**Genetic Influence of *ABCG2, UGT1A1 and NR1I2* on Dolutegravir Plasma Pharmacokinetics**

Emilie R ELLIOT\*,1,2, Megan NEARY2, Laura ELSE2, Saye KHOO2, Graeme MOYLE1, Daniel F. CARR2, Xinzhu WANG3, Myra MCCLURE3, Marta BOFFITO1,3 Andrew OWEN2

*1Chelsea and Westminster Hosp, London, UK, 2University of Liverpool, Liverpool, UK, 3Imperial College, London*

*Corresponding author:*

*Dr Emilie Elliot*

*St. Stephen’s Centre – Chelsea and Westminster Hospital*

*369 Fulham road, London SW10 9NH*

*tel: +44(0)20 33156506*

*fax: +44(0)20 33155628*

*email:* *emilieelliot@doctors.co.uk*

*RUNNING TITLE: Pharmacogenomics of dolutegravir PK*

*WORD COUNT: abstract/manuscript: 217/3500, references: 55*

**ABSTRACT**

**Objectives**

Dolutegravir has replaced efavirenz as first line treatment in universal HIV guidelines. We sought to ascertain the contributory effect of single nucleotide polymorphisms (SNPs) in four key genes linked to dolutegravir disposition (*UGT1A1*, *ABCG2*, *CYP3A* and *NR1I2*) on plasma DTG pharmacokinetics.

**Methods**

Paired pharmacogenetic/pharmacokinetic data from 93 subjects were analyzed for association using multivariate linear regression.

**Results**

Co-occurring *UGT1\*28* and *NR1I2* c.63396C>T homozygosity was associated with a 79% increase in AUC0-24 (p=0.001; 27% if analysed individually) whilst combined *ABCG2* c.421C>A and *NR1I2* c.63396C>T variants were associated with a 43% increase in Cmax (p=0.002) and a 39% increase in AUC0-24 (p=0.002) respectively. When analysed individually, homozygosity for the *NR1I2* c.63396C>T variant alleles was associated with a 28% increase in Cmax (p=0.033) andhomozygosity for the *ABCG2* c.421C> variant alleles was associated with a 28% in Cmax (p=0.047). The *UGT1A1*\*28 (rs8175347) poor metabolizer status (\*28/\*28; \*28/\*37; \*37/\*37) was individually associated with a 27% increase in AUC0-24 (p=0.020). The combination of *UGT1A1*\*28 poor metaboliser and *UGT1A1*\**6* intermediate metaboliser statuses correlated with a 43% increase in AUC0-24 (p=0.023).

**Conclusion**

This study showed a pharmacogenetic association between dolutegravir pharmacokinetics and variants in the *ABCG2*, *UGT1A1* and *NRI1/2* genes, particularly when combined. Further research is warranted to confirm these associations in population-specific studies and to investigate their putative relationship with DTG pharmacodynamics.

**INTRODUCTION**

Dolutegravir is now a preferred agent in major guidelines and a drug of choice for many HIV healthcare providers worldwide.[1-3](#_ENREF_1) Importantly, it has replaced efavirenz as the preferred first-line agent in the WHO antiretroviral (ARV) guidelines and has been recommended by PEPFAR (Emergency Programme on AIDS Research) for rapid introduction in key target countries, meaning that it is a major player in the global ARV scale up.[4](#_ENREF_4) Despite a signal for a possible increased risk of neural tube defects in women who conceive on dolutegravir (0.3%), the WHO still recommends dolutegravir in women, provided that those of childbearing age are well informed and have access to reliable contraception.[4](#_ENREF_4), [5](#_ENREF_5) It is estimated that 15 million people could be taking dolutegravir by 2025, which stresses the importance of understanding how its pharmacology behaves in diverse and wide-ranging populations.[6](#_ENREF_6), [7](#_ENREF_7)

Whilst dolutegravir discontinuation rates secondary to any adverse events (AEs) were low in phase III trials (<2%),[8](#_ENREF_8) real-life data reveal unexpectedly higher frequencies (range 2.3%-13.7%), most commonly due to neuropsychiatric (NP) AEs, with a mean incidence of 3.5% (range 1.4-7.2%).[9-12](#_ENREF_9) Risk factors have been proposed in some cohorts[12-16](#_ENREF_12) but altogether disproved in others.[17-19](#_ENREF_17) Whilst a relation with dolutegravir Cmin is suggested, mechanisms of dolutegravir-related AEs, particularly neurotoxicity, are thought to be more complex than a simple linear or threshold-defined PK relationship and may relate to a combination of factors that include pharmacogenetic, immune and/or functional predispositions.[14](#_ENREF_14), [20](#_ENREF_20)

The pharmacokinetic (PK) and pharmacodynamic (PD) properties of dolutegravir have been extensively described.[21](#_ENREF_21) It is primarily metabolised via the phase II enzyme uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) and cytochrome P450 3A4 (CYP3A4; ~ 15%). It is also a substrate for the efflux transporters breast cancer resistance protein (BCRP; *ABCG2*) and P-glycoprotein (P-gp; *ABCB1*), which are found on gastrointestinal and liver epithelial cells and the blood brain barrier endothelium.[22](#_ENREF_22) Dolutegravir displays no significant CYP enzyme inhibition or induction and thus is a minor drug–drug interaction (DDI) perpetrator.[23](#_ENREF_23) Its PK inter-individual variability was moderate in pre-licencing trials (coefficient of variation, CV%, 24-26%) but was greater in subsequent studies (CV% up to 85%).[20](#_ENREF_20), [24](#_ENREF_24), [25](#_ENREF_25) Furthermore, its Summary of Product Characteristics (SPC) reports no ethnicity or gender differences in exposure[26](#_ENREF_26) but this remains to be confirmed more specifically in large, controlled and diverse populations.[23](#_ENREF_23)

Pharmacogenetics data for dolutegravir, however, are limited. To date, associations between *UGT1A1\*6* (rs4148323), *UGT1A1\*28* (rs8175347) *and ABCG2 c.*421C>A(rs2231142) variants and increased dolutegravir PK parameters have been suggested.[16](#_ENREF_16), [27-29](#_ENREF_27) An association between a variant of the OCT2 encoding gene (*SLC22A2*)and sub-clinical neuropsychiatric pharmacodynamic measurements has also been reported.[30](#_ENREF_30) Dolutegravir, though, is not a substrate of OCT2 and variants would not be expected to impact plasma concentrations.

