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Cytochrome P450 3A5 Genotype Impacts Maraviroc Concentrations in Healthy Volunteers

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Abbreviations:

ALP, Alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; $AUC_{0-\infty}$, area under the concentration-time curve from time 0 to infinity; AUC_{0-32} , area under the curve from time 0 to 32 h; AUC_{τ} , AUC during a dosing interval; BMI, body mass index; CrCL, creatinine clearance; C_{ave} , plasma average concentration; CL/F, apparent oral clearance (clearance divided by bioavailability F); C_{max} , plasma peak concentration; CYP, a cytochrome P450; IQR, interquartile range; OATP1B1, organic anion transporting polypeptide 1B1; PCR, polymerase chain reactions; P-gp, P-glycoprotein; T_{max} , time to reach C_{max} ; UPLC-MS/MS,

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ABSTRACT

Cytochrome P450 3A5 (CYP3A5) plays a prominent role in the metabolism of maraviroc, an approved drug for HIV-1 treatment and a candidate for HIV-1 prevention. We studied the effect of CYP3A5 genotype on pharmacokinetics of maraviroc and a primary CYP3A5-dependent metabolite of maraviroc denoted as metabolite 1 (M1). Volunteers were screened for health status and CYP3A5 genotype (wild-type allele *1 and dysfunctional alleles *2, *3, *6, and *7) to obtain 24 evaluable subjects in 3 groups (N = 8 each): homozygous dysfunctional (two dysfunctional alleles), heterozygous (one *1 allele and one dysfunctional allele), and homozygous wild-type (two *1 alleles). Subjects received 300-mg maraviroc orally followed by blood collection for 32 h. The homozygous wild-type group exhibited lower mean plasma maraviroc concentrations at almost all sampling times. The median (interquartile range) maraviroc area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) was 2099 (1422-2568) ng•h/mL, 1761 (931-2640) ng•h/mL and 1238 (1065-1407) ng•h/mL, respectively, for the homozygous dysfunctional, heterozygous, and homozygous wild-type groups. The homozygous wild-type group had 41% lower maraviroc $AUC_{0-\infty}$ and 66% higher apparent clearance compared to the homozygous dysfunctional group ($P = 0.02$). The $AUC_{0-\infty}$ ratios of maraviroc to M1 in heterozygous and homozygous wild-type subjects were lower by 51% and 64% relative to the homozygous dysfunctional group, respectively ($P < 0.001$). In conclusion, the lower maraviroc concentrations in the homozygous wild-type group indicate that maraviroc may be under-dosed in people homozygous for the CYP3A5*1 allele including almost a half of African Americans.

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INTRODUCTION

Maraviroc is a Food and Drug Administration (FDA)-approved drug used in combination with other anti-HIV drugs for treatment of HIV-1 infection. It inhibits HIV-1 entry by blocking the interaction between viral envelope glycoprotein (gp120) and the chemokine (C-C motif) receptor 5 (CCR5), a coreceptor that HIV-1 uses to enter host cells (Dorr et al., 2005). HIV-1 strains predominantly utilize CCR5 as a coreceptor during transmission stage and in the early stages of HIV disease (Michael et al., 1997; Philpott, 2003). As the only available CCR5 antagonist on the market, maraviroc has been considered as a potential candidate for HIV-1 pre-exposure prophylaxis since maraviroc penetrates into the male and female reproductive tracts and colorectal tissues (Dumond et al., 2009; Brown et al., 2011), the primary sites of transmission through sexual exposure, and has favorable safety profiles (Gulick et al., 2014). Currently, maraviroc is being investigated for reducing the acquisition of HIV-1 infection with once daily oral administration alone or in combination with other anti-HIV drugs in people who are at-risk (ClinicalTrials.gov identifier NCT01505114).

Following oral administration in humans, the absorption of maraviroc has been predicted to be approximately 84% at 300 mg (Abel et al., 2008e; Abel et al., 2009a). Maraviroc undergoes extensive metabolism via N-dealkylation and oxygenation, which produces several oxidative metabolites (Walker et al., 2005). N-dealkylation is mediated by CYP3A4 (Hyland et al., 2008). According to a mass balance study using a ^{14}C -labelled dose of maraviroc, the N-dealkylated product was the major circulating metabolite in blood; and the N-dealkylated metabolite recovered in excreta accounted for 7% of the administered dose (Abel et al., 2008e). Although the abundance of each individual oxidative metabolite in plasma was less than that of the N-dealkylated metabolite, collectively, the oxygenated products accounted for ~ 40% of the

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administered maraviroc dose (Walker et al., 2005; Abel et al., 2008e; Abel et al., 2009a), suggesting that oxidative metabolism plays an important role in maraviroc elimination. Our previous in vitro metabolism study demonstrated that both CYP3A4 and CYP3A5 exhibited activity towards maraviroc (Lu et al., 2012); however, CYP3A5 played a predominant role in the formation of an abundant oxidative metabolite denoted as M1, resulting from oxygen insertion on the difluorocyclohexane ring of maraviroc (Supplemental Figure 1), with 23-fold higher maximum formation rate and 4-fold higher intrinsic clearance when compared to CYP3A4 (Lu et al., 2012).

The involvement of CYP3A5 in maraviroc oxidative metabolism indicates that maraviroc pharmacokinetics may be affected by CYP3A5 genetic polymorphisms that determine the expression and/or activity of CYP3A5. The CYP3A5*1 allele is referred to as wild-type and it is associated with the highest CYP3A5 protein expression while alternative alleles CYP3A5*3, CYP3A5*6, and CYP3A5*7 are considered to be non-functional or dysfunctional since the single-nucleotide alterations in these alleles lead to lower expression or no activity of CYP3A5 (Hustert et al., 2001; Kuehl et al., 2001; Lamba et al., 2002; Lin et al., 2002). In individuals who express CYP3A5, CYP3A5 is present in the intestines (Kolars et al., 1994) and liver with CYP3A5 content comprising up to greater than 50% of total hepatic CYP3A (Kuehl et al., 2001). Further, CYP3A5 expression varies greatly with ethnicity due to differences in allele frequency distribution. CYP3A5*3 is abundantly present in European Americans with 80-90% being CYP3A5 non-expressors (van Schaik et al., 2002; Daly, 2006) while the majority of African Americans are CYP3A5 expressors due to carrying at least one wild-type CYP3A5*1 allele and ~ 45% are homozygous CYP3A5*1 carriers (Xie et al., 2004).

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Maraviroc pharmacokinetic variations may lead to changes in anti-viral efficacy since the currently recommended maraviroc dosing regimen delivers concentrations near the top but still within the steep portion of exposure-response curve (FDA., 2007; Jacqmin et al., 2013).

Maraviroc plasma trough concentrations (C_{\min}) and average concentrations (C_{ave}) have been used for exposure-response analysis. While maximal anti-viral efficacy is achieved when C_{\min} and C_{ave} are above 50 ng/mL and 100 ng/mL (Jacqmin et al., 2013), respectively, most studies had C_{\min} below 43 ng/mL with the recommended regimen (FDA., 2007). Currently, there is limited information on the pharmacokinetics of maraviroc in individuals with different CYP3A5 genotypes, which is not only necessary to understand pharmacokinetic variations among people who are on maraviroc for treatment of HIV-1 infection but also extremely important to know if maraviroc is going to be used alone for HIV pre-exposure prophylaxis among people who are at high risk for HIV-1 infection. Therefore, we performed the current clinical study to investigate the impact of CYP3A5 polymorphism on pharmacokinetics of maraviroc and the primary CYP3A5-dependent metabolite M1.

