n=48$) and TT ($n=6$).

DISCUSSION

In thalassemic patients, regular red blood cell transfusion can lead to iron overload. In the absence of physiological mechanisms to excrete exceding iron, the administration of an iron-chelation therapy is necessary.[22–24]

The first iron chelator available for the iron overload treatment, deferoxamine, has short half-life (~20 min) and patients need a subcutaneous 8–12 h infusion, 5–7 times a week. Moreover, this drug leads to many side effects, such as local reaction at the site of

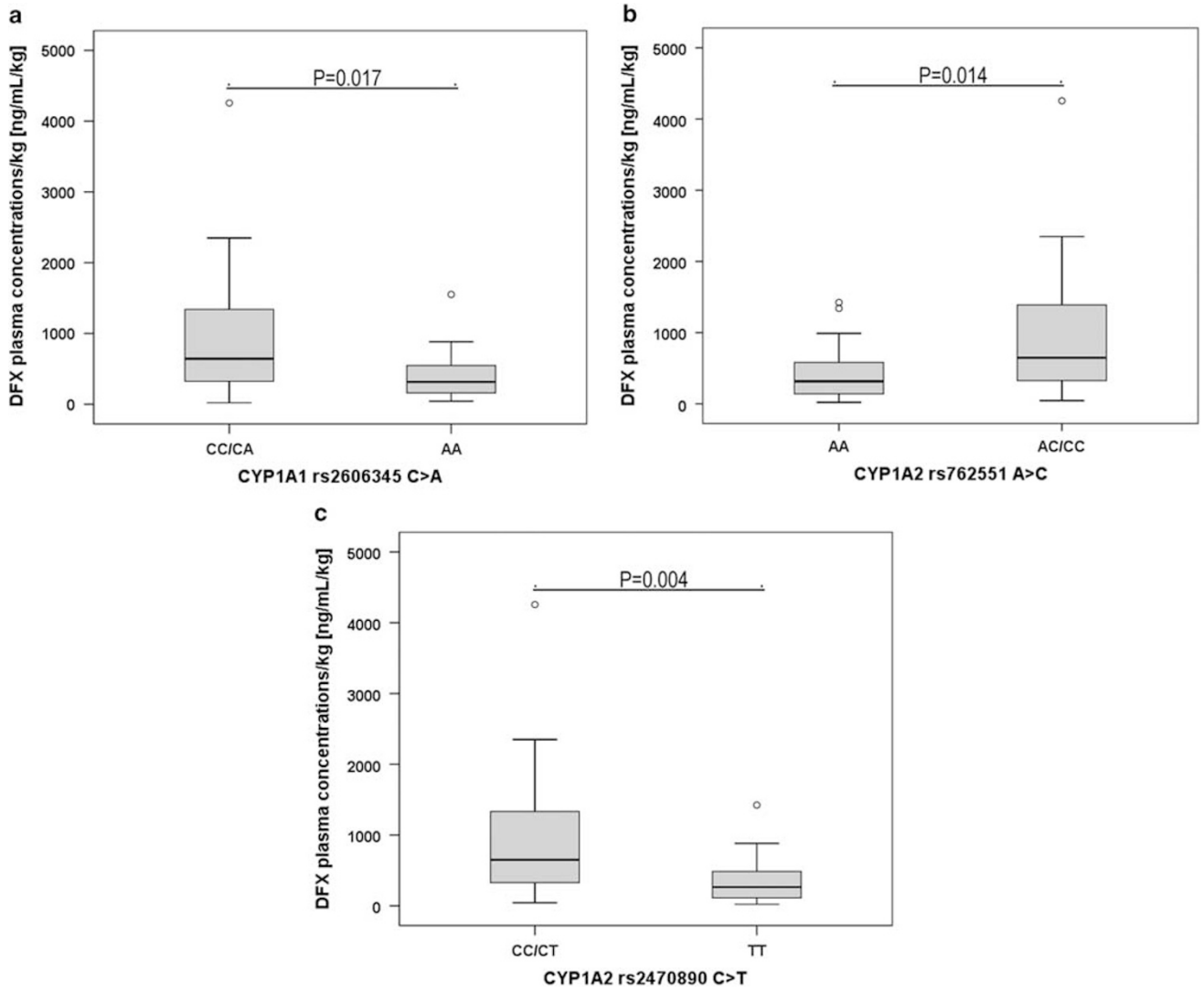

Figure 3. Influence of *CYP1A1C*>A rs2606345, *CYP1A2A*>C rs762551 and *CYP1A2C*>T rs2470890 polymorphisms on deferasirox C_{trough} levels. Box plots of *CYP1A1C*>A rs2606345 (**a**), *CYP1A2A*>C rs762551 (**b**), *CYP1A2C*>T rs2470890 and (**c**) SNPs influences on plasma deferasirox concentration at 24 h after dosing. (**a**) CC/CA ($n=29$) and AA ($n=25$). (**b**) AA ($n=27$) and AC/CC ($n=27$). (**c**) CC/CT ($n=31$) and TT ($n=23$).

infusion, hypoacusia, ocular toxicity, retarded growth and skeletal changes.[25,26] The first oral iron chelator used in clinical practice is deferiprone, with a short half-life of ~45–60 min; thus, it requires at least three-times-per-day administrations. Rare, but severe, agranulocytosis, mild neutropenia, abdominal discomfort and erosive arthritis are reported side effects.[25,27,28]

The urgent need for an effective and safe once-daily orally administered iron chelator leads to the expedited approval of DFX.[1,18] For its clinical and pharmacokinetics properties, DFX is considered the most suitable to be an 'ideal chelator';[29] it has a relatively good toxicity profile, which includes only transient skin rashes and gastrointestinal upset.[30]