Of additional interest, *CYP3A4\*22* (522-191C>T; rs35599367) is associated with lower CYP3A4 expression and activity within the liver,[31](#_ENREF_31) as well as increased lopinavir concentrations [32](#_ENREF_32). The *CYP3A5*\*3 (6986A>G, rs776746) variant allele, whilst not directly involved in dolutegravir metabolism, is known to be in linkage disequilibrium with *CYP3A4*\*1B and has been independently associated with higher nevirapine AUC and reduced atazanavir clearance.[33-35](#_ENREF_33) *NR1I2* encodes the pregnane X receptor (PXR), which regulates the expression and activity of several enzymes, including CYP3A4 and UGT1A1.[33](#_ENREF_33), [36](#_ENREF_36) *NR1I2* c.63396C>T (rs2472677) has been associated with the PK of unboosted atazanavir.[37](#_ENREF_37), [38](#_ENREF_38) Accordingly, the objective of this study was to investigate the role of common *UGT1A1*, *ABCG2, CYP3A and NR1I2* single nucleotide polymorphisms (SNPs) on plasma dolutegravir concentrations in pooled subject data from four clinical trials investigating the PK of 50mg dolutegravir taken once daily (OD). *ABCB1* SNPs were not selected since there are many known compensatory mechanisms for any potential *ABCB1* polymorphism related PK/PD effects.

**MATERIALS AND METHODS**

*Clinical study & participant selection*

Pooled samples from three Phase I (NCT02219217, NCT02509195 and NCT03094507) and one Phase III (NCT02351908)) clinical trials carried out at the St Stephen’s AIDS Trust clinical trial unit, London, between 2014 and 2017 were collected and saved for genetic analysis. All Phase I trials were clinical pharmacology repeat-dose studies involving intensive PK assessments. The Phase III trial included a PK sub-study involving a timed dolutegravir C24 at steady state. All studies used a 50mg dose of dolutegravir OD, taken as a tablet either alone (healthy volunteers) or co-formulated with abacavir/lamivudine in the Phase I studies and with emtracitabine/tenofovir in the Phase III study (HIV infected participants). All clinical studies are registered.

The studies selected for inclusion were conducted in accordance with good clinical practice procedures, all applicable regulatory requirements and the guiding principles of the Declaration of Helsinki. The study protocols for each clinical study were reviewed and approved by the applicable National Research Ethics Service (NRES) committees and the Medicines and Healthcare products Regulatory Agency (MHRA). Pharmacogenetic samples were collected under separate written informed consent to the main clinical study, which was optional for par­ticipants. The respective NRES committees for each study approved the pharmacogenetic sub-study for each trial as part of the main study protocol approval. No individual subject took part in more than one study.

*PK sample and data collection*

Within each of the Phase I studies, subjects underwent steady-state intensive dolutegravir plasma PK determinations, following witnessed drug intake. Blood samples were collected pre-dose, 1, 2, 3, 4, 8, 12- and 24-hours post-dose. The Phase III study involved a one-off PK sample taken 24-hour post-dose. This was carefully timed, with research staff instructing participants over the phone to take the medication the morning of dosing and with participants attending the clinical research unit the following day to allow for sampling exactly 24-hours post-dose. Overall, Medication adherence was assessed through direct questioning and pill count in all studies. Steady-state plasma concentrations were determined using high-pressure liquid chromatography–tandem mass spectrometry methods for samples from three clinical trials (HPLC-MS/MS; Bioanalytical Facility, University of Liverpool) or ultra-performance liquid chromatography coupled with UV detection for one clinical trial (UPLC; Jefferiss Trust Laboratory, Imperial College London). Both have been validated and described including accuracy data.[39](#_ENREF_39), [40](#_ENREF_40) The lower limits of quantification (LLQ) was 10 ng/mL and 80 ng/mL, respectively. For concentrations below the assay LLQ, a value of one-half of the quantification limit was used. The two methods were not crossed validated as their respective calibration ranges vary widely.

The calculated PK parameters for plasma dolutegravir in the three phase I studies were the plasma concentration measured 24 hours after the observed dose (Cmin), the maximum observed plasma concentration (Cmax), AUC0-24 and the half-life (t1/2). For the phase III study participants, only Cmin was determined. PK parameters were calculated using actual blood sampling time and non-compartmental analysis techniques (WinNonlin Phoenix; version 6.1 or above; Pharsight, Mountain View, CA).

*Pharmacogenetics sample collection, DNA extraction and genotyping*

Venous blood was collected at baseline, from subjects con­senting to pharmacogenetics research, into an EDTA vacu­tainer. Samples were then shipped on dry ice to the University of Liverpool Pharmacology Research Laboratories, UK, and stored at -80°C. Genomic DNA was extracted from whole blood using a spin-column based kit according to the manufacturer’s protocol (E.Z.N.A Blood DNA Mini Kit; Omega bio-tek; Norcross, GA). Extracted DNA was quantified using NanoDrop (ThermoFisher Scientific, Wilmington, DE). Genotyping was completed using real time allelic discrimination PCR assays on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA). Taqman Genotyping Master mix and assays *CYP3A4\*22* c.522-191C>T (rs35599367, catalogue number C\_\_59013445\_10), *CYP3A5\*3* c.6986A>G (rs776746, catalogue number C\_26201809\_30), *ABCG2* c.421C>A (rs2231142, catalogue number C\_15854163\_70), *ABCG2* c.34G>A (rs2231137, made to order), *NR1I2* c.63396C>T (rs2472677, catalogue number C\_26079845\_10), *NR1I2* c.44477A>G (rs1523130, catalogue number C\_9152783\_20) and *UGT1A1\*6* c.211G>A (rs4148323, catalogue number C\_559715\_20) were purchased from Thermofisher Scientific (Life Technologies Ltd, Paisley, Renfrewshire, UK). Opticon Monitor V3.1 software (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and identify genotypes. The *UGT1A1* promoter region (*\*1, \*28, \*36* and *\*37*) was genotyped using an Agena MassArray iPLEX assay.

*Covariates*

Subject age, gender, height, weight, ethnicity, HIV status and accompanying drug to dolutegravir were extracted from each study. Covariates were then included in the univariate and multivariate linear regressions described below.

*Statistical analysis*

In order to determine assay performance, genotypes for each marker were evaluated for compliance with Hardy–Weinberg equilibrium (p>0.05) using validated and previously outlined methods.[41](#_ENREF_41) Allele frequencies were also compared to publicly available British and European allele frequencies.[42](#_ENREF_42) Genotypes were coded for regression analyses as 0 for the homozygous common allele, 1 for the heterozygous and 2 for the homozygous variant allele. For SNPs displaying a dominant or recessive allele effect, coding was dichotomized and weighted appropriately (eg: if using a recessive genotypic test model, the homozygote common variant and the heterozygote allele were grouped into a single category coded as 0 whilst the homozygote variant was coded as 2).[43](#_ENREF_43) The SNPs selected for the study were analyzed individually. SNPs found to correlate with any PK parameter were then also combined in pairs to create scoring algorithms consisting of the sum of each genotype code.