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MATERIALS AND METHODS

Study design. This was an open label, single dose study. Inclusion criteria included age 18-65 years, healthy with no acute medical illness, no evidence of hepatic or renal impairment (liver function tests values < 1.5 Upper Limit of Normal (ULN), total bilirubin below ULN, creatinine clearance > 60 mL/min), and negative HIV test results. Any concomitant medications or herbal supplements for which there is a known risk of pharmacokinetic or pharmacodynamics drug interactions, including those that inhibit CYP3A family enzymes, were prohibited for 4 weeks prior to and during the study. The study was reviewed and approved by the institutional review board of the Johns Hopkins Medical Institutions and was conducted in compliance with national and local institutional guidelines. All participants gave written informed consent.

Volunteers were screened for health status and genotyped for CYP3A5 (*1, *2, *3, *6, and *7). Healthy volunteers were enrolled into 3 groups (N = 8 each): homozygous dysfunctional (2 dysfunctional alleles), heterozygous (1 CYP3A5*1 allele and 1 dysfunctional allele), and homozygous wild-type (2 CYP3A5*1 alleles). A single dose of 300 mg maraviroc tablet was given to the volunteers orally along with 200 mL water under direct observation. Ten mL of venous blood was collected before (0 h) and 1, 2, 3, 4, 6, 8, 10, 24 and 32 h after the drug administration. Plasma was prepared by centrifugation of blood at $1,500 \times g$ for 10 min at 4 °C and stored at -80 °C until analysis.

CYP3A5 genotyping procedure. Genomic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit (Qiagen Inc, Valencia, CA). Previously reported primers were used for amplification of polymorphic loci containing fragments: forward primer 5'- CCTGCCTTCAATTTTCACTG-3' and reverse primer 5'- GCAATGTAGGAAGGAGGGCT-

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3' for *3 (Kuehl et al., 2001); forward primer 5'-GCTGCATGTAT AGTGGAAAGGAC-3' and reverse primer 5'-GGAATTGTACCTTTTAAGTGGATG-3' for *6 (Floyd et al., 2003); and forward primer 5'-AAATACTTCA CGAATACTATGATC-3' and reverse primer 5'-CAGGGACATAATTGATTATCTTTG-3' for *7 (Chou et al., 2001). iProof™ High Fidelity DNA polymerase (Bio-Rad, Hercules, CA) was used for polymerase chain reactions (PCR) with the following program: (1) 98 °C for 30 s; (2) 98°C for 10s; (3) 65.2 °C for CYP3A5*3, 59.0°C for CYP3A5*6, 57.8°C for CYP3A5*7; (4) 72 °C for 45 s; repeat (2)-(4) for 35 cycles; and finally 72°C for 10 min. The PCR products were purified using QIAquick PCR purification kit (Qiagen, Inc.) followed by Sanger sequencing for CYP3A5 genotype determination. Non-functional allele CYP3A5*2 with a nucleotide transition from C to A (van Schaik et al., 2002), located in the fragment of PCR product amplified for *7, was also examined from the sequenced PCR product.

Measurement of plasma concentrations of maraviroc and M1. The structures of maraviroc and M1 are shown in supplemental Figure 1. Plasma maraviroc concentrations were determined using a validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method as previously reported (Emory et al., 2014), with inter- and intra-assay precision of $\leq 5.4\%$ and $\leq 6.0\%$, and inter- and intra-assay accuracy within $\pm 10.2\%$ and $\pm 8.4\%$, respectively. The validation results exceeded standards recommended by FDA (FDA., 2013). M1 was quantitated using calibration curve of 4-hydroxyphenyl maraviroc, which had the same precursor/daughter m/z ratios with M1. Fifty microliters of plasma was mixed with 50 μL of 10 ng/mL internal standard $^2\text{H}_6$ -maraviroc followed by addition of 500 μL of acetonitrile and filtration by applying vacuum. Filtrates were dried and reconstituted in 75 μL of mobile phases A (5% acetonitrile-0.1% formic acid in water) and B (5% water-0.1% formic acid in

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acetonitrile). Ten microliters was injected onto a Waters BEH C₈ UPLC column (1.7 μm, 50 × 2.1 mm) with a flow rate of 1 mL/min and analytes were detected over 8 minutes using AB SCIEX QTRAP®5500 mass spectrometer. Mobile phases were programmed at 4% B from 0 to 0.5 min, 6% B at 4.0 min, 15% B from 4.1 to 5 min, 100% B from 5.1 to 6.5 min, and 4% B from 7 to 8 min. The parent/daughter ion transitions monitored were as follows: M1 and 4-hydroxyphenyl maraviroc m/z 530.2 > 405.2; ²H₆-maraviroc m/z 520.6 > 389.1. The calibration range was linear from 0.5-1,000 ng/mL and 0.25-1,000 ng/mL, respectively, for maraviroc and M1.

Pharmacokinetic and statistical analyses. Pharmacokinetic parameters of maraviroc and M1 including area under the concentration-time curve from time 0 to infinity (AUC_{0-inf}), area under the curve from time 0 to 32 h (AUC₀₋₃₂), plasma peak concentration (C_{max}), time to reach C_{max} (T_{max}), half-life (T_{1/2}), and oral apparent clearance (CL/F) were estimated by WinNonlin (version 6.1, Pharsight, Cary, NC) by performing non-compartmental analysis. Previous studies have shown that maraviroc pharmacokinetics is time-independent with the AUC_{0-inf} of maraviroc following single doses having similar values to maraviroc AUC during a dosing interval (AUC_τ), 12 h, at steady-state with the currently recommended dosing regimen (Abel et al., 2008f; Abel et al., 2009b); the predicted C_{ave} values for multiple dosing maraviroc were estimated by AUC_{0-inf} divided by 12 h. The sample size of the study was based on our previous observation of 34% of coefficient of variation for the major oxidative metabolite formation activity in human liver microsomes to detect a 50% difference (with a 5% type I error for a two-sided test and 80% power) in the AUC of maraviroc between any two of the three CYP3A5 genotypic groups. Pharmacokinetic parameters among the three groups were compared using Kruskal-Wallis test in R (version 2.15.1); and differences between the groups with different CYP3A5 genotypes were

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assessed using Mann-Whitney-Wilcoxon test. $P < 0.05$ was considered statistically significant.

Linear regression was performed in SigmaPlot (version 12.3).

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RESULTS

Genotyping results and subject demographics. A total of 34 subjects were evaluated for CYP3A5 genotype screening. Fourteen subjects were found to be homozygous dysfunctional (41.2%, 10 CYP3A5*3/*3, 2 CYP3A5*3/*6, 1 CYP3A5*6/*7, and 1 CYP3A5*7/*7), 10 were heterozygous (29.4%, 7 CYP3A5*1/*3, 2 CYP3A5*1/*6, 1 CYP3A5*1/*7) and 10 were homozygous wild-type (29.4%, CYP3A5*1/*1). No CYP3A5*2 was found. We enrolled 25 healthy volunteers but one had no measurable maraviroc and M1 in all samples. For the 24 evaluable participants, the homozygous dysfunctional group had 5 CYP3A5*3/*3, 2 CYP3A5*3/*6, and 1 CYP3A5*6/*7; the heterozygous group had 7 CYP3A5*1/*3 and 1 CYP3A5*1/*6; and the homozygous wild-type group had 8 CYP3A5*1/*1.

