Class-specific relative genetic contribution for key antiretroviral drugs

Class-specific relative genetic contribution for key antiretroviral drugs

Marco Siccardi, Adeniyi Olagunju, Marco Simiele, Antonio D'Avolio, Andrea Calcagno, Giovanni Di Perri, Stefano Bonora, Andrew Owen

Abstract

Objectives Antiretroviral pharmacokinetics is defined by numerous factors affecting absorption, distribution, metabolism and elimination. Biological processes underpinning drug distribution are only partially characterized and multiple genetic factors generate cumulative or antagonistic interactions, which complicates the implementation of pharmacogenetic markers. The aim of this study was to assess the degree to which heredity influences pharmacokinetics through the quantification of the relative genetic contribution (rGC) for key antiretrovirals.

Methods A total of 407 patients receiving lopinavir/ritonavir, atazanavir/ritonavir, atazanavir, efavirenz, nevirapine, etravirine, maraviroc, tenofovir or raltegravir were included. Intra-patient variability (SDw) and inter-patient (SDb) variability were measured in patients with plasma concentrations available from more than two visits. The rGC was calculated using the following equation: $1-(1/F)$ where $F=\mathrm{SDb}^2/\mathrm{SDw}^2$.

Results Mean (95% CI) rGC was calculated to be 0.81 (0.72–0.88) for efavirenz, 0.74 (0.61–0.84) for nevirapine, 0.67 (0.49–0.78) for etravirine, 0.65 (0.41–0.79) for tenofovir, 0.59 (0.38–0.74) for atazanavir, 0.47 (0.27–0.60) for atazanavir/ritonavir, 0.36 (0.01–0.48) for maraviroc, 0.15 (0.01–0.44) for lopinavir/ritonavir and 0 (0–0.33) for raltegravir.

Conclusions The rank order for genetic contribution to variability in plasma concentrations for the study drugs was efavirenz > nevirapine > etravirine > tenofovir > atazanavir > atazanavir/ritonavir > maraviroc > lopinavir/ritonavir > raltegravir, indicating that class-specific differences exist. The rGC strategy represents a useful tool to rationalize future investigations as drugs with higher rGC scores may represent better candidates for pharmacogenetic–pharmacokinetic studies.

Introduction

The response to ART is characterized by broad heterogeneity, which has been attributed to numerous factors, such as adherence to therapy, viral resistance, status of the immune system, and pharmacokinetic exposure to antiretrovirals (ARVs). The relationship between efficacy and pharmacokinetic exposure has been demonstrated for a large number of ARVs.[1–5]

Plasma exposure to ARVs is affected by the processes of absorption, distribution, metabolism and elimination (ADME) in tissues and organs, mediated by complex interactions between numerous proteins. Most ARVs are administered orally and the first stage in absorption therefore involves the gastrointestinal (GI) tract. The oral absorption is influenced by drug-specific factors (tablet dissolution rate, solubility and substrate specificity for transporters) in addition to patient-specific factors (GI physiology and pH). The distribution and elimination of ARVs is the result of the action of several transporters and metabolizing enzymes. The hepatic tissue is the main site of phase I and II metabolism of all ARVs, except the NRTIs, which are mostly renally excreted unchanged.[6–8]

Environmental and patient-specific factors influence ADME processes, resulting in intra- and inter-patient variability. Environmental factors such as food, concomitant drugs and cultural habits can potentially alter drug distribution. Light meals and high-fat meals, with lower variability under both fed conditions relative to fasting, have been reported to increase atazanavir (with and without ritonavir or cobicistat) bioavailability.[9,10] Similar patterns have been described for darunavir with ritonavir and cobicistat,[11] lopinavir with ritonavir, and rilpivirine.[12] Different meal types can affect raltegravir pharmacokinetics by

altering GI pH, which affects raltegravir solubility.[13,14] Genetic factors influencing the pharmacokinetics of several ARVs have also been described for metabolizing enzymes (CYP2B6, efavirenz and nevirapine; CYP3A4 and lopinavir; CYP2C19 and etravirine), nuclear receptors (PXR and atazanavir; CAR and efavirenz) and transporters (ABCC10 and tenofovir/nevirapine; SLCO1B1 and maraviroc/lopinavir).[6,15–29] Two main strategies have been applied to identify the influence of genetic variants on pharmacokinetics. Candidate gene studies are based on *in vitro* investigation of molecular mechanisms, and a small number of functional polymorphisms are subsequently assessed in patient cohorts. Conversely, genome-wide association studies (GWAS) involve hypothesis-free identification of specific genetic determinants of phenotypes and are based on a scan of genetic markers across the whole genome or exons in large cohorts of patients. Irrespective of the strategy used, the cost of these investigations can be high and data need to be replicated in multiple independent cohorts.