Categorical variables were described using relative frequencies, while continuous variables were described using median and IQR. Drug PK parameters were described using Geometric Means (GM) and 95% CI. Inter-individual variability in PK parameters was expressed as a percentage coefficient of variation [CV, (standard deviation/mean)×100].

The Shapiro–Wilk test was applied to test continuous variables for normality, with *p*<0.05 considered statistically significant; variables were Log10 transformed if the normality test failed.

Associations between participant covariate characteristics or genotypes and dolutegravir concentrations were determined through univariate and multivariate linear regressions. Univariate linear regressions with a p value of <0.2 were carried through to multivariate linear regression analysis where a value of <0.05 was classed as statistically significant and checked with the Benjamini-Hochberg procedure to account for multiple comparisons, using a false positivity rate (*Q* value) of 10%. All statistical analyses were carried out using IBM SPSS Statistics v.22 (IBM, Armonk, NY). Charts were produced using GraphPad Prism 8 (GraphPad Software, La Jolla, CA).

**RESULTS**

*Participants*

One hundred participants attended the baseline visit of one of the four clinical trials. Two subjects declined participation to a genomic sub-study and 5 withdrew from their trial before PK data were collected. 93 subjects with paired pharmacogenetic and PK data were pooled for analysis (57 HIV-infected and 36 healthy volunteers; 67 men and 26 women). Subject characteristics and genotype frequencies are summarised in **table 1**. The median (IQR) age and weight were 51 years (35–64 years) and 77 kg (67-84); 71% self-described as Caucasian and 17% as Black African or Black Caribbean.

*Dolutegravir* *pharmacokinetics*

76 participants provided intensive PK data collected over 24 hours and 17 provided a single PK sample 24 hours post-dose. 53 samples were analysed using HPLC-MS/MS and 40 using UPLC. All the participants received 50mg OD dolutegravir, taken in the morning of the intensive PK day or the morning before the one-off PK measurement. Dolutegravir GM (95% CI) for Cmax, AUC0-24, Cmin and t1/2 were 3974 ng/mL (3864 – 4357), 51846 ng\*h/mL (48607- 55085), 1182 ng/mL (994 – 1371) and 13.0 hours (12.1 – 14.0). Dolutegravir PK parameters are summarised by SNP in **table S1**.

 *Dolutegravir pharmacogenetics*

All SNPs were in Hardy–Weinberg equilibrium, except for *CYP3A5\*3* c.6986A>G (rs776746; *X*2 = 33.36; p=0.001) and *CYP3A4\*22* c.522-191C>T (rs35599367; *X*2 = 33.13; p=0.001), which may compromise their interpretation (although both still mirrored European genotype distribution). Genomic data were missing in 1 case for *CYP3A5\*3*, 1 case for *UGT1A1\*6* and 9 cases for *UGT1A1\*28* due to assay failure. Genotype distributions are summarised in table 1. Univariate and multivariate regression analyses with significant associations for dolutegravir PK parameters are presented in **table 2** whilst **figure 1** shows scatter plots for each statistically significant genotype, plotting dolutegravir plasma PK data (GM) *versus* genotype for each SNP. The totality of the regression results can also be found in **table S2.**

***Covariates***

Weight and Log10 height were associated with lower dolutegravir Log10Cmax (β=-1.649; p =0.012 and β=-0.003; p=0.009, respectively)whilst dolutegravir administration within abacavir/lamivudine was associated with a higher dolutegravir Log10Cmax than intake alone (GM Cmax (95%CI) 4246 (3872-4620) *versus* 3692 (3414-3971) ng/mL, p=0.001). Tenofovir/emtricitabine co-administration with dolutegravir was associated with a higher Log10 Cmin than administration alone or with abacavir/lamivudine (GM Cmin (95%): 1791 (975-2607) *versus* 1106 (976-1236) & 1052 (876-1228) ng/mL, respectively; β=0.069; p=0.034). Finally, increased weight was also associated with a decrease in dolutegravir Log10 AUC0-24 (β=-0.002; p=0.02), with an 8-10% decrease in GM AUC0-24 for every 10kg increase in weight bracket between 40 and 80kg.

***ABCG2 c.*421C>A (rs2231142)**

After multivariate analysis*, ABCG2 c.*421C>A (rs2231142) was independently associated with a 28% increase in dolutegravir Cmax (β=0.053, p=0.047) in the homozygous variant. GM Cmax (95%CI) was 3893 (3774-4240), 4346 (3629-5531) and 4994 (single value) ng/mL in the CC, CA and AA genotype groups, respectively.

***NR1I2 c.*63396C>T (rs2472677)**

*NR1I2* c.63396C>T(rs2472677) was associated with higher dolutegravir Log10 Cmax (β=0.032; *p*=0.033) and higher dolutegravir Log10AUC0-24, (β= 0.042; *p*=0.029). GM Cmax (95% CI) was 3445 (3176-3822), 3938 (3705-4480) and 4278 (3992-4817) ng/mL and GM AUC0-24 (95% CI)was 42750 (38002-52263), 54138 (50998-61344) and 54170 (51019-60413) ng\*h/mL in the CC, CT and CC genotype groups, respectively. This represents a 24% increase in Cmax and a 27% increase in AUC0-24 between homozygote groups.

***UGT1A1\*28* (rs8175347)**

The *UGT1A1*\*28 variant allele displayed a recessive allele effect (**figure 1**) and coding was therefore dichotomized and weighted appropriately (extensive and intermediate metabolisers grouped as a single category coded as 0 and poor metabolisers coded as 2). The *UGT1A1*\*28 poor metaboliser genotype was independently associated with higher dolutegravir Log10 AUC0-24 (β=0.042; p=0.02). GM AUC0-24 (95% CI)were 52639 (47956-57321), 51818 (46866-56771) and 66281 (57162-75401) ng\*h/mL for the extensive, intermediate and poor metaboliser genotypes respectively (27% difference between homozygote groups). When *UGT1A1*\*28 was combined with *UGT1A1*\*6, genotypic scores ≥ three/4 were associated with a 36% increase in AUC0-24 and a 44% increase in Cmin (β=0.041; p=0.023 and β=0.042; p=0.009 respectively). GM AUC0-24 (95%CI) was 48500(43417-53583) ng\*h/mL in participants who scored 0 and 66085 (54917-77253) ng\*h/mL in those who scored three/4 (no individual scored four/4) and GM Cmin (95%CI) was 1109 (885-1334) and 1594 (1247-1941) ng/mL respectively.