Mean \pm SD of age and weight of the subjects was 41 ± 11 years and 78.2 ± 15.1 kg, respectively. Among the 24 volunteers, 17 of them were African Americans, 6 were European Americans, and one was Asian. Age, weight, body mass index (BMI), creatinine clearance (CrCL), total protein, albumin, bilirubin, and aspartate transaminase (AST) activity were similar among different genotypic groups (Table 1). Alkaline phosphatase (ALP) and alanine transaminase (ALT) were lower in homozygous wild-type group compared with the other two groups but the differences were not statistically significant. There were 6 females with 2, 1, and 3 of them distributing in homozygous dysfunctional, heterozygous and homozygous wild-type groups, respectively.

Effect of CYP3A5 polymorphism on the pharmacokinetics of maraviroc and M1. The maraviroc concentration-time profiles of the homozygous dysfunctional group were similar to those of the heterozygous group (Fig. 1A). In contrast, the homozygous wild-type group exhibited lower mean plasma maraviroc concentrations at almost all blood collection times. Compared with the homozygous dysfunctional group, the homozygous wild-type group had 41%

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lower maraviroc AUC_{0-inf} ($P = 0.02$, Fig. 1B), 66% higher CL/F ($P = 0.02$, Fig. 1C), and 33% lower but not significantly different C_{max} ($P = 0.33$). The heterozygous group exhibited large inter-individual variability in AUC_{0-inf} and CL/F, which was not statistically different from either of the other two groups. The medians (interquartile range, IQR) of pharmacokinetic parameters of maraviroc and M1 are shown in Table 2. T_{max} and $T_{1/2}$ were not statistically different among the three groups. The percentage of extrapolated AUC from the time of the last measurable concentration to infinity is small, as reflected by the values of AUC_{0-32} being very close to values of AUC_{0-inf} . Using values of AUC_{0-inf} from this current single dose maraviroc study, we estimated values of maraviroc C_{ave} at steady state if maraviroc were used for multiple doses using AUC_{0-inf} . The medians of C_{ave} in the homozygous dysfunctional and heterozygous groups were well above 100 ng/mL, the threshold value below which there is an increased risk of virological failure. In comparison, the median of predicted C_{ave} in the CYP3A5 homozygous wild-type group dropped to the threshold at a value of 103 ng/mL, and a half of people in this group (4 out of 8) had C_{ave} falling below 100 ng/mL (Table 2).

For metabolite M1, the plasma concentrations are lower in the homozygous dysfunctional group compared with the other two groups (Fig. 2A). The homozygous wild-type group exhibited 57% higher M1 AUC_{0-inf} when compared to the homozygous dysfunctional group ($P = 0.01$) while the heterozygous wild-type group was not statistically different from the homozygous dysfunctional group ($P = 0.10$) (Fig. 2B). The M1 C_{max} was not different among the three genotypic groups. Median T_{max} in the homozygous wild-type group was 1 h earlier than in the homozygous dysfunctional group, although not statistically significant ($P = 0.06$).

The influence of the CYP3A5 genotype was more evident in the AUC_{0-inf} ratio of maraviroc to metabolite M1 ($AUC_{maraviroc}/AUC_{M1}$). The values of $AUC_{maraviroc}/AUC_{M1}$ were statistically

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significant among the three groups (Kruskal-Wallis test, $P < 0.001$). Compared to the homozygous dysfunctional group that had median (IQR) of $AUC_{\text{maraviroc}}/AUC_{\text{M1}}$ at 49.5 (35.3-64.4), the homozygous wild-type group and the heterozygous group were 64% and 52% lower with median (IQR) at 17.8 (15.7-22.5) and 24.0 (19.8-25.7), respectively, and the differences were both statistically significant ($P < 0.001$, Table 2 and Fig. 3). To identify a simple method to assess the maraviroc/M1 ratio using a single blood sample, the correlation between $AUC_{0-\text{inf}}$ ratios and plasma concentration ratios of maraviroc to M1 was examined at all sample collection times and the strongest correlation was observed at 4 hr ($R^2 = 0.94$, $P < 0.001$, Fig. 4A). Similar to the $AUC_{0-\text{inf}}$ ratio, 4-hr plasma concentration ratio in the homozygous wild type and heterozygous groups were 46% ($P = 0.001$) and 56% ($P < 0.001$) lower when compared to the homozygous dysfunctional group (Fig. 4B).

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DISCUSSION

Since we previously showed that CYP3A5 played a substantial role in the oxidative metabolism of maraviroc (Lu et al., 2012), we conducted this study in order to evaluate the impact of CYP3A5 genotype on the pharmacokinetics of maraviroc and the primary CYP3A5 metabolite M1 following an oral dose of maraviroc in healthy volunteers. Maraviroc has time-independent pharmacokinetics with minimal accumulation in AUC at steady-state compared to that following a single dose (Abel et al., 2008f; Abel et al., 2009b) and HIV-1 infection status does not change maraviroc pharmacokinetics (FDA., 2007). Therefore, data from this study are relevant to the clinical situations where maraviroc is administered twice daily in HIV-1 infected patients for treatment and for the potential use in HIV-1 prevention. The values of maraviroc C_{max} and AUC in the CYP3A5 homozygous dysfunctional group from the current study are comparable to those reported in previous maraviroc pharmacokinetic studies where most participants were of European ancestry (Abel et al., 2008a; Abel et al., 2008f; Pozniak et al., 2008).

Here, we found that, compared with the CYP3A5 homozygous dysfunctional group, maraviroc AUC_{0-inf} in the CYP3A5 homozygous wild-type group decreased by 41%. The reduction is similar in magnitude to the decrease (45%) in maraviroc AUC caused by the concomitant use of CYP3A4 inducer, efavirenz, because of which, the maraviroc package inserts recommend doubling the maraviroc dose when co-administered with efavirenz (FDA., 2007; Abel et al., 2008a; Abel et al., 2009a). Furthermore, the median maraviroc C_{ave} in the CYP3A5 homozygous wild-type, 103 ng/mL, was very near the targeted C_{ave} value associated with virological success, 100 ng/mL. This indicates nearly half of those with wild-type homozygous CYP3A5 alleles fall below the target concentration for treatment.

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CYP3A including both CYP3A4 and CYP3A5 played a predominant role in maraviroc metabolism (Lu et al., 2012; Tseng et al., 2014). Hepatic metabolism has been thought to be responsible for non-renal clearance, which accounts for 77% of maraviroc total clearance (Abel et al., 2008e; Abel et al., 2009a). Previous clinical studies have shown that CYP3A inhibitors such as ketoconazole and HIV-1 protease inhibitors significantly increased maraviroc exposure (Abel et al., 2008b). Among the HIV-1 protease inhibitors, ritonavir, saquinavir, and nelfinavir exhibit non-selective inhibition towards CYP3A4 and CYP3A5 while amprenavir and indinavir preferentially inhibit CYP3A4 with a weaker inhibitory effect on CYP3A5 (Granfors et al., 2006). If maraviroc is used in combination with drugs that are not CYP3A inhibitors for HIV-1 treatment or used alone for HIV-1 pre-exposure prophylaxis, a difference in maraviroc exposure between the CYP3A5 homozygous dysfunctional and homozygous wild-type groups is expected based on our study results; if maraviroc is used concurrently with drugs that selectively inhibit CYP3A4, the difference in maraviroc exposure between the CYP3A5 homozygous dysfunctional and homozygous wild-type groups could be even greater than what we observed in the present study. Lower maraviroc exposure in the CYP3A5 homozygous wild-type group may indicate the potential for failure of HIV-1 treatment or prevention and the development of drug resistance.