However, De Francia *et al.*[16] showed an high interindividual variability in plasma levels, confirmed in our cohort: median DFX plasma levels was 420 ng ml^{-1} kg^{-1} (IQR 171–989 ng ml^{-1} kg^{-1}). Daar *et al.*[31] showed an inverse correlation between labile plasma iron, target of the drug and DFX C_{trough}. This suggests the role of DFX C_{trough} in predicting the therapeutic response.[16,31]

As previously reported, genetic factors may have a sustained role in drug metabolism and transport.[4,6,17] We therefore investigated the involvement of some SNPs with DFX C_{trough} levels as a potential therapeutic tool to plan DFX therapy at the dose based on genotype. Pharmacogenetics aim is the 'personalized treatment': maximize therapy efficacy and therapeutic failure. It could be integrated and it completes the therapeutic drug monitoring information to better clarify the single patient condition. These issues were frequently used integrated in HIV-therapy (efavirenz therapeutic drug monitoring and *CYP-2B6* SNPs, for example) and in HCV therapy (ribavirin therapeutic drug monitoring and *IL-28B* and/or *ITPA* SNPs).

Moreover, the causality between interindividual variable response to treatment and interindividual variable exposure of DFX could be due to genetic factors.

Our results showed that *UGT1A1 C*>*T* rs887829 ($P=0.045$), *CYP1A1 C*>*A* rs2606345 ($P=0.017$), *CYP1A2 A*>*C* rs762551 ($P=0.014$), *CYP1A2 C*>*T* rs2470890 ($P=0.004$) and *MRP2 G*>*A* rs2273697 ($P=0.032$) affected DFX C_{trough} levels.

UGT1A1 C>*T* rs887829 SNP is located in the core promoter region of the *UGT1A1* gene.[32] Several studies reported an association between this SNP and bilirubin levels.[33–35] Our

study demonstrated a correlation between this SNP and DFX C_{trough}: CC and CT genotypes had lower concentrations (441.48 ng ml^{-1} kg^{-1}) than the TT one (1306.14 ng ml^{-1}) ($P = 0.045$) (Figure 2).