The importance of genetic factors in pharmacokinetic variability or other phenotypes can be estimated using the relative genetic contribution (rGC) score.[30–32] The rGC is determined using the repeated drug administration method, which compares the intra-patient (SDw) and inter-patient (SDb) variation in pharmacokinetic parameters following repeated administrations of a drug. The SDw is assumed to be determined only by environmental factors that influence ADME processes since the DNA sequence does not change in individuals. Conversely, SDb can be influenced by host genetic factors in addition to environmental factors, both of which are known to influence ADME processes. This comparison can be used to identify drugs with a strong genetic contribution to variability in pharmacokinetic parameters (or pharmacodynamics). In addition, it can be used to identify the pharmacokinetic parameters, routes of administration, drug dose or patient population most prone to genetic variability. Recently, an rGC of 0.904 (0.64–0.97) was reported for nevirapine AUC_{0-6}.[33]

The aim of this study was to assess the rGC of a panel of key ARVs to determine the degree to which genetic factors influence their pharmacokinetics.

Methods

Patients

Patients were recruited in the Department of Infectious Diseases, University of Torino, Amedeo di Savoia Hospital, Torino, Italy. This retrospective study was conducted according to the Declaration of Helsinki and the European Guidelines on Good Clinical Practice. Written informed consent was signed by patients for therapeutic drug monitoring of ARVs (allowing data collection and publication) and data were collected retrospectively. Inclusion criteria were as follows: >18 years old; not receiving interfering drugs; in good general medical condition; good adherence record as assessed by analyses of clinical and pharmacy records; and not underweight (<40 kg) or obese (>110 kg). Patients receiving the following as part of combination ART were included: 600 mg of efavirenz once daily; 400 mg of nevirapine once daily; 200 mg of etravirine twice daily; 400 mg of raltegravir twice daily; 300 mg of maraviroc twice daily; 400 mg/100 mg of lopinavir/ritonavir twice daily; 300 mg/100 mg of atazanavir/ritonavir once daily; 400 mg of atazanavir once daily; or 300 mg of tenofovir once daily.

Quantification of ARV concentrations

Plasma concentrations were quantified using validated bioanalytical methods. For efavirenz, nevirapine, etravirine, lopinavir, atazanavir and raltegravir an HPLC–photodiode array (PDA) method was used, following a solid-phase extraction procedure as previously described.[34] Briefly, 500 µL of plasma diluted with 500 µL of solvent A (50 mM KH_2PO_4 with orthophosphoric acid, final pH 3.23) was loaded on SPE C-18 cartridges previously activated with 1 mL of methanol and washed with 1 mL of solvent A. Subsequently, the cartridge was washed with 500 µL of solvent A and 250 µL of deionized water. Finally, analytes were eluted with 500 µL of methanol and acetonitrile solution (90 : 10, v/v). The eluate was dried with a vortex

vacuum system at 60°C, reconstituted in 150 µL of mobile phase and injected. Chromatographic separation was obtained using a gradient run (mobile phase A, 50 mM KH_2PO_4 with orthophosphoric acid, final pH 3.23; mobile phase B, acetonitrile) and a reverse-phase Luna 5 µm column (150 × 3 4.6 mm ID; Phenomenex, CA, USA). Maraviroc was quantified using an HPLC–UV method following a protein precipitation extraction procedure.[35] A 600 µL aliquot of plasma was treated with 1400 µL of acetonitrile + 0.1% trifluoroacetic acid (TFA), vortexed and centrifuged at 12 000 rpm for 10 min. The supernatant was dried by vortex vacuum evaporation at 60°C and subsequently reconstituted in 100 µL of mobile phase and injected.

