

**ABCG2 Deficiency Does Not Alter Dolutegravir Metabolism and  
Pharmacokinetics**

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**Abbreviations:** ABCB1, ATP-binding cassette subfamily B member 1; ABCG2, ATP-binding cassette subfamily G member 2; AUC, area under concentration-time curve; CL, clearance; CYP, cytochrome P450; DTG, dolutegravir; HIV, human immunodeficiency virus; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PPIX, protoporphyrin IX; SNP, single nucleotide polymorphisms;  $t_{1/2}$ , elimination half-life; UGT, UDP-glucuronosyltransferase; UPLC-QTOFMS, ultra-performance liquid chromatography and quadrupole time-of-flight mass spectrometry;  $V_z$ , volume of distribution; WT, wild-type

**Topic Category:** Metabolism, Transport and Pharmacogenomics

## ABSTRACT

Dolutegravir (DTG) is a potent integrase inhibitor of human immunodeficiency virus. Because DTG is a substrate of the efflux transporter ABCG2 and ABCG2 is highly polymorphic, we asked whether dose adjustment of DTG is needed for ABCG2-deficient individuals. Using Abcg2-null mice, the current work investigated the impact of ABCG2 deficiency on DTG metabolism and pharmacokinetics. Compared to WT mice, no statistically significant difference was found in the systemic and tissue specific (liver, kidney, and brain) pharmacokinetics of DTG in Abcg2-null mice. In addition, ABCG2 deficiency had no statistically significant impact on the production and excretion of DTG metabolites. In summary, this study demonstrated that deficiency of ABCG2 does not alter DTG metabolism and pharmacokinetics, suggesting that dose adjustment of DTG is not needed for individuals with ABCG2 deficiency.

## **SIGNIFICANCE STATEMENT**

The current work demonstrated that deficiency of ABCG2 does not alter DTG metabolism and pharmacokinetics, suggesting that dose adjustment of DTG is not needed for individuals with ABCG2 deficiency.

## Introduction

Dolutegravir (DTG) is an integrase inhibitor of human immunodeficiency virus (HIV) with potent antiretroviral activity (Cahn et al., 2013; Raffi et al., 2013). DTG overcomes HIV drug resistance to the first generation of integrase inhibitors (Hightower et al., 2011; Eron et al., 2013). In addition, DTG inhibits the replication of viral strains resistant to other marketed anti-HIV drugs, including nucleoside and non-nucleoside reverse transcriptase inhibitors (Kobayashi et al., 2011). Therefore, DTG-containing regimens are highly recommended for antiretroviral therapy (Phillips et al., 2017).

DTG is rapidly absorbed after oral administration (Min et al., 2010). The predominant circulating component in plasma is DTG, while major metabolites found in urine and feces are glucuronide M2, cysteine adduct M4, and the hydroxylated metabolite M3 and its hydrolytic products M1 and M5 (Castellino et al., 2013; Zhu et al., 2018a) (Fig. 1). Among these metabolites, M2 and M3 are produced by UDP-glucuronosyltransferase (UGT) 1A1 and cytochrome P450 (CYP) 3A4, respectively (Reese et al., 2013). The identification of M4 indicates the production of a reactive intermediate that interacts with glutathione. In addition to the Phase I and II metabolism of DTG, a cell-based assay suggests that DTG is a substrate of ATP-binding cassette subfamily B member 1 (ABCB1) and ATP-binding cassette subfamily G member 2 (ABCG2) (Fig. 1) (Reese et al., 2013).

ABCG2 is an efflux transporter expressed in multiple organs including the liver, kidney, and brain (Maliepaard et al., 2001; Fetsch et al., 2006). In the liver, ABCG2 is expressed on the bile canalicular membrane of hepatocytes, and plays a vital role in biliary excretion of a broad range of drugs (Maliepaard et al., 2001; Fetsch et al., 2006). In the brain, ABCG2 is one of the primary components in blood-brain barrier extruding xenobiotics and endobiotics from the brain (Eisenblätter et al., 2003; Nicolazzo and Katneni, 2009). Therefore, blockage and/or deficiency of ABCG2 can increase the hepatic, cerebral and systemic drug exposure and potentially lead to adverse drug reactions (Hirano et al., 2005). For instance, a high exposure of rosuvastatin and atorvastatin has been observed in individuals with ABCG2 deficiency (Keskitalo et al., 2009; Wan et al., 2015). Because ABCG2 is important in modulating drug disposition, dose adjustment should be considered for ABCG2-deficient individuals receiving drugs as ABCG2 substrates (Hira and Terada, 2018).