The intron located *CYP1A1 C>A* rs2606345 polymorphism determines enhanced gene expression in the presence of specific substrates (C allele) or in their absence (A allele).[36,37] A allele was associated with an increased risk of seizures in epileptic woman, but not in man, treated with carbamazepine, phenobarbital, phenytoin or valproic acid, compared with the C allele.[38] The smoking excess odds ratio per pack-years in current smokers compared with never smokers was directly proportional to the number of cigarettes per day, with a plateau for subjects carrying the CC alleles and an exponentially increase for those with CA and AA genotypes.[36] This suggested a gain of function in AA carriers, and hence lower concentrations, all confirmed in our population: in fact, CC and CA genotypes had higher DFX C_{trough} of 644.67 ng ml^{-1} kg^{-1} compared with AA one, which had 317.06 ng ml^{-1} kg^{-1} ($P = 0.017$) (Figure 3a).

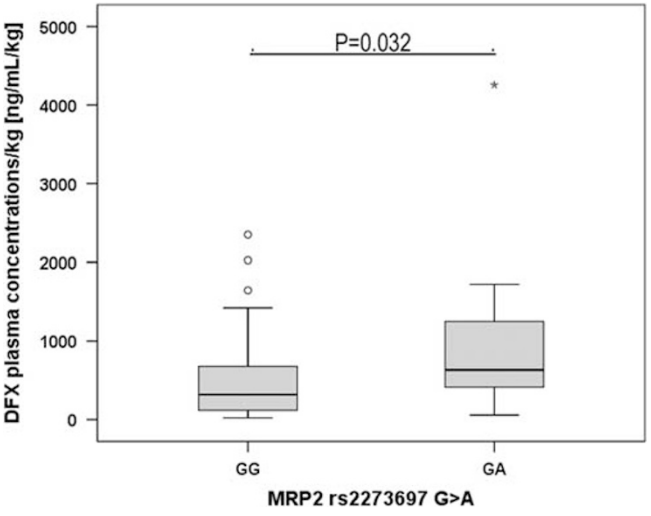

Figure 4. Influence of *MRP2G>A* rs2273697 polymorphism on deferasirox C_{trough} levels. Box plot of *MRP2G>A* rs2273697 polymorphism influence on plasma deferasirox concentration at 24 h after dosing. GG ($n = 34$) and GA ($n = 20$).

CYP1A2 A>C rs762551 polymorphism is located in the intron 1 of *CYP1A2* gene[39,40] and is related with reduced enzyme activity.[41,42] Several papers reported the influence of this SNP on response and side effects with antipsychotic drugs use: the A allele leading to lower plasma drug concentration and higher risk for non-response; the presence of C allele causes an increase of plasma exposure and risk for adverse effects.[43–47] Also in our study, patients with AA genotype had lower drug concentrations (317.06 ng ml^{-1} kg^{-1}) compared with AC and CC ones (648.68 ng ml^{-1} kg^{-1}) ($P = 0.014$) (Figure 3b).

For *CYP1A2 C>T* rs2470890 exonic variant, patients with TT genotype had a stronger coffee-Parkinson disease inverse correlation than patients with CC and CT genotypes, but the biological significance of this synonymous variant is not actually known.[48] rs2470890 determines a synonymous substitution affecting mRNA stability.[49] In our population, CC and CT genotypes had higher DFX C_{trough} (648.68 ng ml^{-1} kg^{-1}) compared with TT one (265.68 ng ml^{-1} kg^{-1}) ($P = 0.004$) (Figure 3c).

MRP2 G>A rs2273697 non-synonymous variant was associated with a reduction in carbamazepine transport rate across cell membrane in Corean epileptic patients and, in Zimbabwe, high-exposed miners who were A variant carriers had lower Hg urinary concentrations than those with no copies of the A allele.[50,51] These studies suggested that the A allele determines a reduced enzyme function, resulting in a lower transport activity. In our study, patients with GG genotype had lower drug concentrations (318.49 ng ml^{-1} kg^{-1}) than the GA one (633.00 ng ml^{-1} kg^{-1}) ($P = 0.032$) (Figure 4).

Our second aim was to identify a DFX C_{trough} cutoff value predicting therapeutic efficacy, based on two different definitions:[6,21] the identified value was 20 000 ng ml^{-1} for both definitions.