Tenofovir was quantified using liquid chromatography coupled with mass spectrometry.[36] The solid-phase extraction procedure involved 100 µL of plasma diluted with 100 µL of a solution of 0.6% TFA in HPLC-grade water. SPE cartridges (C-18) were activated with 1 mL of methanol, followed by 1 mL of 0.6% TFA in HPLC-grade water before loading of the samples. Loading was carried out under gravity. Then the cartridges were washed with 250 µL of 0.6% TFA in HPLC-grade water, and elution was carried out using 500 µL of a solution of methanol and acetonitrile (90 : 10, v/v). Eluted solutions were evaporated to dryness at 50°C. Extracts were reconstituted with 200 µL of water/acetonitrile (95 : 5) solution and 30 µL was injected into the column. Chromatographic separation was performed at 35°C on an Atlantis dC-18 3 µm column (150 × 4.6 mm i.d.) (Waters, Milan, Italy). Tenofovir was detected at *m*/*z* of 288.20 with a cone voltage of 20 V.

Data analysis

SDw and SDb were determined in patients with C_{24} (tenofovir and atazanavir) or C_{12} (nevirapine, etravirine, atazanavir/ritonavir, maraviroc, lopinavir/ritonavir and raltegravir) available from more than two visits. The rGC was calculated using the following equation: $1-(1/F)$, where $F = SDb^2/SDw^2$. Statistical significance for genetic contribution was calculated using the *F*-test ($\alpha = 0.05$). Data were processed in Microsoft Excel 2007 and statistical analyses were performed using SPSS 21. Results for categorical data are expressed as mean and SD or mean with 95% CI. All data were assessed for normality using the Shapiro–Wilk test and categorical data were compared using the Mann–Whitney or Kruskal–Wallis test. To investigate continuous data, Spearman's rank correlation was used.

Results

A total of 460 patients were included. Patient demographics are summarized in Table 1. The distribution of mean plasma concentrations for the ARVs within the study population is represented in Figure S1 (available as Supplementary data at *JAC* Online) and Table 1.

Table 1. Patient demographics, ARV concentrations, SDb, SDw and rGC

ARV (number of patients)	Age (years), mean ± SD	Weight (kg), mean ± SD	Height (cm), mean ± SD	Male, %	Number of quantifications, median (range)	Plasma concentrations (ng/mL), mean ± SD	Intra-patient variability (SDw), %	Inter-patient variability (SDb), %	rGC, mean (95% CI)
Efavirenz (*n* = 41)	44 ± 10	74 ± 20	174 ± 10	70	3 (3–7)	2838 ± 1826[a]	25	58	0.81 (0.72–0.88)
Nevirapine (*n* = 31)	45 ± 8	72 ± 12	170 ± 7	60	3 (3–9)	5524 ± 2351[a]	21	43	0.74 (0.61–0.84)
Etravirine (*n* = 42)	45 ± 12	69 ± 10	171 ± 6	90	4 (3–21)	575 ± 307[a]	45	79	0.67 (0.49–0.78)
Tenofovir (*n* = 39)	41 ± 8	71 ± 14	173 ± 8	68	4 (3–12)	95 ± 82[b]	50	86	0.65 (0.41–0.79)

ARV (number of patients)	Age (years), mean ± SD	Weight (kg), mean ± SD	Height (cm), mean ± SD	Male, %	Number of quantifications, median (range)	Plasma concentrations (ng/mL), mean ± SD	Intra-patient variability (SDw), %	Inter-patient variability (SDb), %	rGC, mean (95% CI)
Atazanavir (*n* = 63)	46 ± 10	66 ± 13	169 ± 8	60	4 (3–16)	361 ± 465[b]	75	128	0.59 (0.38–0.74)
Atazanavir/ritonavir (*n* = 31)	44 ± 8	69 ± 14	170 ± 9	63	4 (3–8)	795 ± 526[a]	56	86	0.47 (0.27–0.60)
Maraviroc (*n* = 32)	47 ± 9	73 ± 5	170 ± 8	75	4 (3–17)	95.8 ± 97.8[a]	81	102	0.36 (0.01–0.48)
Lopinavir/ritonavir (*n* = 61)	42 ± 8	66 ± 12	172 ± 9	78	6 (3–13)	8925 ± 4556[a]	47	51	0.15 (0.01–0.44)
Raltegravir (*n* = 67)	48 ± 9	72 ± 12	172 ± 9	80	4 (3–25)	369 ± 362[a]	91	90	0.00 (0.00–0.33)