ABCG2 deficiency is usually caused by loss-of-function mutations of ABCG2. Forty-three single nucleotide polymorphisms (SNP) have been identified in genetic analysis of ABCG2 (Iida et al., 2002). SNP rs2231142 resulted in a replacement of glutamine by lysine, leading to a lower expression level of ABCG2 and influencing the clinical outcomes of drugs that are ABCG2 substrates (Imai et al., 2002; Hira and Terada, 2018). Although cell-based assays revealed ABCB1 and ABCG2 as transporters of DTG (Reese et al., 2013), high plasma DTG concentrations were only observed in individuals with ABCG2 SNP rs2231142 (Tsuchiya et al., 2017).

Neurotoxicity is one of the major adverse effects of DTG (de Boer et al., 2016; Elzi et al., 2017), which leads to DTG discontinuation in ~6% patients (Hoffmann et al., 2017). Although the mechanism of DTG neurotoxicity remains unclear, high plasma DTG concentrations are associated with DTG neurotoxicity (Yagura et al., 2017; Parant et al., 2018). All these data raised the concern whether dose adjustment of DTG is needed in ABCG2-deficient individuals.

The current study aimed to investigate the impact of ABCG2 deficiency on DTG metabolism and disposition (Fig. 1). Abcg2-null mice were used to mimic the ABCG2-deficient condition in humans. Systemic and tissue specific (liver, kidney, and brain) pharmacokinetics of DTG were compared between WT and Abcg2-null mice. In addition, the excretions of DTG and its metabolites were investigated in the urine and feces of WT and Abcg2-null mice.

## Materials and Methods

**Chemicals and Reagents.** DTG and protoporphyrin IX (PPIX) were purchased from Sigma-Aldrich (St. Louis, MO). Solvents for metabolite analysis were purchased from Fischer Chemical (Fair Lawn, NJ).

**Animals and Treatments.** Abcg2-null mice were originally generated in Dr. Schinkel's group (Jonker et al., 2002) and purchased from Taconic Biosciences (Hudson, NY). Abcg2-null mice were verified by polymerase chain reaction (PCR) genotyping. The primers used for genotyping were: 5'-CTTCTCCATTCATCAGCCTCG-3'; 5'-CAGTCGATGGATCCACTTAGG-3'; and 5'-GGAGCAAAGCTGCTATTGGC-3'. A PCR band at 368 bp indicated the WT allele, while the ABCG2-null allele was evident with a 594 bp band. To validate ABCG2 function in Abcg2-null mice, PPIX, an endogenous substrate of ABCG2 was measured in liver samples collected from WT and Abcg2-null mice (male, 7-10 weeks, n = 4 for each group) without any treatment. For the pharmacokinetic study, WT and Abcg2-null mice (male, 7-10 weeks, n = 4 for each group) were treated with DTG (20 mg/kg, p.o.). Blood, liver, kidney, and brain were collected at different time points (0-48 h) after DTG treatment. Another two groups of WT and Abcg2-null mice (n = 4 for each group) were treated with DTG (20 mg/kg, p.o.) and housed separately in metabolic cages overnight to collect urine and feces. All animal studies were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.