***Composite Scores***

* *UGT1A1*\*28 *+ NR1I2* c*.*63396C>T

Participants carrying the homozygous variant alleles for both *NR1I2* c*.*63396C>T (rs2472677) and *UGT1A1\*28* displayed a statistically significant 79% increase in AUC0-24 (β=0.42; p=0.005). GM AUC0-24 (95% CI) in those who carried the common allele for both genotypes was 42306 (36990-52278) *versus* 75807 (69714-82166) ng\*h/mL in those who carried the variant allele for both. This was the largest effect size seen in this study. Variability in the two groups as reflected by IQR was 41921-47692 and 73180-74542 ng\*h/mL. There was also a significant 47% increase in Cmax and a 78% increase in GM Cmin but the latter was not statistically significant (p=0.436).

* *ABCG2* c.421 C>A (rs2231142) and *NR1I2* c*.*63396C>T

When combined into a scoring algorithm, a statistically significant 43% increase in Cmax and a39% increase in AUC0-24 were seen in participants who scored ≥three/4 relative to participants who scored 0 (β=0.038; p=0.002 and β=0.038; p=0.002 respectively). GM Cmax (95% CI) were 3450 (3102-3799) *versus* 4924 (3555-6293) ng/mL and GM AUC0-24 (95% CI) were 42768 (35078-50457) *versus* 59335 (48362-70308) ng\*h/mL in the two groups respectively. A genotypic score-dose effect was seen.

Of note, there were no significant differences in genotype distribution for *ABCG2, UGT1A1 and NR1I2* between PK sample groups analysed with either HPLC-MS/MS or UPLC.

***Remaining genotypes***

No other clinically significant association was found with the remaining genotypes studied (**Table S2**)

**DISCUSSION**

The impact on dolutegravir PK parameters of key common SNPs coding for the four main enzymes and transporters involved in its disposition were investigated.[22](#_ENREF_22) For the first time, to the best of our knowledge, these were brought together in a multivariate analysis model, which controlled for important demographic covariates and were combined into scoring algorithms.

A statistically significant increase in dolutegravir exposure was found in carriers of the UGT*1A1\*28* (rs8175347) poor metaboliser genotype. Our results are in keeping with findings from the Chen *et al.* study albeit with a smaller effect size (28% *versus* 46%).[27](#_ENREF_27) Yagura *et al.* reported an association between the *\*28* heterozygote status (intermediate metaboliser) and an increase in Cmin but not for the homozygote (poor metabolizer) genotype, which the authors related to a lack of statistical power.[16](#_ENREF_16) Contrastingly, our study demonstrated higher dolutegravir PK parameters in *the UGT1A1\*28* poor metabolisers group and not the intermediate group, consistent with a recessive genotype model. Whilst this may also relate to a potential lack of statistical power, other studies have reported a lack of PK or PD effect in the intermediate metaboliser genotype, with drugs such as irinotecan or raloxifene.[44](#_ENREF_44), [45](#_ENREF_45) Of note, the impact of *UGT1A1\*28* alone on dolutegravir concentrations seemed modest compared to that seen for raltegravir concentrations, where C12 was 110% higher in individuals carrying the poor metaboliser genotype.[46](#_ENREF_46) However, when *UGT1A1\*28* and *NR1I2* c.63396 C>Twere combined in this study, a 79% increase in AUC0-24 and a 47% increase in Cmax, were seen in those with a higher score. Additionally, when *UGT1A1*\*28 and *UGT1A1*\*6were combined, genotypic scores ≥three/4 were associated with a 36% increase in dolutegravir AUC0-24 and a 44% increase in Cmin. Overall, this indicates that genomic biomarkers of dolutegravir plasma exposure may be better based on carefully defined sets of SNPs or scoring algorithms rather than on single SNP characterisation.[47](#_ENREF_47), [48](#_ENREF_48)

A statistically significant, but moderate, 28% increase in dolutegravir Cmax was found in subjects carrying the *ABCG2* c.421C>A *(*rs2231142) homozygous variant genotype (AA) compared to those homozygous for the common allele (CC). This therefore confirms, results previously reported by Tsuchiya *et al.* but in averydifferent population (75% Caucasian and 17% Black participants (n=76) *versus* 100% Japanese participants (n=42) respectively).[28](#_ENREF_28) The increase in Cmax may reflect a decrease in first pass metabolism, for instance through reduced expression of efflux BRCP transporters in intestinal epithelial cells leading to increased absorption and/or through reduced hepatic clearance.[28](#_ENREF_28), [49](#_ENREF_49) Higher exposures to sunitinib, rosuvastatin and atorvastatin have similarly been described in individuals carrying the *ABCG2* c.421C>A(rs2231142) variant.[50](#_ENREF_50), [51](#_ENREF_51) Interestingly, when *ABCG2* c.421C>A was combined with *NR1I2* c.63396C>T (rs2472677), participants homozygous for the variant in both genes showed a significant 43% increase in dolutegravir Cmax, once again suggesting a carefully defined set of SNPs may be a more useful genomic biomarker of dolutegravir PK parameters.

The *NR1I2* c.63396C>T (rs2472677) variant was independently associated with increases in Cmax (24%) and AUC0-24 (27%). This is converse to the effects seen with unboosted atazanavir[37](#_ENREF_37), [38](#_ENREF_38) and is surprising since the TT genotype is thought to increase expression of the nuclear receptor PXR, which in turn would be expected to result in higher UGT1A1, BCRP and CYP3A4 expression and lower dolutegravir concentrations.[33](#_ENREF_33), [52](#_ENREF_52) Therefore, this observation should be interpreted with caution and needs to be confirmed.

There are limitations to this work. The use of pooled data means drug intake conditions, such as time of day, accompanying food and backbone regimen were standardised within but not necessarily across trials. Moreover, a number of studies involved healthy volunteers whilst others investigated HIV infected individuals, though this was included as a covariate in the multivariate analyses. Whilst the majority of studies contributing data were Phase I clinical trials, one study was a Phase III trial (n=17) and only provided dolutegravir trough concentrations rather than intensive PK data, meaning the sample sizes for Cmax/AUC0-24 and for Cmin differed (N=76 and N=93 respectively). Additionally, two different assay methodologies were used for the PK analysis, potentially introducing variability. However, reassuringly, there was no significant difference in genotypic distribution for *ABCG2, UGT1A1 and NR1I2* between groups analysed by either method*.* Findings need to be interpreted in the context of the limited population size and statistical power of this study. Finally, our population was predominantly Caucasian and whilst the genetic associations found were preserved when the analyses were restricted to Caucasians only, we could not conduct any other ethnicity sub-analyses due to the small numbers representing other ethnic groups, therefore clinical findings should be verified further in population-specific studies.