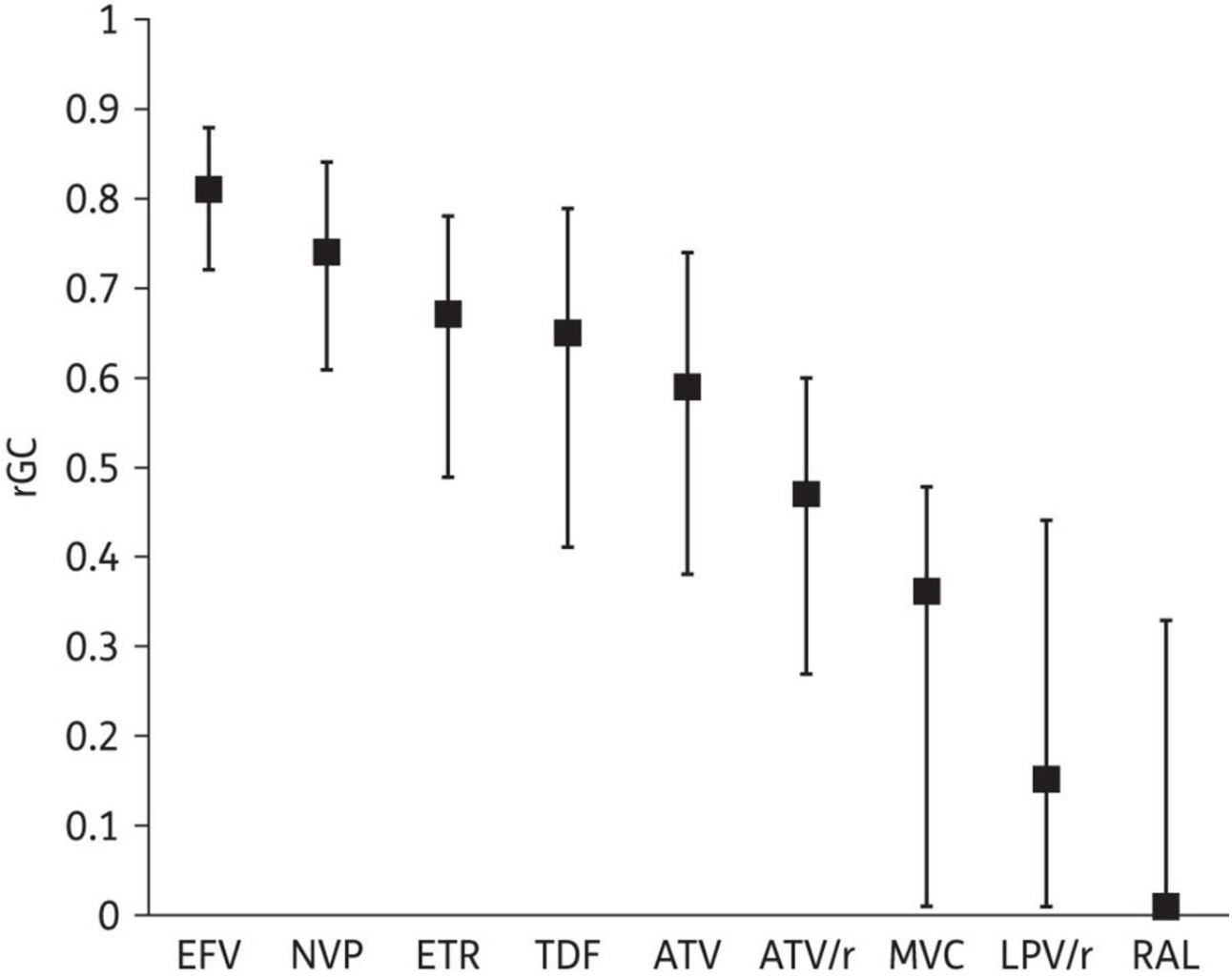
[a]C_{12}. [b]C_{24}.

For efavirenz, 41 patients were included and the mean (95% CI) rGC was calculated to be 0.81 (0.72–0.88). For nevirapine, 31 patients were considered in our study and the rGC was calculated to be 0.74 (0.61–0.84). For etravirine, 42 patients were included in our study and the rGC was calculated to be 0.67 (0.49–0.78). For tenofovir, 39 patients were included and the rGC was calculated to be 0.65 (0.41–0.79).

For atazanavir, 63 patients were included in the analysis and the rGC was calculated to be 0.59 (0.38–0.74). For atazanavir/ritonavir, 31 patients were included and the rGC was calculated to be 0.47 (0.27–0.60). For lopinavir/ritonavir, in 61 patients the rGC was calculated to be 0.15 (0.01–0.44).