**Sample Preparation for Metabolite Analysis.** The extraction of DTG and its metabolites from sera, tissues, urines and feces was performed according to previous reports with minor modification (Bennetto-Hood et al., 2014; Liu et al., 2017; Zhu et al., 2018b). For serum, 15  $\mu$ L of serum sample was mixed with 90  $\mu$ L of acetonitrile followed by vortexing and centrifugation to precipitate the protein. For the liver, 100 mg of liver sample was homogenized in 600  $\mu$ L of water/methanol (1:2, v/v), and 600  $\mu$ L of acetonitrile was added to the resulting suspension. Then the mixture was vortexed for 2 min followed by centrifugation at 15000 rpm for 10 min. For the urine, 100  $\mu$ L of urine was mixed with 400  $\mu$ L acetonitrile followed by centrifugation at 15,000 rpm for 10 min. Feces were homogenized in water (50 mg feces in 500  $\mu$ L water). Four hundred  $\mu$ L of acetonitrile was added to 200  $\mu$ L of the resulting suspension, and vortexed for 1 min. Subsequently, the resulting mixture was centrifuged at 15,000 rpm for 10 min. PPIX in mouse liver was extracted according to a previous report (Wang et al., 2019). Briefly, 50 mg of liver sample was homogenized in 250  $\mu$ L of water, and the resulting 100  $\mu$ L of suspension was mixed with 200  $\mu$ L of methanol/acetonitrile (1:1, v/v) followed by centrifugation at 15,000 rpm for 10 min. Metabolites in the final supernatants were analyzed by ultra-performance liquid chromatography and quadrupole time-of-flight mass spectrometry (UPLC-QTOFMS, Waters Corporation, Milford, MA).

**UPLC-QTOFMS Analysis and pharmacokinetic (PK) analysis.** The analyses of

DTG and its metabolites were performed on an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm) coupled with a SYNAPT G2 mass spectrometer (Waters Corporation, Milford, MA). Column temperature was maintained at 50 °C. The mobile phases for metabolite analysis were A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile), and were delivered at a flow rate of 0.5 mL/min with a gradient elution (0.0-1.0 min, 2% B; 1.0-12.0 min, 2-95% B; 12.0-20.0 min, 95% B; 20.0-20.5 min, 95-2% B; 20.5-24 min, 2% B). PPIX assay was conducted using a previously reported method (Wang et al., 2019). In brief, PPIX analysis was performed on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) and acetonitrile/water containing 2 mM ammonium bicarbonate and 0.05% aqueous ammonia was used as the mobile phase. The QTOFMS system was operated in both positive and negative modes with electrospray ionization. The detailed mass parameters were the same as previously reported (Zhu et al., 2018b). Collision energy ramping from 15 to 45 eV was used for structural elucidations of DTG metabolites. DTG levels in serum, liver, kidney, brain, feces, and urine were determined by the calibration curve made by different concentrations of DTG standard spiked in the corresponding blank tissue matrix. The quantification method of DTG was validated by accuracy, linearity and recovery. DTG metabolites were identified based on accurate mass measurement (mass errors less than 10 ppm) and MS/MS fragmental analysis, and were relatively qualified using their peak areas of the mass signals using MassLynx (Milford, MA). Systemic PK parameters were calculated by non-compartmental model analysis on Phoenix WinNonlin. Tissue PK

parameters were calculated by Graphpad Prism.

**Statistical Analysis.** Data are expressed as means  $\pm$  SD. Statistical analyses were performed with two-tailed Student's t-test (for two groups) or two-way ANOVA (for multiple groups).  $P < 0.05$  was considered as statistically significant.

## Results

### Systemic Pharmacokinetics of DTG in WT and Abcg2-null Mice

Abcg2-null mice were confirmed by PCR genotyping (Fig. 2A). In addition, we used PPIX, a well-known substrate of ABCG2 (Jonker et al., 2002; Jonker et al., 2007), to validate the function of ABCG2 in Abcg2-null mice. As expected, PPIX level was statistically significantly increased in the liver of Abcg2-null mice when compared to WT mice (Fig. 2B), indicating that ABCG2 is functionally deficient in Abcg2-null mice. After DTG treatment, it was quickly absorbed in both WT and Abcg2-null mice (Fig. 2C). Because DTG is a substrate of ABCG2 (Reese et al., 2013), we hypothesized that deficiency of ABCG2 slows down DTG excretion and leads to its accumulation in the body. However, no statistically significant difference was found in the area under serum DTG concentration-time curve ( $AUC_{0-48}$ ) of WT and Abcg2-null mice (Fig. 2C and 2D). In addition, no genotype-related differences were found in other pharmacokinetic parameters including clearance (CL), elimination half-life ( $t_{1/2}$ ), or volume of distribution ( $V_z$ ) (Fig. 2E-2G). These results indicate that deficiency of ABCG2 does not statistically significantly alter the

systemic pharmacokinetics of DTG.