The reduced maraviroc exposure in homozygous carriers for CYP3A5*1 is an impactful finding since CYP3A5 is more frequently expressed in people with African ancestry, especially when combined with the fact that African Americans are disproportionately infected with HIV-1 and at greater risk of acquiring HIV-1 infection compared to European Americans (Moore, 2011).

Maraviroc was approved based on the MOTIVATE trials comprised of 85% individuals with European ancestry (Gulick et al., 2008) and most previous maraviroc pharmacokinetic studies were conducted in participants with European Ancestry, with the median percentage of

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participants with European Ancestry at 78.5% (Abel et al., 2008a; Abel et al., 2008b; Abel et al., 2008d; Abel et al., 2008c; Abel et al., 2008f; Chan et al., 2008; Pozniak et al., 2008; Dumond et al., 2009; Andrews et al., 2010; Ramanathan et al., 2010; Brown et al., 2011; Kakuda et al., 2011; Gruber et al., 2013; Mora-Peris et al., 2013; Taiwo et al., 2013; Vourvahis et al., 2013). A few studies compared maraviroc concentrations in individuals with African ancestry to that with European ancestry concluding no differences or higher concentrations in individuals with African ancestry (FDA., 2007; Okoli et al., 2012), which may be due to the use of CYP3A inhibitors such as ritonavir in the optimized background therapy where CYP3A5 activity may have been inhibited in individuals who are carrying CYP3A5*1 allele. Nevertheless, a recent maraviroc exposure-response relationship assessment suggested that ethnicity is a prognostic factor of virologic success in the final model with an odds ratio of 0.35 for people with African ancestry versus people with European ancestry when controlling for other prognostic factors (Jacqmin et al., 2013). Our finding of lower maraviroc exposure in homozygous CYP3A5*1 allele carriers indicates that people with this genotype may be at risk of HIV-1 treatment failure. Maraviroc concentrations associated with HIV-1 prevention have not been established, but this pharmacokinetic difference causing lower maraviroc concentrations in many African-Americans warrants caution in future development of maraviroc as HIV-1 pre-exposure prophylaxis. The absolute bioavailability of a 100 mg oral dose of maraviroc was 23% and has been predicted to be 33% at 300 mg (Abel et al., 2009a). The low bioavailability may be due to extensive pre-systemic first-pass intestine and hepatic metabolism. In this study, we found that oral apparent clearance increased but half-life did not change in the CYP3A5 homozygous wild-type group compared with the CYP3A5 homozygous dysfunctional group. Since CYP3A5 is present in the gut (Kivisto et al., 1996) and represents up to more than 50% of total CYP3A content in liver

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(Kuehl et al., 2001) in individuals who express CYP3A5, we suspect that the reduced maraviroc AUC in the CYP3A5 homozygous wild-type group may be a result of decreased bioavailability due to enhanced CYP3A5-mediated maraviroc oxidative metabolism in gut and liver of those with this genotype which was suggested by the lower AUC_{0-inf} ratio of maraviroc to M1 when compared to the homozygous dysfunctional group. The higher AUC_{0-inf} ratio of maraviroc to M1 in the homozygous dysfunctional group is concordant with our previous in vitro observation that CYP3A5 was the major enzyme responsible for M1 formation. Besides M1, CYP3A5 was involved in the formation of several other oxidative metabolites (M2-M6) (Lu et al., 2012), which we did not measure in this study. Therefore, the CYP3A5 activity towards maraviroc may, at least partially, contribute to the reduced maraviroc AUC_{0-inf} in the CYP3A5 homozygous wild-type group. Investigations of the pharmacokinetics of other CYP3A5 substrate drugs such as tacrolimus have also suggested that CYP3A5 polymorphism could affect drug bioavailability (Asberg et al., 2013). In addition, we observed a strong correlation between AUC_{0-inf} ratios and 4-hr plasma concentration ratios of maraviroc to M1 in this study, which indicates that measuring concentration ratios of maraviroc/M1 at 4 hr following an oral maraviroc dose may be used as a simple method to predict the AUC_{0-inf} ratios of maraviroc to M1 in order to evaluate the CYP3A5 genotypic effect on maraviroc pharmacokinetics if intensive samplings are impractical under certain circumstances.

Previous maraviroc clinical studies demonstrated large inter-patient pharmacokinetic variability (50% coefficient of variation) for maraviroc (FDA., 2007), however, the reasons were not clear. Our results suggest that the CYP3A5 genotype contributes to the variability of maraviroc exposure. We observed large variability in maraviroc AUC_{0-inf} in the heterozygous group. The assignment of a wild-type allele was based on the absence of the common dysfunctional alleles

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CYP3A5*2, *3, *6, and *7. Besides these variants, other CYP3A5 non-functional alleles have been found with much lower frequency (van Schaik et al., 2002; Lee and Goldstein, 2005); however, these alleles were not screened for in this study. Maraviroc pharmacokinetic variations could also be introduced by CYP3A4*22, an allele with 5.3% frequency in European Americans correlating with decreased CYP3A4 activity (Wang et al., 2011; Elens et al., 2013), or induction/inhibition of CYP3A activity by food, juice, unknown herbal supplements, or previous medications. In addition, maraviroc is a substrate for P-glycoprotein (P-gp; also known as MDR1) (Walker et al., 2005), a drug efflux pump located on intestinal epithelial cells which limits drug absorption, and organic anion transporting polypeptide 1B1 (OATP1B1) (Siccardi et al., 2010), an influx transporter located on the basolateral membrane of hepatocytes mediating hepatic uptake of drugs. The polymorphism-dependent expression of these transporters has been reported to affect maraviroc plasma concentrations (Hoffmeyer et al., 2000; Schaeffeler et al., 2001; Walker et al., 2005; Fung and Gottesman, 2009; Siccardi et al., 2010). The polymorphisms in these genes, which we did not evaluate in this study, may also contribute to the maraviroc pharmacokinetic variations.

The present investigation involved a small number of subjects in each of the three CYP3A5 genotypic groups (N = 8). The results merit confirmation via studies with larger sample size. In conclusion, we observed a reduction of maraviroc AUC_{0-inf} almost by half and 66% higher maraviroc oral clearance in the CYP3A5 homozygous wild-type group, raising concern that maraviroc may be under-dosed in people who are homozygous for the CYP3A5*1 allele, which includes nearly half of all African Americans, a population with a disproportionately large impact of HIV-1 infection.

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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Lu, Hendrix, Bumpus, Fuchs

Conducted study and experiments: Lu, Hendrix, Bumpus, Fuchs

Performed data analysis: Lu, Hendrix, Bumpus

Wrote or contributed to the writing of the manuscript: Lu, Hendrix, Bumpus, Fuchs

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FOOTNOTES

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FIGURE LEGENDS

Fig. 1. Reduced maraviroc exposure and increased clearance in individuals homozygous for wild-type CYP3A5*1 allele. (A) Mean (\pm SE) plasma concentrations of maraviroc after administration of a single oral dose of 300 mg maraviroc. Box plots of (B) maraviroc $AUC_{0-\infty}$ and (C) oral apparent clearance ($N = 8$ in each group).