For maraviroc, 32 patients were included and the rGC was calculated to be 0.36 (0.01–0.48). For raltegravir, in 67 patients the rGC was calculated to be 0 (0–0.33). Mean rGC for the ARVs is presented in Figure 1 and Table 1.

Figure 1.



rGC for ARVs (mean with 95% CI). EFV, efavirenz; NVP, nevirapine; ETR, etravirine; TDF, tenofovir; ATV, atazanavir; ATV/r, atazanavir/ritonavir; LPV/r, lopinavir/ritonavir; RAL, raltegravir; MVC, maraviroc.

All patients were of Caucasian ancestry and demographics were not significantly associated with C_{24} or C_{12}. Insufficient females were included to robustly assess the influence of gender. An inverse correlation between rGC and SDw ($r = -0.68$; $P = 0.045$) but not SDb ($r = -0.167$; $P = 0.651$) was observed.

Discussion

This investigation represents the first systematic description of SDw and SDb of ARV pharmacokinetics in the clinical setting. The theoretical relevance of genetics and other patient-specific factors was derived considering the rGC score in a cohort of patients with multiple quantifications of C_{24} or C_{12}. The rank order for rGC score for the included ARVs was efavirenz > nevirapine > etravirine > tenofovir > atazanavir > atazanavir/ritonavir > maraviroc > lopinavir/ritonavir > raltegravir. An indication of class-specific differences was also evident (NRTIs and NNRTIs > PIs > integrase inhibitors) and these findings are in good agreement with existing pharmacogenetic knowledge.

Efavirenz (rGC = 0.81) is metabolized primarily by CYP2B6, which is characterized by polymorphisms such as 516G>T (rs3745274). More recently studies have also demonstrated that in the presence of defective CYP2B6 metabolism, there is a significant association between efavirenz exposure and *CYP3A4*, *CYP2A6* and *UGT2B7* genetic variants.[37,38] This illustrates the simultaneous effects of major and minor metabolic pathways and their genetic variants on efavirenz pharmacokinetics. The combined effect of *CYP2B6*,

CYP3A4, *CYP2A6* and *UGT2B7* genetic variants has been estimated to account for 65% of the SDb in efavirenz pharmacokinetics. The potential effect of concomitant food on inter-occasion variability is minimized due to the fact that patients are advised to take efavirenz at night and on an empty stomach. Nevirapine (rGC = 0.74) is metabolized by CYP2B6 and CYP3A4 and the influence of *CYP2B6* 516G>T (rs3745274) on clearance has been described.[23] Moreover, transporter genetics represents a relevant candidate to explain observed pharmacokinetic variability in patients. The findings of our study are in agreement with a recent investigation in which an rGC of 0.904 (0.64–0.97) was reported for nevirapine AUC_{0-6}.[33] Etravirine (rGC = 0.67) is mainly metabolized by CYP2C19. The *CYP2C19* gene is polymorphic, with variants reported at high frequencies in different populations. The effect of CYP2C9*2 (rs1799853) has been previously described through a population pharmacokinetic approach and has been estimated to explain 16% of the variation observed in patients.[29] Our finding suggests that a significant portion of the genetic contribution to etravirine pharmacokinetics remains unexplained.

Tenofovir pharmacokinetics (rGC = 0.65) is the product of complex oral absorption during which the prodrug tenofovir disoproxil fumarate is enzymatically degraded.[39] The enzymes mediating this process have not been fully characterized and this ADME process could represent a relevant step defining inter-patient variability, with a potential role for pharmacogenetic factors.[40] Tenofovir disoproxil fumarate is a substrate for ABCB1 and possibly ABCG2, and therefore genetic variability of these transporters could potentially contribute to the inter-patient variability in tenofovir disoproxil fumarate absorption. Furthermore, several transporters have been identified as important actors in tenofovir renal clearance, constituting good candidates for pharmacogenetic studies.[41]

The metabolism of PIs and maraviroc is mainly mediated in the hepatic and intestinal tissues by CYP3A4, whose expression and activity are thought to be regulated by environmental factors and by genetics. This seems to be confirmed in our analysis, in which PIs and maraviroc present lower rGCs compared with NNRTIs. Other determinants of PI and maraviroc disposition are ABCB1 and SLCO1B1, with polymorphic genes previously investigated in pharmacogenetic studies for these classes of drugs.[6,19] A higher rGC was observed for unboosted atazanavir (rGC = 0.59) compared with atazanavir/ritonavir (rGC = 0.47). These data indicate that ritonavir reduces the genetic contribution to variability in atazanavir concentrations, presumably through its inhibition potential. Ritonavir is a strong inhibitor of gene products such as CYP3A4, ABCB1 and SLCO1B1, acting on several ADME processes and consequently resulting in enhanced absorption and lower clearance. The ritonavir inhibitory activity reduces the role of transporters and phase I metabolism enzymes and therefore moderates the contribution of genetic factors defining SDb, besides altering inter-occasion variability.