To date, the literature for the aetiology of NP-AEs remains inconclusive. A number of cohort studies report an increased risk with abacavir co-administration, although this has been refuted in others, as have implications of gender and age.[12-19](#_ENREF_12) Borghetti *et al*. reported a synergy between dolutegravir PK and *SLC22A2* variant-mediated neurological susceptibility to NP-AEs.[30](#_ENREF_30) Yagura *et al*. similarly described a Cmin-mediated association between *UGT1A1*\*28 and *UGT1A1*\*6 gene polymorphisms and a higher cumulative incidence of selected NP-AEs.[16](#_ENREF_16) Meanwhile, there is a current paradigm shift in the field of HIV pharmacotherapy towards treatment simplification and individualization, applicable to clinically and genetically predefined sub-populations. Therefore, since there is evidence of concentration dependent dolutegravir side effects and dolutegravir is becoming a key player in the global ARV scale up, searching for genomic biomarkers of plasma exposure may help tailor dolutegravir-based HIV therapy at individual and population levels.

In conclusion, this study showed a pharmacogenetic association between dolutegravir pharmacokinetics and variants in the *ABCG2*, *UGT1A1* and *NRI1/2* genes, particularly when combined. Further studies in large and diverse populations are warranted, particularly examining pharmacodynamic endpoints such as NP-AEs, in order to further determine the clinical validity and population impact of pharmacogenetic testing for dolutegravir.[53-55](#_ENREF_53)

**Acknowledgements**

This data was presented in part at the annual Conference on Retroviruses and Opportunistic Infections (CROI), 3-6 March 2018, Boston, USA. Abstract number 467.

**Funding**

The work was funded by the St Stephens AIDS Trust and was supported by the Liverpool institute of Translational Medicine and the NIHR Imperial BRC

**Transparency declarations**

EE**,** MN, LE, XW, MM: none to declare

GM has received speaker's and adviser's fees from Gilead Sciences, Merck Sharp & Dohme, Janssen, and Bristol-Myers Squibb and has served as a member of the board of directors and on the scientific advisory board of Tobira Therapeutics.

MB had received travel and research grants from and has been advisor/speaker for Janssen, Roche, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, Gilead, Mylan, Cipla, Teva.

SK has received support from ViiV Healthcare, Merck, Janssen, Gilead and Bristol Myers Squibb for the HIV drug interactions website, and research grants from Merck, Janssen and ViiV Healthcare.

AO has received research funding from ViiV Healthcare, Merck, Janssen, AstraZeneca, and consultancy from ViiV Healthcare and Merck. He is also co-inventor of patents relating to drug delivery technologies.

.

**REFERENCES**

1. Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the Use of Antiretroviral Agents in Adults and Adolescents Living with HIV. Department of Health and Human Services. Available at <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.

2. European AIDS Clinical Society Guidelines. Version 9.0 October 2017. English. Available: <http://www.eacsociety.org/files/guidelines_9.0-english.pdf>.

3. Elliot E, Chirwa M and Boffito M. How recent findings on the pharmacokinetics and pharmacodynamics of integrase inhibitors can inform clinical use. *Curr Opin Infect Dis* 2017; **30**: 58-73. DOI: 10.1097/QCO.0000000000000327.

4. World Health Organization. Updated Recommendations On First-Line And Second-Line Antiretroviral Regimens And Post-Exposure Prophylaxis And Recommendations On Early Infant Diagnosis Of HIV. Published July 2018. Accessed August 2018. Available at: <http://apps.who.int/iris/bitstream/handle/10665/273632/WHO-CDS-HIV-18.18-eng.pdf?ua=1>.

5. Zash R, Holmes L, Makhema J, et al. Surveillance for neural tube defects following antiretroviral exposure from conception, the Tsepamo study (Botswana). Program and abstracts of the 22nd International AIDS Conference; July 23-27, 2018; Amsterdam, The Netherlands. Session TUSY15.

6. Hill A, Clayden P, Thorne C, et al. Safety and pharmacokinetics of dolutegravir in HIV-positive pregnant women: a systematic review. *J Virus Erad* 2018; **4**: 66-71.

7. World Health Organization. Transition to new antiretrovirals in HIV programmes; 2017. Available at: <http://apps.who.int/iris/bitstream/10665/255888/1/WHOHIV-2017.20-eng.pdf?ua=1> (accessed May 2018).

8. Patel DA, Snedecor SJ, Tang WY, et al. 48-week efficacy and safety of dolutegravir relative to commonly used third agents in treatment-naive HIV-1-infected patients: a systematic review and network meta-analysis. *PLoS One* 2014; **9**: e105653. DOI: 10.1371/journal.pone.0105653.

9. Bonfanti P, Madeddu G, Gulminetti R, et al. Discontinuation of treatment and adverse events in an Italian cohort of patients on dolutegravir. *AIDS* 2017; **31**: 455-457. DOI: 10.1097/QAD.0000000000001351.

10. Hoffmann C and Llibre JM. Neuropsychiatric Adverse Events with Dolutegravir and Other Integrase Strand Transfer Inhibitors. *AIDS Rev* 2019; **21**: 4-10. 2019/03/23. DOI: 10.24875/AIDSRev.19000023.

11. Hoffmann C, Welz T, Sabranski M, et al. Higher rates of neuropsychiatric adverse events leading to dolutegravir discontinuation in women and older patients. *HIV Med* 2017; **18**: 56-63. DOI: 10.1111/hiv.12468.

12. de Boer MG, van den Berk GE, van Holten N, et al. Intolerance of dolutegravir-containing combination antiretroviral therapy regimens in real-life clinical practice. *AIDS* 2016; **30**: 2831-2834. DOI: 10.1097/QAD.0000000000001279.

13. Capetti AF, Di Giambenedetto S, Latini A, et al. Morning dosing for dolutegravir-related insomnia and sleep disorders. *HIV Med* 2017. DOI: 10.1111/hiv.12540.

14. Hoffmann C, Welz T, Sabranski M, et al. Reply to Letter 'Morning dosing for dolutegravir-related insomnia and sleep disorders' by Capetti et al. *HIV Med* 2017. DOI: 10.1111/hiv.12539.

15. Menard A, Montagnac C, Solas C, et al. Neuropsychiatric adverse effects on dolutegravir: an emerging concern in Europe. *AIDS* 2017; **31**: 1201-1203. DOI: 10.1097/QAD.0000000000001459.

16. Yagura H, Watanabe D, Kushida H, et al. Impact of UGT1A1 gene polymorphisms on plasma dolutegravir trough concentrations and neuropsychiatric adverse events in Japanese individuals infected with HIV-1. *BMC Infect Dis* 2017; **17**: 622. DOI: 10.1186/s12879-017-2717-x.