Fig. 2. The CYP3A5 homozygous dysfunctional group has reduced plasma M1 concentrations. (A) Mean (\pm SE) plasma concentrations of M1 after maraviroc administration. (B) $AUC_{0-\infty}$ of metabolite M1. $N = 8$ for each of the three groups.

Fig. 3. Carrying CYP3A5*1 allele caused reduction of $AUC_{0-\infty}$ ratio of maraviroc to metabolite M1. Statistical test was performed using Wilcoxon rank sum test in R. Bonferroni post hoc test significance level $P < 0.017$.

Fig. 4. Four-hour plasma concentration ratios were strongly correlated with plasma $AUC_{0-\infty}$ ratios of maraviroc to M1. (A) Scatter plot of $AUC_{0-\infty}$ ratios against 4-hour plasma concentration ratios with fitted linear regression line (middle thick black line), 95% confidence interval (next outer curved lines) and the prediction band (broken lines). An extreme value ($x = 310$, $y = 142$) from an individual with genotype of CYP3A5*3/*3 was excluded ($R^2 = 0.87$ when included). (B) CYP3A5 genotype effect on 4-hour plasma concentration ratio of maraviroc to M1. The horizontal lines represent medians.

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TABLE 1. Demographic information of the healthy volunteers.

Group	No CYP3A5*1 (N=8)	One CYP3A5*1 (N=8)	Two CYP3A5*1 (N=8)
Age[#] (year)	45.1 ± 12.7	39.4 ± 11.7	38.0 ± 8.8
Weight[#] (Kg)	84.0 ± 15.6	76.7 ± 14.9	73.8 ± 14.5
BMI^{†#} (Kg/m²)	26.4 ± 4.5	24.9 ± 3.6	23.8 ± 3.6
CrCL^{‡#} (mL/min)	114.3 ± 28.8	103.2 ± 15.2	100.1 ± 30.2
Total Protein[#] (g/dL)	7.0 ± 0.4	7.5 ± 0.4	7.1 ± 0.3
Albumin[#] (g/dL)	4.6 ± 0.3	4.6 ± 0.1	4.5 ± 0.3
Bilirubin[#] (mg/dL)	0.5 ± 0.2	0.4 ± 0.3	0.5 ± 0.2
ALP[#] (IU/L)	73.3 ± 16.7	74.9 ± 17.0	54.9 ± 14.3
ALT^{‡#} (IU/L)	17.5 ± 4.5	18.5 ± 6.8	13.9 ± 3.4
AST[#] (IU/L)	19.6 ± 2.9	22.4 ± 4.9	20.0 ± 2.9
No. of Female/male	2/6	1/7	3/5

Abbreviations: BMI, body mass index; CrCL, creatinine clearance; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase.

[#]Data are shown as mean ± SD.

[†]Calculated as weight/(height × height).

[‡]Calculated as [(140-age(year)) × weight (kg)]/[72 × serum creatinine concentration (mg/dL)]
 (multiply by 0.85 for female).

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TABLE 2. Pharmacokinetic parameters (medians [interquartile range]) of maraviroc and metabolite M1.

Group	No CYP3A5*1 (N=8)	One CYP3A5*1 (N=8)	Two CYP3A5*1 (N=8)
Maraviroc			
C_{max} (ng/mL)	505(348-959)	435(210-889)	339 (237-552)
T_{max} (h)	2.5(2.0-3.8)	2.0(1.2-3.0)	2.0(1.0-3.5)
AUC₀₋₃₂ (ng·h/mL)	2056(1454-2416)	1718(1200-2513)	1180(1038-1350)*
AUC_{0-inf} (ng·h/mL)	2099(1422-2568)	1761(931-2640)	1238(1065-1407)*
T_{1/2} (h)	7.6(7.1-8.7)	7.4(6.5-10.8)	8.7(8.0-9.7)
CL/F (L/h)	146(117-212)	173(114-343)	243(213-282)*
C_{ave} (ng/mL)^a	175(126-207)	147(103-214)	103 (90-117)*
C_{ave} < 100 ng/mL^b	0% (0/8)	25%(2/8)	50%(4/8)
M1			
C_{max} (ng/mL)	10.1(6.5-18.5)	20.7(7.6-30.1)	15.9(15.2-18.9)
T_{max} (h)	3.0(2.3-3.8)	2.0(1.3-3.0)	2.0(1.0-2.0)
AUC₀₋₃₂ (ng·h/mL)	40.4(36.0-48.2)	90.1(49.2-117.9)	61.0(56.2-70.6)*
AUC_{0-inf} (ng·h/mL)	41.1(32.2-53.1)	91.9(38.7-133.8)	64.5(57.4-82.9)*
AUC_{0-inf} ratio (maraviroc/M1)	49.5(35.3-64.4)	24.0(19.8-25.7)***	17.8(15.7-22.5)***

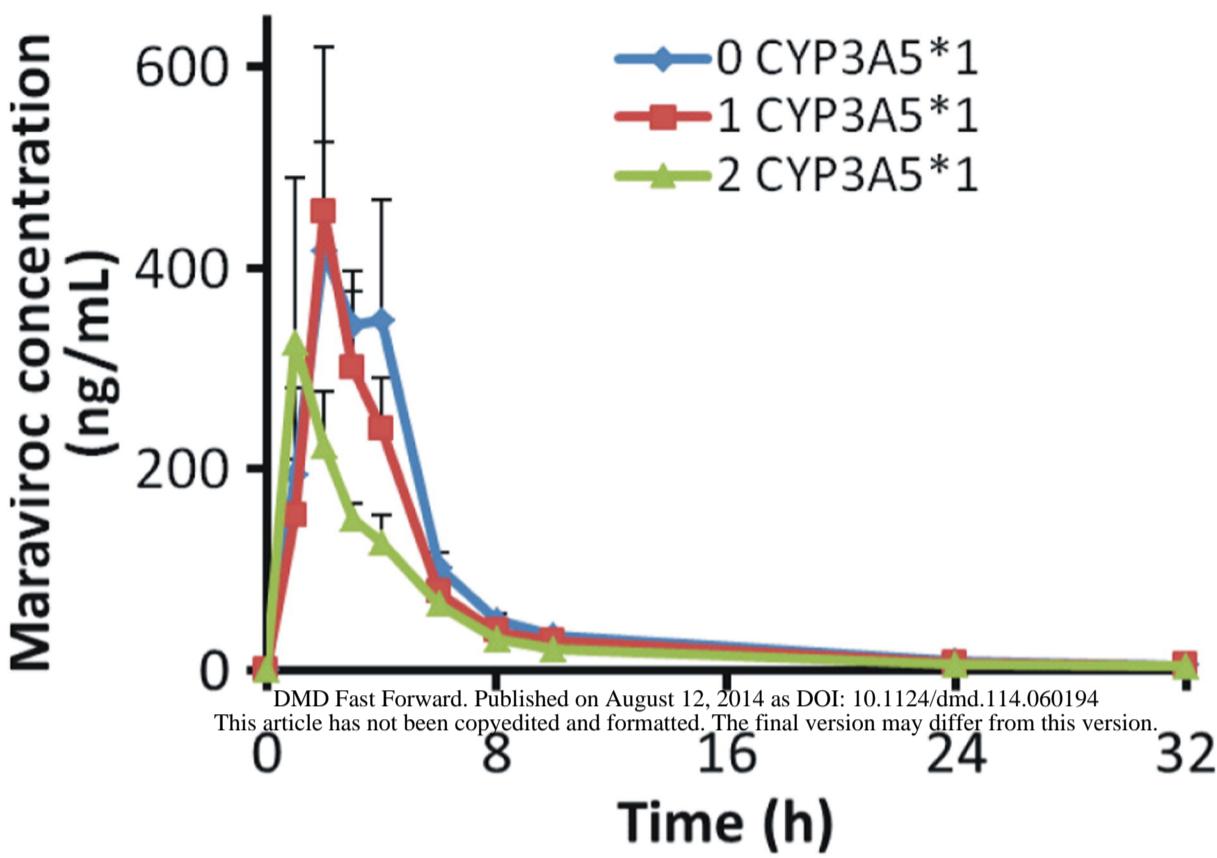
^aPlasma average concentrations (C_{ave}) values for multiple dosing maraviroc at steady state were estimated as AUC_{0-inf} of maraviroc following a single dose of 300 mg maraviroc divided by the dosing interval (12 hr) of the current recommended maraviroc dosing regimen for HIV-1 treatment.