The pharmacokinetics of raltegravir (rGC = 0) is characterized by elevated SDw and is thought to be defined by gastrointestinal pH and physiology, concomitant food and formulation characteristics.[13,14,42] Moreover, clinical studies have described only a minor role for *UGT1A1* polymorphisms in raltegravir pharmacokinetics.[43,44]

The correlation of SDw with rGC illustrates a higher genetic contribution for drugs with lower environmental influences. In fact SDw is thought to be influenced by environmental factors that influence ADME mechanisms since genetic factors do not change within individuals. This investigation indicates that SDw could be significant for different classes of ARVs and investigations of ARV pharmacokinetics using a single measure of plasma concentrations may underestimate pharmacokinetic variability. The absence of clear bimodal distributions of plasma concentrations could be observed for all ARVs, suggesting an influence of multiple factors. This hypothesis could be particularly relevant for ARVs that are substrates of different metabolizing enzymes and transporters.

The current study is based on retrospective evaluation of a single pharmacokinetic variable, either C_{12} or C_{24}. Although this strategy allowed us to evaluate relevant pharmacokinetic data for several patients receiving ART and represents an effective screening approach, several limitations can be identified. C_{12} and

C_{24} do not represent specific markers of a single ADME mechanism but result from a combination of absorption, distribution, metabolism and elimination processes. The effects of genetic factors on a specific ADME mechanism could be underestimated or masked by concomitant factors influencing pharmacokinetic patterns through other ADME processes. The inclusion of other pharmacokinetic variables could define a more precise quantification of rGC for specific ADME processes. For example, C_{max} represents a more specific pharmacokinetic variable for the determination of the absorption rGC, while the measurement of the full AUC might allow a better characterization of the rGC for metabolism and elimination. C_{12} or C_{24} could be a suboptimal marker of exposure considering how even a minor fluctuation in the time post-dose could result in a relevant change in drug concentrations. This phenomenon is mitigated for ARVs with long half-lives, such as efavirenz (40–55 h), nevirapine (40–50 h) and etravirine (30–40 h). Additionally, relevant factors (e.g. adherence, food effect and comorbidities) could not be controlled due to the retrospective design of the study and could potentially influence SDw and SDb, limiting the quantification of rGC. Although the current study is based on a retrospective analysis of a therapeutic drug monitoring cohort, the observed SDw and SDb were in good agreement with previous studies, especially for efavirenz, nevirapine, etravirine, tenofovir, atazanavir, maraviroc and raltegravir (Table 1 and Table S1).

Genetics is one of the many variables influencing drug distribution, which is an intricate phenotype resulting from numerous molecular, physiological and anatomical processes. Multiple genetic factors in different genes generate cumulative or antagonistic interactions, which complicate the characterization of relevant pharmacogenetic markers. Moreover, biological processes underpinning drug distribution are partially characterized and extensive knowledge gaps exist, hindering pharmacogenetic research. Consequently, the identification of clinically relevant pharmacogenetic markers is a complex research area based on challenging *in vitro* research to better describe ADME mechanisms, the inclusion of large and well-characterized cohorts of patients and costly experimental approaches. Therefore, the rGC strategy represents a useful approach to the rationalization of future pharmacogenetic studies; considering that pharmacokinetic data are available in the first stages of clinical studies, the potential role of genetics could be estimated before drug commercialization. The integration of the rGC approach in early stages of clinical research could support a more comprehensive understanding of drug pharmacokinetics and define a rational evaluation of the potential role of pharmacogenetic factors for therapy optimization. Through this approach, drugs with high rGC scores may represent optimal candidates for future genetic studies and their investigation should be prioritized.