### **Tissue Specific Pharmacokinetics of DTG in WT and Abcg2-null Mice**

Because ABCG2 is expressed in the liver, kidney, and brain (Maliepaard et al., 2001; Fetsch et al., 2006), we examined DTG levels in these organs of Abcg2-null mice. After DTG treatment, high levels of DTG were observed in the liver and kidney of both WT and Abcg2-null mice (Fig. 3A-D). The levels of DTG in the brain were much lower than that in the liver and kidney (Fig. 3E-F). However, no genotype-related difference was found in DTG pharmacokinetics in the liver, kidney, or brain of WT and Abcg2-null mice (Fig. 3A-F). These data indicate that ABCG2 is not critical in modulating DTG excretion from the liver, kidney, and brain.

### **Excretion of DTG in WT and Abcg2-null Mice**

In humans, DTG is mainly excreted through feces (Castellino et al., 2013). We observed a similar pattern of DTG excretion in mice as a high level of DTG was found in feces, but not in urine (Fig. 4 A, B). If ABCG2 is important in DTG excretion, DTG level should decrease in feces and may increase in urine of Abcg2-null mice. However, DTG level in feces of Abcg2-null mice did not decrease when compared to WT mice and it actually slightly increased (Fig. 4A). In addition, no statistically significant differences in DTG levels were observed in the urine of WT and Abcg2-null mice (Fig. 4B). These data suggest that ABCG2 deficiency does not alter DTG excretion.

### **Metabolism of DTG in WT and Abcg2-null Mice**

DTG metabolites were identified in both feces and urine, although their abundances were much lower than the parent drug (Fig. 4C-F). We hypothesized that deficiency of ABCG2 will retain DTG in the liver of Abcg2-null mice, which will increase DTG metabolism because DTG metabolizing enzymes, including CYP3A and UGT, are highly expressed in the liver (Guengerich, 1999; Nakamura et al., 2008). However, no statistically significant increase was found among the four major metabolites of DTG (M1, M2, M3 and M4) in Abcg2-null mice (Fig. 4G, H). These data indicate that ABCG2 deficiency does not statistically significantly alter DTG metabolism.

### **Discussion**

Abcg2-null mice have been used to explore the impact of ABCG2 deficiency on drug metabolism and pharmacokinetics. Both pitavastatin and nitrofurantoin are ABCG2 substrates, and their excretion was statistically significantly decreased in Abcg2-null mice (Hirano et al., 2005; Merino et al., 2005). The plasma AUC of sunitinib, a substrate of ABCG2, was ~4 fold higher in Abcg2-null mice than that in WT mice, consistent with human data showing high AUCs in individuals with ABCG2 deficiency (Mizuno et al., 2012). These data demonstrate that the Abcg2-null mouse model is an ideal tool to determine the impact of ABCG2 deficiency on drug metabolism and pharmacokinetics. The current work utilized Abcg2-null mice to investigate the role of ABCG2 in DTG metabolism and pharmacokinetics. We found

that ABCG2 deficiency had no statistically significant impact on DTG metabolism and pharmacokinetics.

Since DTG is a substrate of ABCG2 (Reese et al., 2013), we expected a high AUC of DTG in Abcg2-null mice when compared to WT mice. However, no genotype-related difference was found in either systemic or tissue specific AUCs of DTG, indicating that ABCG2 is not critical in modulating DTG distribution and disposition. In addition, we found that ABCG2 deficiency had no statistically significant impact on DTG metabolism and excretion. Our data did not support the finding in a previous report showing high plasma concentrations of DTG in ABCG2-deficient individuals (Tsuchiya et al., 2017). In this clinical report, the number of ABCG2-deficient individuals with SNP rs2231142 homozygous is 3, which might be too small to evaluate group differences. In addition, this clinical study only used a limited time window to monitor DTG concentrations instead of a full pharmacokinetic study. Furthermore, plasma concentrations of DTG in individuals with SNP rs2231142 heterozygous genotype (n = 17) did not increase when compared to WT genotype (n = 22) (Tsuchiya et al., 2017), suggesting that ABCG2 is not critical in DTG excretion.