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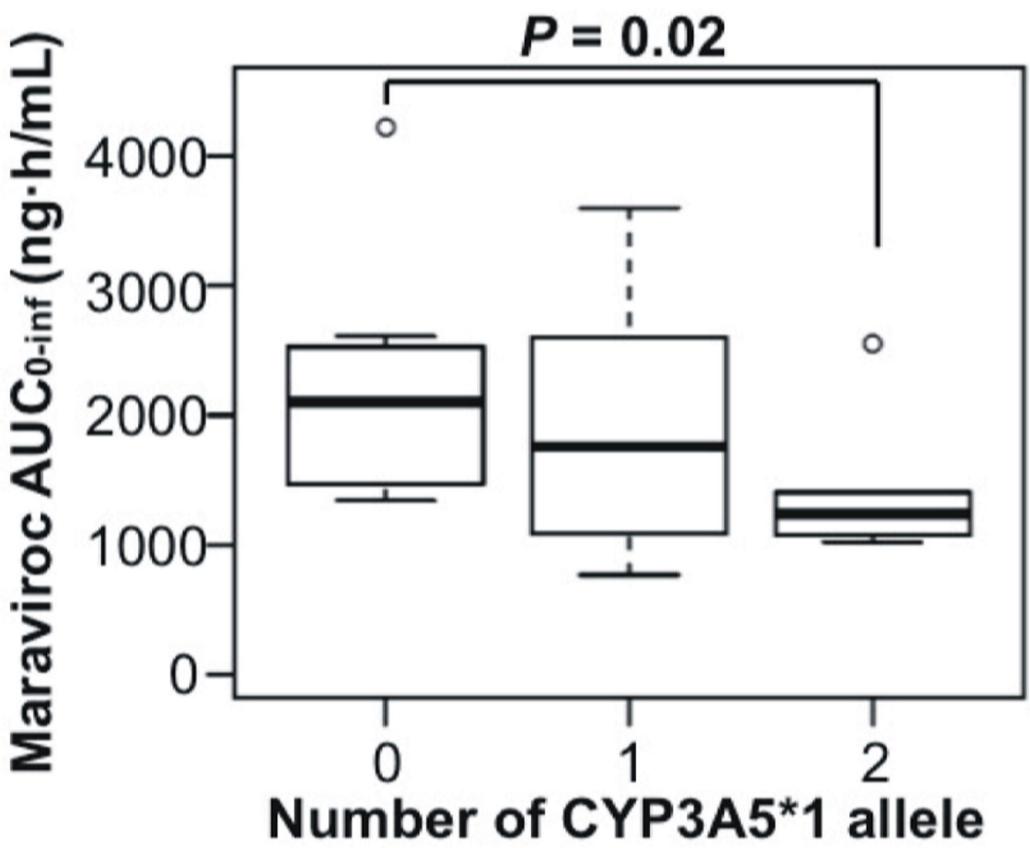
^bPercentage of individuals who had C_{ave} below 100 ng/mL.

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with homozygous dysfunctional group of individuals who did not carry a wild-type allele CYP3A5*1.