Finally, using this threshold value we performed regression analysis to determine which factors, not just genetics, could predict this cutoff (Table 3): both genotypes *CYP1A1 C>A* rs2606345 AA (Figure 5a) and *CYP1A2 C>T* rs2470890 TT (Figure 5b) may predict drug concentrations below 20 000 ng ml^{-1}. This observation was confirmed with data not corrected per kilogram. We reported Benjamini–Hochberg correction in Table 3, but we have not considered it in our analysis; next, it is only reported here to make the data more complete; this type of

Table 3. Factors, in univariate and multivariate analyses, able to predict DFX concentration above the cutoff toxicity of 20 000 ng/ml

	Univariate		*Corrected* P-*value*	*Multivariate*	
	P-*value*	*OR*		P-*value*	*OR*
Age >34.27 years	**0.114**	0.37 (0.11–1.27)	0.228	0.243	0.42 (0.10–1.82)
Gender	0.365	0.57 (0.17–1.92)	0.465		
BMI at baseline >22.25 kg m^{-2}	0.811	1.16 (0.35–3.89)	0.811		
UGT1A1 TT, rs887829	0.213	3 (0.53–16.89)	0.331		
UGT1A1 GG, rs3806596	0.627	1.41 (0.35–5.62)	0.732		
UGT1A3 CT/TT, rs1983023	**0.093**	0.29 (0.07–1.22)	0.326	0.968	1.04 (0.16–6.59)
CYP1A1 AA, rs2606345	**0.007**	0.11 (0.02–0.54)	0.098	**0.017**	0.13 (0.02–0.70)
CYP1A1 TT/TC, rs4646903	**0.093**	3.4 (0.82–14.15)	0.260	0.640	1.52 (0.26–8.77)
CYP1A2 AC/CC, rs762551	**0.04**	3.95 (1.07–14.65)	0.187	0.966	1.07 (0.04–25.8)
CYP1A2 TT, rs2470890	**0.014**	0.13 (0.03–0.66)	0.098	**0.037**	0.17 (0.03–0.90)
CYP 2D6 GG, rs1135840	**0.114**	2.68 (0.79–9.10)	0.266	0.768	1.26 (0.27–5.86)
MRP2 GG/GA, rs2273697	0.780	1.19 (0.35–4.04)	0.840		
BCRP1 GG/GA, rs2231142	**0.193**	0.24 (0.03–2.07)	0.338	0.201	0.21 (0.02–2.27)
BCRP1 CC, rs13120400	0.348	2.19 (0.43–11.21)	0.487		

Abbreviations: BMI, body mass index; DFX, deferasirox; OR, odds ratio. [a]Corrected (Benjamini–Hochberg–false discovery rate) *P*-value for univariate analysis.