Our data are supported by a previous report showing that concomitant use of DTG with lopinavir and ritonavir, two potent inhibitors of ABCG2 and ABCB1 (Kis et al., 2010), did not cause statistically significant changes in DTG pharmacokinetics (Song et al., 2011). Interestingly, co-administration of DTG with darunavir and ritonavir

leads to a statistically significant reduction in DTG exposure (Song et al., 2011), which suggests that DTG may be a substrate of certain uptake transporter(s), such as organic anion transporting polypeptide transporters (OATPs), since darunavir is an inhibitor of OATPs (Kis et al., 2010). Further studies are needed to determine the specific transporters in DTG uptake and efflux.

In summary, the current work revealed that ABCG2 deficiency did not alter DTG metabolism and pharmacokinetics, suggesting that dose adjustment of DTG is not needed for individuals with ABCG2 deficiency.

## **Authorship Contributions**

Participated in research design: JZ, DKM and XM

Conducted experiments: JZ and XT

Contributed new reagents or analytic tools: JZ and XM

Performed data analysis: JZ, XT, AIS, and XM

Wrote or contributed to the writing and editing of the manuscript: JZ, DKM and XM



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## Footnotes

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## Figure Legends

**Fig. 1. DTG metabolism and disposition in hepatocytes.** M1, M2, M3, M4, and M5 are the metabolites of DTG. M2 and M3 are formed by UGT1A1 and CYP3A4, respectively. ABCG2 and ABCB1 are the potential transporters mediating the efflux of DTG from hepatocytes to the biliary system. The current work investigated the impact of ABCG2 deficiency on DTG metabolism and pharmacokinetics in mice.

**Fig. 2. Systemic pharmacokinetics of DTG in WT and Abcg2-null mice.** (A, B) Genetic and functional validation of Abcg2-null mice. PPIX was used as a substrate of ABCG2. (C) Serum concentration-time curves of DTG in WT and Abcg2-null mice. The mice were administrated with a single dose of DTG (20 mg/kg, p.o.). (D-G) Comparison of major pharmacokinetic parameters of DTG between WT and Abcg2-null mice. PPIX and DTG were measured by UPLC-QTOFMS. Pharmacokinetic parameters were calculated by non-compartmental model analysis on Phoenix WinNonlin. All data are expressed as means  $\pm$  SD (n = 4). \*\*\* $P < 0.001$  vs WT.

**Fig. 3. Tissue specific pharmacokinetics of DTG in WT and Abcg2-null mice.**

The mice were administrated with a single dose of DTG (20 mg/kg, p.o.). (A, C, and E) Concentration-time curves of DTG in the liver, kidney, and brain of WT and Abcg2-null mice. (B, D, and F) AUCs of DTG in the liver, kidney, and brain of WT