A



B



C

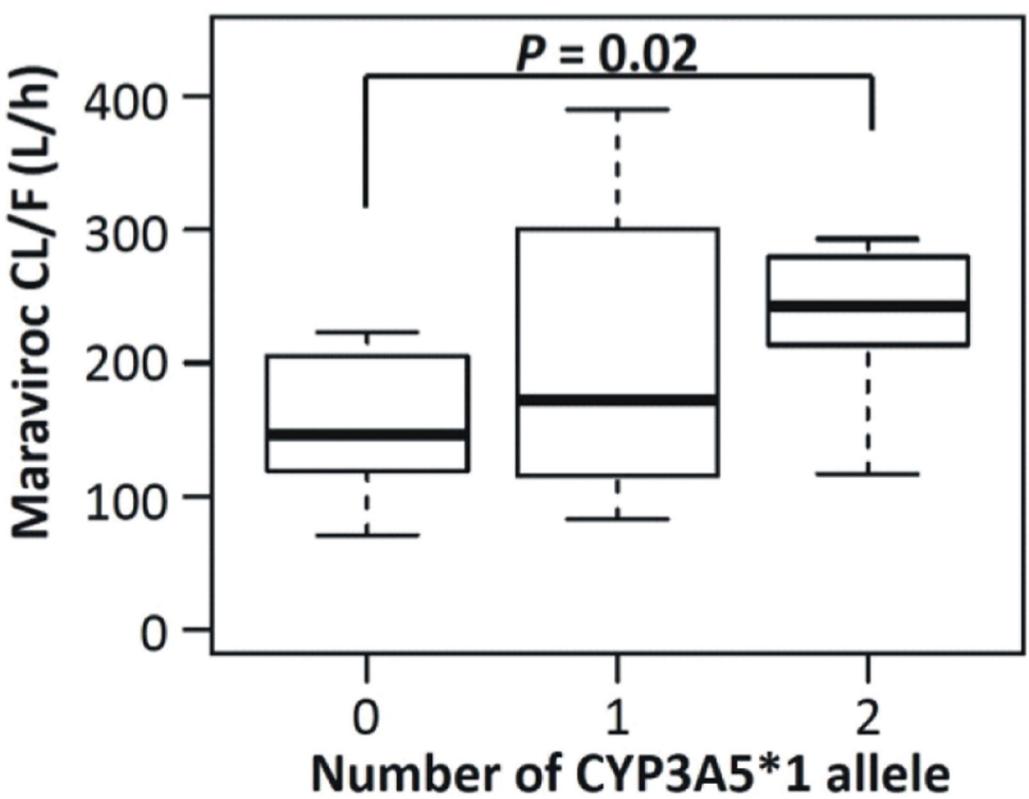
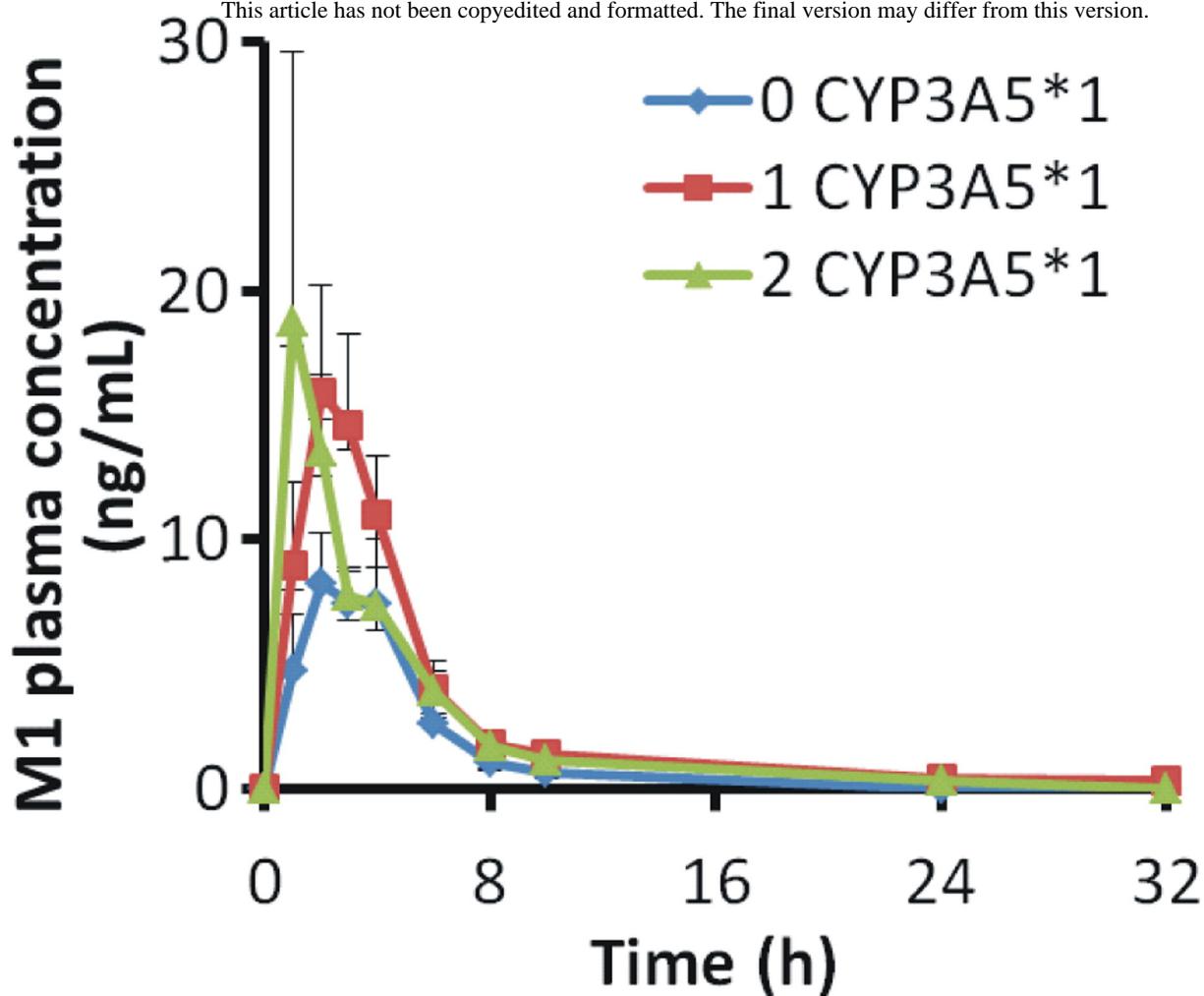