17. Cattaneo D, Rizzardini G and Gervasoni C. Intolerance of dolutegravir-containing combination antiretroviral therapy: not just a pharmacokinetic drug interaction. *AIDS* 2017; **31**: 867-868. DOI: 10.1097/QAD.0000000000001394.

18. Cailhol J, Rouyer C, Alloui C, et al. Dolutegravir and neuropsychiatric adverse events: a continuing debate. *AIDS* 2017; **31**: 2023-2024. DOI: 10.1097/QAD.0000000000001596.

19. Eisner M, Fernandez C, Michie K, et al. Response letter to SEJ Todd et al. - Early clinical experience of dolutegravir in an HIV cohort in a larger teaching hospital. *Int J STD AIDS* 2017; **28**: 1051-1052. DOI: 10.1177/0956462417718759.

20. Elliot ER, Wang X, Singh S, et al. Increased dolutegravir peak concentrations in people living with HIV aged 60 and over and analysis of sleep quality and cognition. *Clin Infect Dis* 2018. DOI: 10.1093/cid/ciy426.

21. Podany AT, Scarsi KK and Fletcher CV. Comparative Clinical Pharmacokinetics and Pharmacodynamics of HIV-1 Integrase Strand Transfer Inhibitors. *Clin Pharmacokinet* 2017; **56**: 25-40. DOI: 10.1007/s40262-016-0424-1.

22. Reese MJ, Savina PM, Generaux GT, et al. In vitro investigations into the roles of drug transporters and metabolizing enzymes in the disposition and drug interactions of dolutegravir, a HIV integrase inhibitor. *Drug Metab Dispos* 2013; **41**: 353-361. DOI: 10.1124/dmd.112.048918.

23. Cottrell ML, Hadzic T and Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. *Clin Pharmacokinet* 2013; **52**: 981-994. DOI: 10.1007/s40262-013-0093-2.

24. Capetti AF, Astuti N, Cattaneo D, et al. Pharmacokinetic drug evaluation of dolutegravir plus rilpivirine for the treatment of HIV. *Expert Opin Drug Metab Toxicol* 2017; **13**: 1183-1192. 2017/09/01. DOI: 10.1080/17425255.2017.1361929.

25. Cattaneo D, Capetti A and Rizzardini G. Drug-drug interactions of a two-drug regimen of dolutegravir and lamivudine for HIV treatment. *Expert Opin Drug Metab Toxicol* 2019; **15**: 245-252. 2019/02/02. DOI: 10.1080/17425255.2019.1577821.

26. Tivicay film-coated tablets 10/25/50mg. ViiV Healthcare UK Ltd, Stockley Park West, Uxbridge, Middlesex,, UB11 1BT, UK. Revised March 2018. Accessed May 208. Available: <https://www.medicines.org.uk/emc/product/5248/smpc#companyDetails>.

27. Chen S, St Jean P, Borland J, et al. Evaluation of the effect of UGT1A1 polymorphisms on dolutegravir pharmacokinetics. *Pharmacogenomics* 2014; **15**: 9-16. DOI: 10.2217/pgs.13.190.

28. Tsuchiya K, Hayashida T, Hamada A, et al. High plasma concentrations of dolutegravir in patients with ABCG2 genetic variants. *Pharmacogenet Genomics* 2017; **27**: 416-419. DOI: 10.1097/FPC.0000000000000308.

29. Yagura HW, H.; Nakauchi, T. et al. Discontinuation of long-term dolutegravir treatment is associated with UGT1A1 gene polymorphisms. 10th IAS Conference on HIV Science, Mexico City, abstract MOPEB239, 2019.

30. Borghetti A, Calcagno A, Lombardi F, et al. SLC22A2 variants and dolutegravir levels correlate with psychiatric symptoms in persons with HIV. *J Antimicrob Chemother* 2019; **74**: 1035-1043. 2018/12/19. DOI: 10.1093/jac/dky508.

31. Wang D, Guo Y, Wrighton SA, et al. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. *Pharmacogenomics J* 2011; **11**: 274-286. DOI: 10.1038/tpj.2010.28.

32. Olagunju A, Schipani A, Siccardi M, et al. CYP3A4\*22 (c.522-191 C>T; rs35599367) is associated with lopinavir pharmacokinetics in HIV-positive adults. *Pharmacogenet Genomics* 2014; **24**: 459-463. DOI: 10.1097/FPC.0000000000000073.

33. Calcagno A, Cusato J, D'Avolio A, et al. Genetic Polymorphisms Affecting the Pharmacokinetics of Antiretroviral Drugs. *Clin Pharmacokinet* 2017; **56**: 355-369. DOI: 10.1007/s40262-016-0456-6.

34. Russo G, Paganotti GM, Soeria-Atmadja S, et al. Pharmacogenetics of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in resource-limited settings: Influence on antiretroviral therapy response and concomitant anti-tubercular, antimalarial and contraceptive treatments. *Infect Genet Evol* 2016; **37**: 192-207. 2015/11/26. DOI: 10.1016/j.meegid.2015.11.014.

35. Savic RM, Barrail-Tran A, Duval X, et al. Effect of adherence as measured by MEMS, ritonavir boosting, and CYP3A5 genotype on atazanavir pharmacokinetics in treatment-naive HIV-infected patients. *Clin Pharmacol Ther* 2012; **92**: 575-583. 2012/10/04. DOI: 10.1038/clpt.2012.137.

36. Sugatani J, Uchida T, Kurosawa M, et al. Regulation of pregnane X receptor (PXR) function and UGT1A1 gene expression by posttranslational modification of PXR protein. *Drug Metab Dispos* 2012; **40**: 2031-2040. DOI: 10.1124/dmd.112.046748.

37. Siccardi M, D'Avolio A, Baietto L, et al. Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C-->T) with reduced concentrations of unboosted atazanavir. *Clin Infect Dis* 2008; **47**: 1222-1225. DOI: 10.1086/592304.

38. Schipani A, Siccardi M, D'Avolio A, et al. Population pharmacokinetic modeling of the association between 63396C->T pregnane X receptor polymorphism and unboosted atazanavir clearance. *Antimicrob Agents Chemother* 2010; **54**: 5242-5250. DOI: 10.1128/AAC.00781-10.

39. Penchala SD, Fawcett S, Else L, et al. The development and application of a novel LC-MS/MS method for the measurement of Dolutegravir, Elvitegravir and Cobicistat in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 2016; **1027**: 174-180. DOI: 10.1016/j.jchromb.2016.05.040.