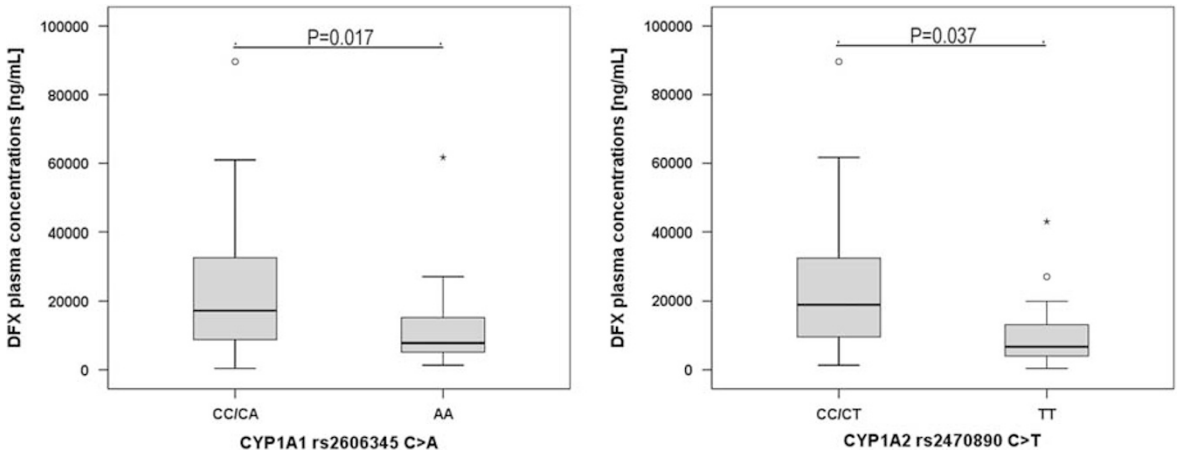

Figure 5. Influence of *CYP1A1C*>A rs2606345 and *CYP1A2C*>T rs2470890 polymorphisms on deferasirox C_{trough} levels not correct per kilogram. Box plots of *CYP1A1C*>A rs2606345 (**a**) and *CYP1A2C*>T rs2470890 (**b**) polymorphisms influences on plasma deferasirox concentration at 24 h after dosing, not corrected per kilogram. (**a**) CC/CA ($n=29$) and AA ($n=25$). (**b**) CC/CT ($n=31$) and TT ($n=23$).

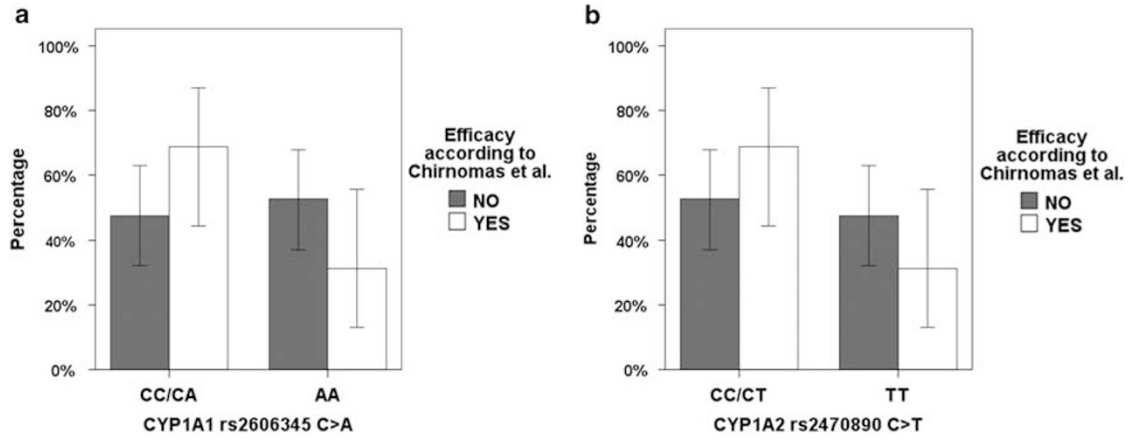

Figure 6. Frequency of non-responders and responders according to Chirnomas *et al.*[6] in the different genotypes of *CYP1A1* rs2602345 (**a**) and *CYP1A2* rs2470890 (**b**) SNPs. Gray columns indicate non-responders, white are responders; each bar represents the interval at 95% confidence level. (**a**) CC/CA non-responders ($n=18$) and responders ($n=11$); AA non-responders ($n=20$) and responders ($n=5$). (**b**) CC/CT non-responders ($n=20$) and responders ($n=11$); TT non-responders ($n=18$) and responders ($n=5$).