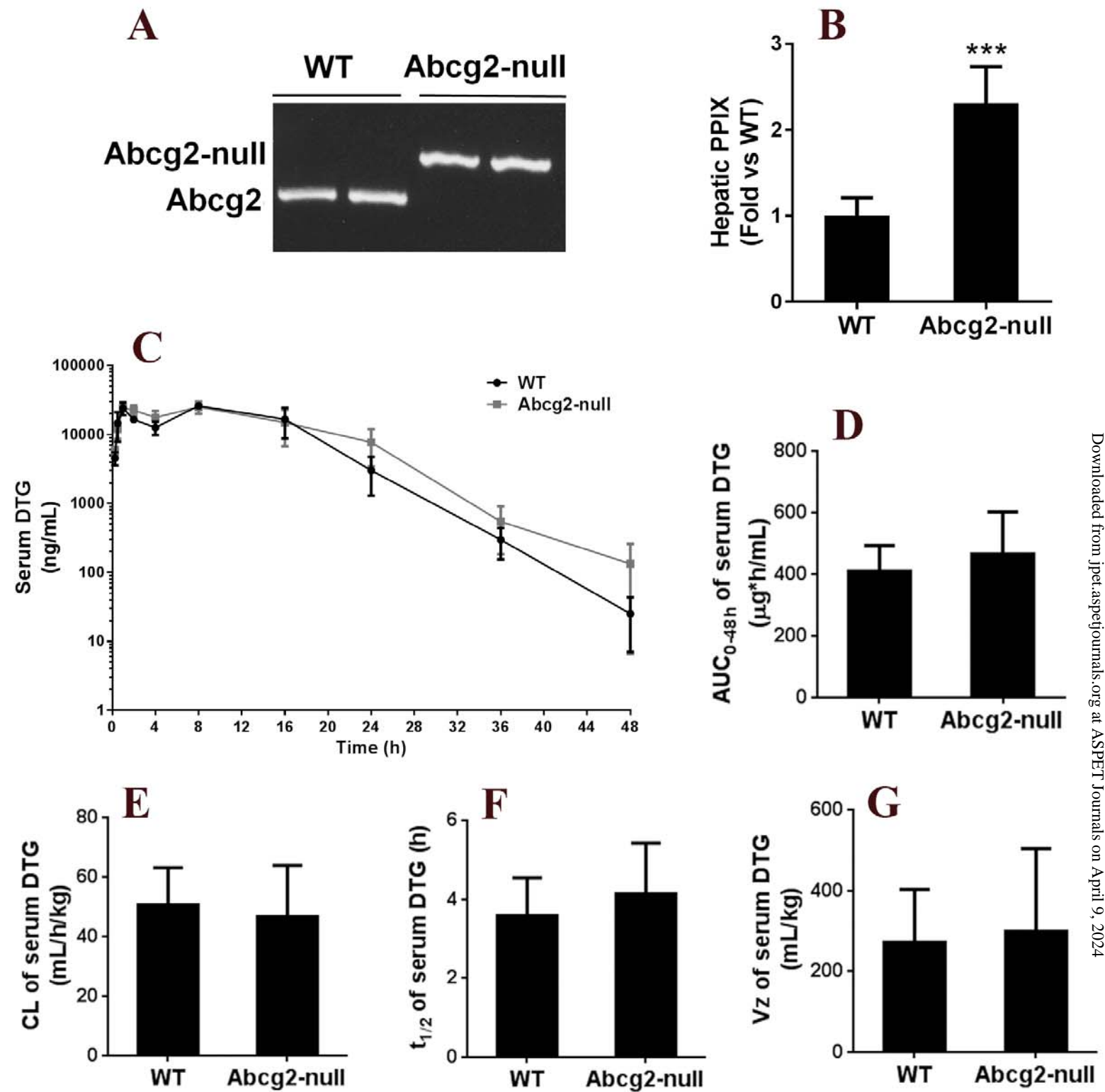
and Abcg2-null mice. AUCs were calculated by Graphpad Prism. All data are expressed as means  $\pm$  SD (n = 4). N.D., not detected.

**Fig. 4. Excretion of DTG and its metabolites in feces and urine of WT and Abcg2-null mice.** Feces and urine were collected overnight from mice treated with a single dose of DTG. (A, B) The abundance of DTG in feces and urine. (C-F) Comparison of DTG and its metabolites in feces and urine. (G, H) Comparison of DTG metabolites between WT and Abcg2-null mice. N.D., not detected. The data (A, B, G, H) are expressed as means  $\pm$  SD (n = 4).