40. Wang X, Penchala SD, Amara A, et al. A Validated Method for Quantification of Dolutegravir Using Ultra Performance Liquid Chromatography Coupled With UV Detection. *Ther Drug Monit* 2016; **38**: 327-331. DOI: 10.1097/FTD.0000000000000286.

41. Rodriguez S, Gaunt TR and Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009; **169**: 505-514. DOI: 10.1093/aje/kwn359.

42. <https://www.ensembl.org/index.html>. Accessed May 2018.

43. Zhao F, Song M, Wang Y, et al. Genetic model. *J Cell Mol Med* 2016; **20**: 765. 2016/01/15. DOI: 10.1111/jcmm.12751.

44. Trontelj J, Marc J, Zavratnik A, et al. Effects of UGT1A1\*28 polymorphism on raloxifene pharmacokinetics and pharmacodynamics. *Br J Clin Pharmacol* 2009; **67**: 437-444. 2009/04/18. DOI: 10.1111/j.1365-2125.2009.03363.x.

45. Hu ZY, Yu Q, Pei Q, et al. Dose-dependent association between UGT1A1\*28 genotype and irinotecan-induced neutropenia: low doses also increase risk. *Clin Cancer Res* 2010; **16**: 3832-3842. 2010/06/22. DOI: 10.1158/1078-0432.CCR-10-1122.

46. Wenning LA, Petry AS, Kost JT, et al. Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. *Clin Pharmacol Ther* 2009; **85**: 623-627. DOI: 10.1038/clpt.2009.12.

47. Horsfall LJ, Zeitlyn D, Tarekegn A, et al. Prevalence of clinically relevant UGT1A alleles and haplotypes in African populations. *Ann Hum Genet* 2011; **75**: 236-246. DOI: 10.1111/j.1469-1809.2010.00638.x.

48. Lankisch TO, Behrens G, Ehmer U, et al. Gilbert's syndrome and hyperbilirubinemia in protease inhibitor therapy--an extended haplotype of genetic variants increases risk in indinavir treatment. *J Hepatol* 2009; **50**: 1010-1018. DOI: 10.1016/j.jhep.2008.12.030.

49. Kondo C, Suzuki H, Itoda M, et al. Functional analysis of SNPs variants of BCRP/ABCG2. *Pharm Res* 2004; **21**: 1895-1903.

50. Birmingham BK, Bujac SR, Elsby R, et al. Impact of ABCG2 and SLCO1B1 polymorphisms on pharmacokinetics of rosuvastatin, atorvastatin and simvastatin acid in Caucasian and Asian subjects: a class effect? *Eur J Clin Pharmacol* 2015; **71**: 341-355. DOI: 10.1007/s00228-014-1801-z.

51. Mizuno T, Fukudo M, Terada T, et al. Impact of genetic variation in breast cancer resistance protein (BCRP/ABCG2) on sunitinib pharmacokinetics. *Drug Metab Pharmacokinet* 2012; **27**: 631-639.

52. Tolson AH and Wang H. Regulation of drug-metabolizing enzymes by xenobiotic receptors: PXR and CAR. *Adv Drug Deliv Rev* 2010; **62**: 1238-1249. DOI: 10.1016/j.addr.2010.08.006.

53. Tonk ECM, Gurwitz D, Maitland-van der Zee AH, et al. Assessment of pharmacogenetic tests: presenting measures of clinical validity and potential population impact in association studies. *Pharmacogenomics J* 2017; **17**: 386-392. DOI: 10.1038/tpj.2016.34.

54. Gharani N, Keller MA, Stack CB, et al. The Coriell personalized medicine collaborative pharmacogenomics appraisal, evidence scoring and interpretation system. *Genome Med* 2013; **5**: 93. DOI: 10.1186/gm499.

55. Khoury MJ. Genetics and genomics in practice: the continuum from genetic disease to genetic information in health and disease. *Genet Med* 2003; **5**: 261-268. DOI: 10.1097/01.GIM.0000076977.90682.A5.

**TABLES AND FIGURES**

**Table 1:** Characteristics of participant population are shown as medians (interquartile range) or count (N), percentage of population (%). PK values are shown as geometric means (GM) (95%CI). CV% = percentage coefficient variation. \*\*Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolizers (\*1/\*1; \*1/\*36; \*36/\*36), intermediate metabolizers (\*1/\*28; \*1/\*37; \*36/\*28; \*36/\*37; \*1/\*6) and poor metabolizers (\*28/\*28; \*28/\*37; \*37/\*37; \*6/\*6) DTG: dolutegravir, ABC: abacavir, 3TC: lamivudine, TDF: tenofovir disoproxil fumarate, FTC: emtracitabine

**Table 2:** Significant results from univariate (p<0.2) and multivariate (p<0.5) linear regression analysis per PK parameter

**Table S1:** Dolutegravir (DTG) pharmacokinetic parameters shown as Geometric Means (GM) (95%CI), summarized by single or combined genotype

**Table S2**: Univariate and Multivariate regressions – complete analysis. Results in bold = above cut off; \*\* UGT1A1\*28 dichotomised throughout, using a recessive genotypic test model

**Figure 1:** Scatter plots showing statistically significant relationships between genotypes and DTG plasma PK parameter

|  |  |
| --- | --- |
|  | **Total N** |
|  | 93 |
|  | **Median (IQR)** |
| **Age (years)** | 51 (36-64) |
| **Weight (kg)** | 77.6 (67-84.4) |
| **Height (cm)** | 173 (168-177) |
| **ARV Regimen** | **N (%)** |
| * **ABC/3TC/DTG**
 | 40 (43) |
| * **TFV/FTC + DTG**
 | 17 (18) |
| * **DTG alone**
 | 36 (39) |
| **Ethnicity** | **N (%)** |
| * **Caucasian**
 | 70 (75) |
| * **Black**
 | 16 (17) |
| * **Asian**
 | 3 (3) |
| * **Mixed race**
 | 1 (1) |
| * **Other**
 | 3 (3) |
| **Female gender** | 26 (28) |
|  | **PK parameters GM (95% CI) – IQR** |
| **DTG GM Cmax (ng/mL; N=76)** | 3974 (3864 – 4357) – IQR 3462-4611 |
| **DTG GM AUC0-24 (hr\*ng/mL, N=76)** | 51846 (48607- 55085) – IQR 53190-57191 |
| **DTG GM Cmin (ng/mL, N=93)** | 1182 (994 – 1371)) – IQR 873-1612 |
| **DTG GM t1/2 (hrs; N=76)** | 13 (12.0- 14.0) – IQR 11.0-15.3 |
|  | **Genotypic frequencies %** |
| ***UGT1A1*\*28 (rs8175347)\*\*** |  **Extensive metaboliser**  | **Intermediate metaboliser** | **Poor metaboliser** |
|  | 46 | 43 | 11 |
| ***UGT1A1*\*6 c.211G>A (rs4148323)** | **Extensive metaboliser** | **Intermediate metaboliser** | **Poor metaboliser** |
|  | 37 | 63 | 0 |
| ***CYP3A4*\*22 G>A (rs35599367)** | **GG** | **GA** | **AA** |
|  | 88 | 6 | 5 |
| ***CYP3A5*\*3 C>T (rs776746)** | **CC** | **TC** | **TT** |
| 76 | 12 | 12 |
| ***ABCG2* 421 C>A (rs2231142)** | **CC** | **CA** | **AA** |
| 82 | 17 | 1 |
| ***ABCG2* 34 C>T (rs2231137)** | **CC** | **CT** | **TT** |
| 83 | 17 | 0 |
| ***NR1I2* 63396 C>T (rs2472677)** | **CC** | **CT** | **TT** |
| 17 | 42 | 41 |
| ***NR1I2* 44477 T>C (rs1523130)** | **TT** | **CT** | **CC** |
| 19 | 39 | 42 |