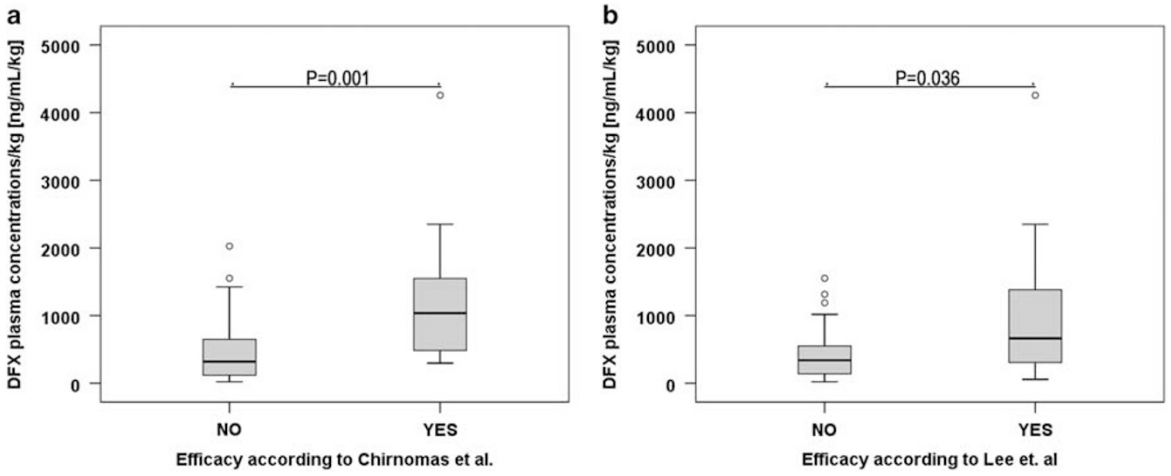

Figure 7. DFX Ctrough levels in non-responders versus responders according to Chirnomas *et al.*[6] (**a**) and Lee (**b**) efficacy definitions. (**a**). Non responders ($n=16$) and responders ($n=38$). (**b**) Non-responders ($n=26$) and responders ($n=28$).

statistical correction is still under discussion by researchers and they believe it should not be used in clinical field as yet.

Responders and non-responders patients and the different genotypes (rs2606345AA and rs2470890TT for A and B, respectively), which were expressed in each group, are shown in Figure 6, while DFX concentrations of responders versus non-responders, according to Chirnomas *et al.*[6] (A) and Lee *et al.*[17] (B) efficacies, are plotted in Figure 7.

In conclusion, these two genotypes can be considered genetically negative predictive factors of therapy efficacy.

Drug plasma concentrations are generally influenced by many factors. Then, an effect directly attributable to a single polymorphism is very difficult to highlight. When it is possible to show an influence, probably this SNP has a very important impact on drug elimination, absorption, metabolism and/or distribution. We chose to analyze SNPs of the main possible enzymes involved in DFX metabolism, those with minor impact too. We have selected SNPs before enrollment, thus before knowing patients outcome.

We chose polymorphisms that were considered important in other contests: in pharmacokinetics or in drug outcomes. Although BCRP is important for intestinal drug absorption and hepatic elimination, rs2231142 and rs13120400 SNPs are not associated with pharmacokinetics in this study; these SNPs could be related with hepatotoxicity, as other *BCRP* ones.[6,17,52,53]

In our cohort, all the patients were GG for *UGT1A1*6 G>A*; thus, statistical analysis was useful. *UGT1A1 A>G* (rs3806596) in literature is associated with hyperbilirubinemia;[6] thus, it could be associated with drug-related toxicity, as *UGT1A3 C>T* (rs1983023). *UGT1A1 G>A* (rs887829) is located in the core promoter region of the *UGT1A1* gene and probably the variation in this region could interfere with the correct one bound with the polymerase, consequently with the gene transcription; moreover, this SNP is associated with bilirubin increased concentrations.[54] *CYP1A1 T>C* (rs4646903) is associated with the highly active antiretroviral therapy efficacy; hence, we aimed to know whether it is related to DFX therapy effectiveness. *CYP2D6 C>G* (rs1135840) is not statistically significantly associated with DFX plasma levels, perhaps because it is responsible for only the 2% of DFX metabolism. *ABCC2 G>A* (rs2273697), as *CYP1A1 C>A* (rs2606345), are related to drug response in patients with epilepsy and hence we thought it was interesting to understand its involvement in DFX outcome.[55,56] *CYP1A2* A>C (rs762551) and C>T (rs2470890) are associated with paroxetine outcome and dose at week 4, respectively.[57]

Sample size is one of this study limitations; we analyzed a relative small number of patients, but it can be considered a relative large number for a rare pathology as β-thalassemia. Moreover, this study is monocentric; thus, more studies are needed to confirm our data in different cohorts.

These pharmacokinetic–pharmacogenetic data are the first obtained from adult patients and they seem to show the usefulness of pharmacogenetic-based DFX dosing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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