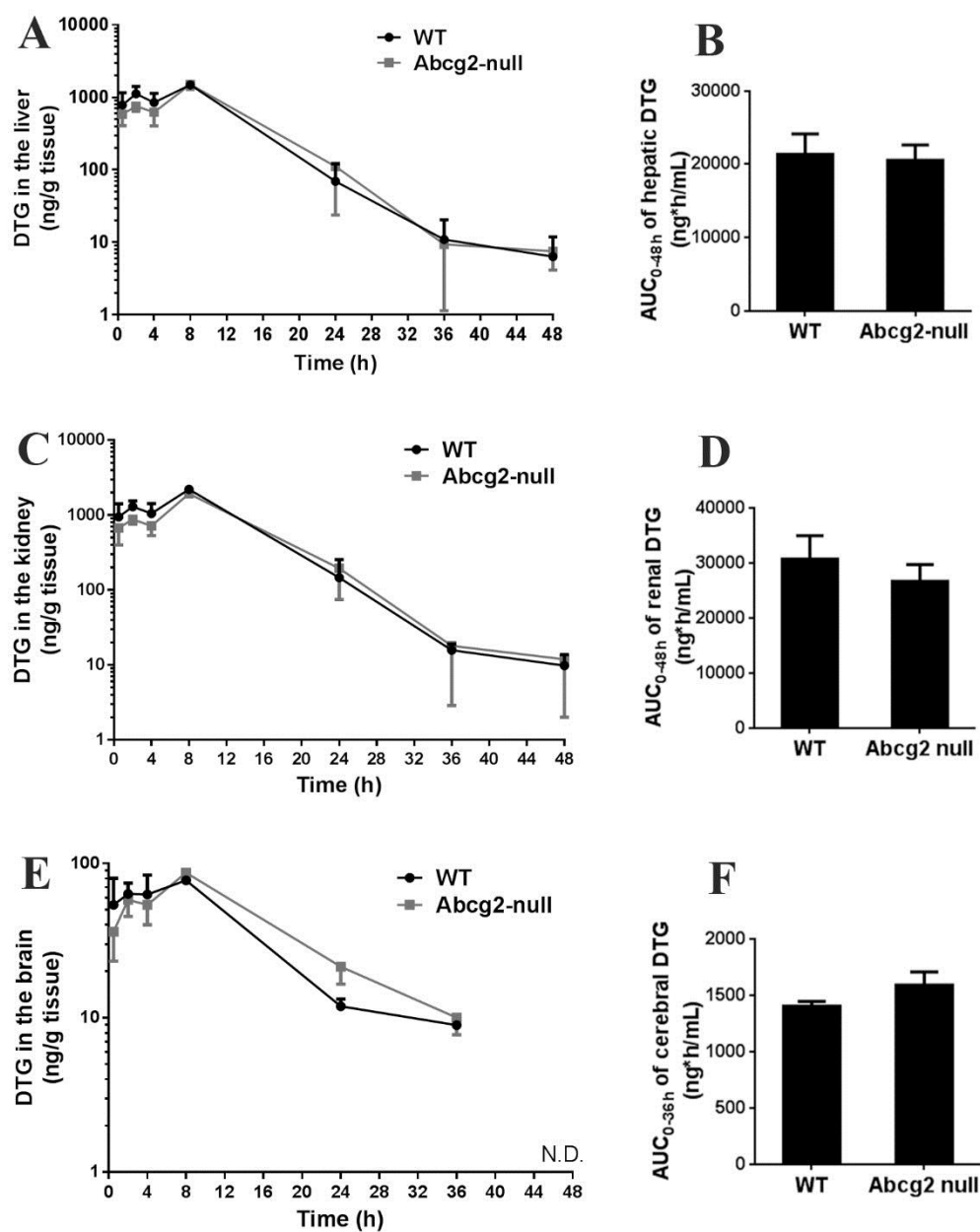
The diagram illustrates the metabolic pathways of Dolutegravir (DTG) within a Hepatocyte. DTG is the central molecule. It can be converted to M3 by the enzyme CYP3A4, which then leads to M1 and finally M5. Alternatively, DTG is converted to M2 by UGT1A1. DTG is also converted to M4 by an unknown enzyme (indicated by a question mark). Furthermore, DTG is transported into the Biliary system by ABCG2 and ABCB1, where it is eventually excreted in Feces. The chemical structures shown are: DTG (a pyrimidine nucleoside with a 4-fluorophenyl group), M3 (DTG with a 4-fluorophenyl group and a hydroxyl group on the sugar), M2 (DTG with a 4-fluorophenyl group and a hydroxyl group on the sugar), M1 (DTG with a 4-fluorophenyl group and an amino group on the sugar), M5 (DTG with a 4-fluorophenyl group and a carboxylic acid group on the sugar), and M4 (DTG with a 4-fluorophenyl group and a thioether group on the sugar).



## Fig 2



**Fig 3**



**Fig 4**