**Table 1**: Characteristics of participant population are shown as medians (interquartile range) or count (N), percentage of population (%). PK values are shown as geometric means (GM) (95%CI). CV% = percentage coefficient variation

\*\*Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolizers (\*1/\*1; \*1/\*36; \*36/\*36), intermediate metabolizers (\*1/\*28; \*1/\*37; \*36/\*28; \*36/\*37; \*1/\*6) and poor metabolizers (\*28/\*28; \*28/\*37; \*37/\*37; \*6/\*6). DTG: dolutegravir, ABC: abacavir, 3TC: lamivudine, TDF: tenofovir disoproxil fumarate, FTC: emtracitabine

|  |  |  |
| --- | --- | --- |
| **Log10 Cmax (N=93)** | **Univariate Linear Regressions** | **Multivariate Linear Regressions** |
| ***P* value** | **β value (95% CI)** | **r2** | ***P* value** | **β value (95% CI)** | **r2** |
| Log10Height (Log10cm) | 0.008 | -1.716 | 0.092 | **0.012** | -1.649 | 0.394 |
| Weight (kg) | 0.000 | -0.004 | 0.175 | **0.009** | -0.003 | 0.394 |
| Accompanying Drug | 0.019 | 0.061 | 0.072 | **0.001** | 0.074 | 0.394 |
| *UGT1A1\*6* (rs4148323) | 0.091 | -0.044 | 0.038 | 0.355 | 0.039 | 0.402 |
| ***ABCG2* c.421C>A (rs2231142)** | 0.111 | 0.050 | 0.034 | **0.047** | 0.053 | 0.394 |
| ***NR1I2* c.63396C>T (rs2472677)** | 0.010 | 0.045 | 0.086 | **0.033** | 0.032 | 0.394 |
| **Combined *NRI1I2* and UGT1A1\*28 scores** | 0.030 | 0.029 | 0.071 | **0.023** | 0.026 | 0.311 |
| **Combined *ABCG2* and *NRI1I2* scores** | 0.011 | 0.057 | 0.291 | **0.005** | 0.054 | 0.377 |
| **Log10 Cmin (N=76)** | **Univariate Linear Regressions** | **Multivariate Linear Regressions** |
| ***P* value** | **β value (95% CI)** | **r2** | ***P* value** | **β value (95% CI)** | **r2** |
| Log10 Age (Log10years) | 0.125 | -0.236 | 0.026 | **0.029** | -0.310 | 0.104 |
| Accompanying drug | 0.008 | 0.084 | 0.076 | 0.114 | 0.048 | 0.133 |
| ***UGT1A1\*28* (rs8175347)\*\*** | 0.045 | 0.070 | 0.049 | **0.083** | 0.059 | 0.140 |
| **Combined *UGT1A1\*6* and *\*28* scores** | 0.009 | 0.067 | 0.082 | **0.009** | 0.067 | 0.082 |
| **AUC0-24 (N=93)** | **Univariate Linear Regressions** | **Multivariate Linear Regressions** |
| ***P* value** | **β value (95% CI)** | **r2** | ***P* value** | **β value (95% CI)** | **r2** |
| Log10Height (Log10cm) | 0.011 | -1.866 | 0.066 | 0.323 | -0.871 | 0.282 |
| Weight (kg) | 0.017 | 0.003 | 0.075 | **0.03** | -0.002 | 0.228 |
| Ethnicity | 0.044 | 0.036 | 0.054 | 0.143 | 0.025 | 0.256 |
| *CYP3A4*\*22 (rs35599367) | 0.059 | 0.053 | 0.047 | 0.295 | 0.027 | 0.270 |
| ***CYP3A5*\*3 (rs776746)** | **0.097** | **-0.034** | **0.037** | **0.033** | **-0.040** | **0.228** |
| ***NR1I2* c.63396C>T (rs2472677)** | 0.033 | 0.043 | 0.060 | **0.029** | 0.042 | 0.228 |
| ***UGT1A1\*28* (rs8175347)\*\*** | 0.020 | 0.058 | 0.060 | **0.020** | 0.116 | 0.228 |
| **Combined *UGT1A1\*6* and *\*28* scores** | 0.075 | 0.046 | 0.048 | **0.041** | 0.050 | 0.231 |
| **Combined *UGT1A1\*28* and *NRI1I2* c.63396C>T scores** | 0.011 | 0.039 | 0.095 | **0.002** | 0.048 | 0.025 |
| **t1/2 (N=93)** | **Univariate Linear Regressions** | **Multivariate Linear Regressions** |
| ***P* value** | **β value (95% CI)** | **r2** | ***P* value** | **β value (95% CI)** | **r2** |
| Ethnicity | **0.057** | 0.031 | 0.058 |  |  |  |

**Table 2**: Significant results from univariate (p<0.2) and multivariate (p<0.5) linear regression analysis per PK parameter (significant SNP associations are boxed in bold. \*\*Clinical Pharmacogenetics Implementation Consortium (CPIC) classification for UGT1A1 genotype-predicted phenotypic function: extensive metabolizers (\*1/\*1; \*1/\*36; \*36/\*36), intermediate metabolizers (\*1/\*28; \*1/\*37; \*36/\*28; \*36/\*37; \*1/\*6) and poor metabolizers (\*28/\*28; \*28/\*37; \*37/\*37; \*6/\*6).



**This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC***

**Figure 1:** Scatter plots showing statistically significant genotypes. DTG plasma PK data (geometric mean) are plotted by genotype groups for each SNP.