References

1. Panel on Antiretroviral Guidelines for Adult and Adolescents. Guidelines for the Use of Antiretroviral Agents in HIV-Infected Adults and Adolescents. Department of Health and Human Services, 2010; 1–166.
2. Gonzalez de Requena D, Bonora S, Vigano O et al. Comparative evaluation of seven resistance interpretation algorithms and their derived genotypic inhibitory quotients for the prediction of 48 week virological response to darunavir-based salvage regimens. J Antimicrob Chemother 2011; 66: 192–200.
3. Rosario MC, Poland B, Sullivan J et al. A pharmacokinetic-pharmacodynamic model to optimize the phase IIa development program of maraviroc. J Acquir Immune Defic Syndr 2006; 42: 183–91.
4. McFadyen L, Jacqmin P, Wade JR et al. Maraviroc (MVC) exposure–efficacy relationship in treatment-experienced HIV-1-infected patients. In: AIDS Conference/EACS, Madrid, Spain, 2007. Poster P4.1/06.
5. la Porte C. Inhibitory quotient in HIV pharmacology. Curr Opin HIV AIDS 2008; 3: 283–
7.Hartkoorn RC, Kwan WS, Shallcross V et al. HIV protease inhibitors are substrates for

OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. Pharmacogenet Genomics 2010; 20: 112–20.
6. Minuesa G, Huber-Ruano I, Pastor-Anglada M et al. Drug uptake transporters in antiretroviral therapy. Pharmacol Ther 2011; 132: 268–79.
7. Kassahun K, McIntosh I, Cui D et al. Metabolism and disposition in humans of raltegravir (MK-0518), an anti-AIDS drug targeting the human immunodeficiency virus 1 integrase enzyme. Drug Metab Dispos 2007; 35: 1657–63.
8. Child M, Agarwala S, Boffito M et al. Effect of food on the pharmacokinetics of atazanavir when administered with food in healthy subjects. In: Eighth International Workshop on Clinical Pharmacology of HIV Therapy, Budapest, Hungary, 2007. Abstract 25.
9. Giguere P, Burry J, Beique L et al. The effect of food on the pharmacokinetics of atazanavir/ritonavir 300/100mg daily in HIV-infected patients. In: Abstracts of the Eleventh International Workshop on Clinical Pharmacology of HIV Therapy Sorrento, Italy, 2010. Abstract 30.
10. Kakuda TN, Van De Casteele T, Petrovic R et al. Bioequivalence of a darunavir/cobicistat fixed-dose combination tablet versus single agents and food effect in healthy volunteers. Antivir Ther 2014; 19: 597–606.
11. Crauwels HM, van Heeswijk RP, Buelens A et al. Impact of food and different meal types on the pharmacokinetics of rilpivirine. J Clin Pharmacol 2013; 53: 834–40.
12. Moss DM, Siccardi M, Back DJ et al. Predicting intestinal absorption of raltegravir using a population-based ADME simulation. J Antimicrob Chemother 2013; 68: 1627–34.
13. Moss DM, Siccardi M, Murphy M et al. Divalent metals and pH alter raltegravir disposition in vitro. Antimicrob Agents Chemother 2012; 56: 3020–6.
14. Mahungu T, Smith C, Turner F et al. Cytochrome P450 2B6 516G→T is associated with plasma concentrations of nevirapine at both 200 mg twice daily and 400 mg once daily in an ethnically diverse population. HIV Med 2009; 10: 310–7.
15. Rotger M, Colombo S, Furrer H et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 2005; 15: 1–5.
16. Saitoh A, Sarles E, Capparelli E et al. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. AIDS 2007; 21: 2191–9.
17. Klein K, Lang T, Saussele T et al. Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. Pharmacogenet Genomics 2005; 15: 861–73.
18. Siccardi M, D'Avolio A, Nozza S et al. Maraviroc is a substrate for OATP1B1 in vitro and maraviroc plasma concentrations are influenced by SLCO1B1 521 T>C polymorphism. Pharmacogenet Genomics 2010; 20: 759–65.
19. 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: 1222–5.
20. Schipani A, Egan D, Dickinson L et al. Estimation of the effect of SLCO1B1 polymorphisms on lopinavir plasma concentration in HIV-infected adults. Antivir Ther 2012; 17: 861–8.
21. Wyen C, Hendra H, Siccardi M et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. J Antimicrob Chemother 2011; 66: 2092–8.