Figure 2

A

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B

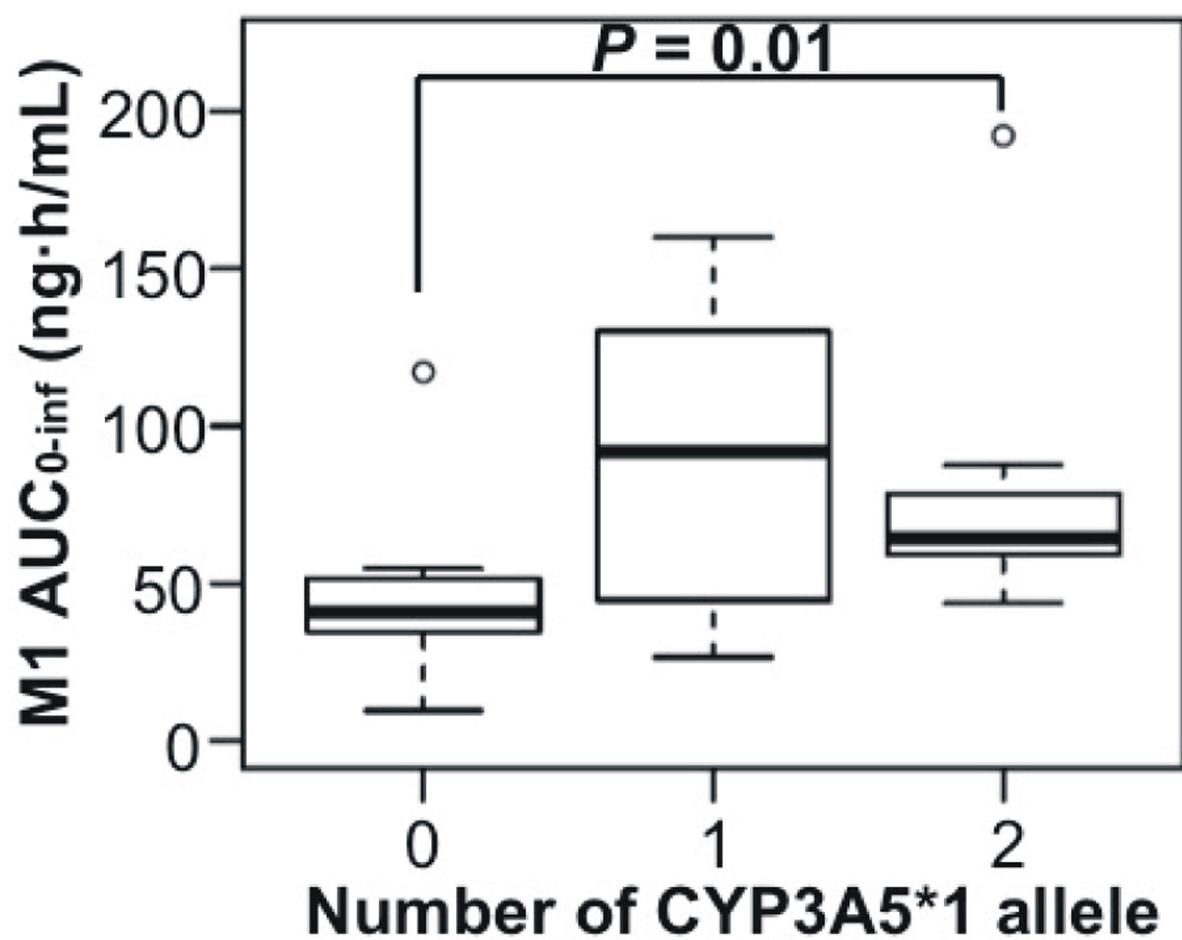
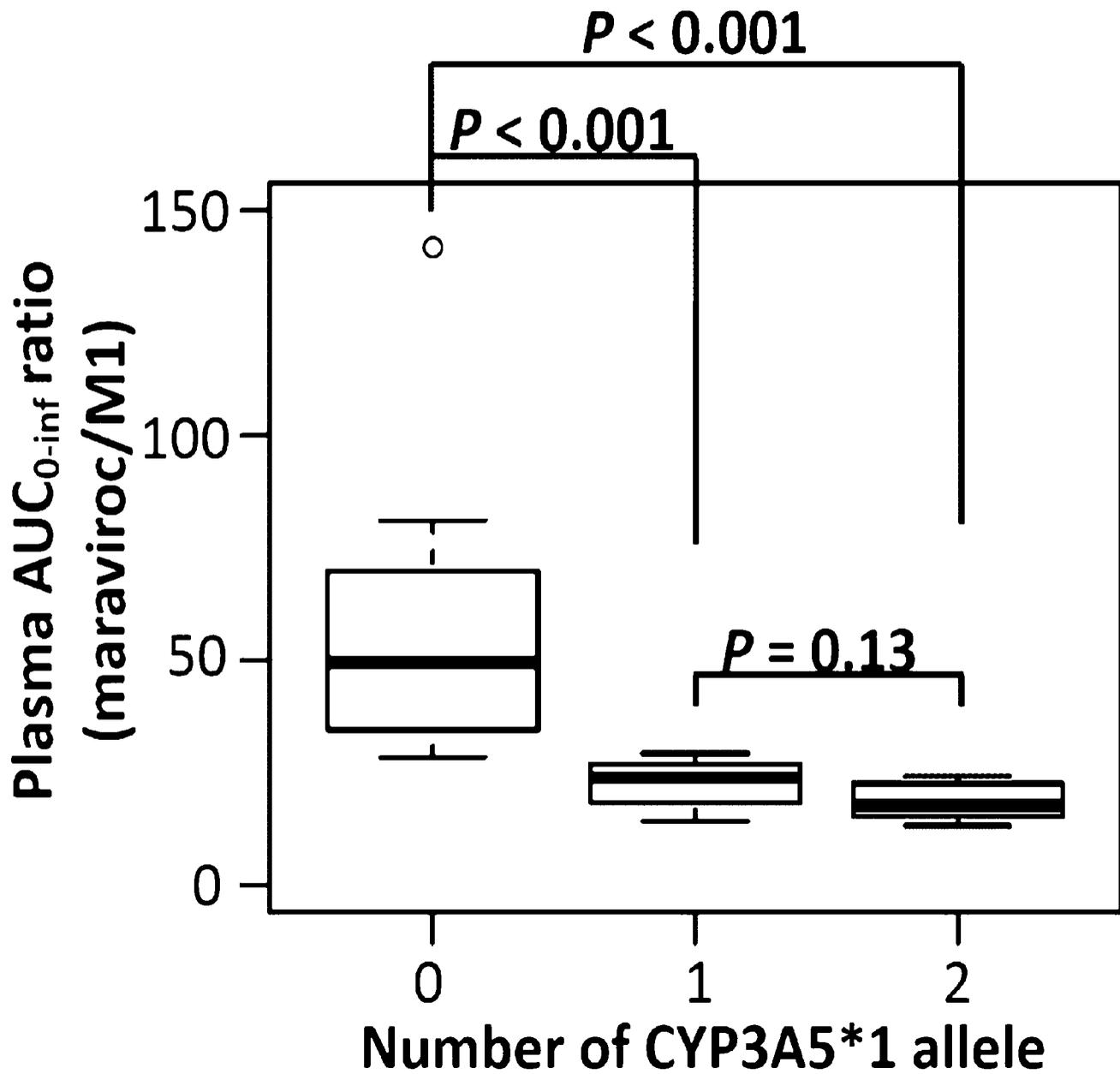
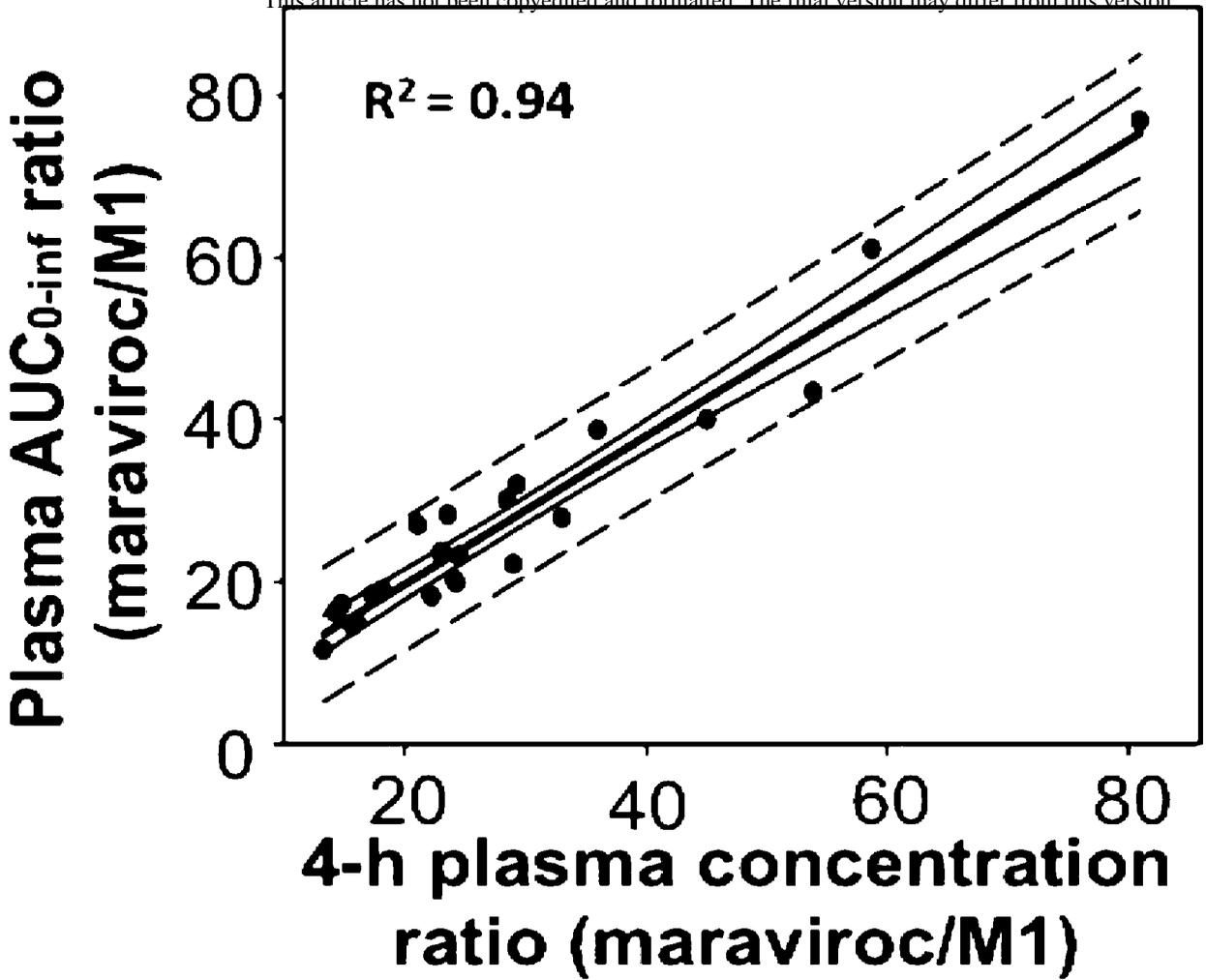


Figure 3



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A



B