22. Schipani A, Wyen C, Mahungu T et al. Integration of population pharmacokinetics and pharmacogenetics: an aid to optimal nevirapine dose selection in HIV-infected individuals. J Antimicrob Chemother 2011; 66: 1332–9.
23. Liptrott NJ, Pushpakom S, Wyen C et al. Association of ABCC10 polymorphisms with nevirapine plasma concentrations in the German Competence Network for HIV/AIDS. Pharmacogenet Genomics 2012; 22: 10–9.
24. Pushpakom SP, Liptrott NJ, Rodriguez-Novoa S et al. Genetic variants of ABCC10, a novel tenofovir transporter, are associated with kidney tubular dysfunction. J Infect Dis 2011; 204: 145–53.
25. Cortes CP, Siccardi M, Chaikan A et al. Correlates of efavirenz exposure in Chilean patients affected with human immunodeficiency virus reveals a novel association with a polymorphism in the constitutive androstane receptor. Ther Drug Monit 2013; 35: 78–83.
26. Kohlrausch FB, de Cassia Estrela R, Barroso PF et al. The impact of SLCO1B1 polymorphisms on the plasma concentration of lopinavir and ritonavir in HIV-infected men. Br J Clin Pharmacol 2010; 69: 95–8.
27. 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: 459–63.
28. Lubomirov R, Arab-Alameddine M, Rotger M et al. Pharmacogenetics-based population pharmacokinetic analysis of etravirine in HIV-1 infected individuals. Pharmacogenet Genomics 2013; 23: 9–18.
29. Kalow W, Tang BK, Endrenyi L. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. Pharmacogenetics 1998; 8: 283–9.
30. Ozdemir V, Kalow W, Tang BK et al. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics 2000; 10: 373–88.
31. Leabman MK, Giacomini KM. Estimating the contribution of genes and environment to variation in renal drug clearance. Pharmacogenetics 2003; 13: 581–4.
32. Micheli JE, Chinn LW, Shugarts SB et al. Measuring the overall genetic component of nevirapine pharmacokinetics and the role of selected polymorphisms: towards addressing the missing heritability in pharmacogenetic phenotypes? Pharmacogenet Genomics 2013; 23: 591–6.
33. D'Avolio A, Baietto L, Siccardi M et al. An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other antiretroviral agents in the plasma of HIV-infected patients. Ther Drug Monit 2008; 30: 662–9.
34. D'Avolio A, Simiele M, Baietto L et al. A validated high-performance liquid chromatography-ultraviolet method for quantification of the CCR5 inhibitor maraviroc in plasma of HIV-infected patients. Ther Drug Monit 2010; 32: 86–92.
35. D'Avolio A, Sciandra M, Siccardi M et al. A new assay based on solid-phase extraction procedure with LC-MS to measure plasmatic concentrations of tenofovir and emtricitabine in HIV infected patients. J Chromatogr Sci 2008; 46: 524–8.
36. Arab-Alameddine M, Di Iulio J, Buclin T et al. Pharmacogenetics-based population pharmacokinetic analysis of efavirenz in HIV-1-infected individuals. Clin Pharmacol Ther 2009; 85: 485–94.

37. Kwara A, Lartey M, Sagoe KW et al. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. AIDS 2009; 23: 2101–6.
38. Van Gelder J, Deferme S, Annaert P et al. Increased absorption of the antiviral ester prodrug tenofovir disoproxil in rat ileum by inhibiting its intestinal metabolism. Drug Metab Dispos 2000; 28: 1394–6.
39. van Gelder J, Deferme S, Naesens L et al. Intestinal absorption enhancement of the ester prodrug tenofovir disoproxil fumarate through modulation of the biochemical barrier by defined ester mixtures. Drug Metab Dispos 2002; 30: 924–30.
40. Moss DM, Neary M, Owen A. The role of drug transporters in the kidney: lessons from tenofovir. Front Pharmacol 2014; 5: 248.
41. Siccardi M, D'Avolio A, Rodriguez-Novoa S et al. Intrapatient and interpatient pharmacokinetic variability of raltegravir in the clinical setting. Ther Drug Monit 2012; 34: 232–5.
42. Hirano A, Ikemura K, Takahashi M et al. Short communication: lack of correlation between UGT1A1*6, *28 genotypes, and plasma raltegravir concentrations in Japanese HIV type 1-infected patients. AIDS Res Hum Retroviruses 2012; 28: 776–9.
43. Wenning LA, Petry AS, Kost JT et al. Pharmacokinetics of raltegravir in individuals with UGT1A1 polymorphisms. Clin Pharmacol Ther 2009; 85: 623–7.