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**Clopidogrel Carboxylic Acid Glucuronidation is Mediated Mainly by UGT2B7,
UGT2B4 and UGT2B17:
Implications for Pharmacogenetics and Drug-Drug Interactions**

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Abbreviations: AUC₀₋₄, area under the plasma concentration–time curve from 0 h to 4 h; CES, carboxylesterase; CI, confidence interval; CL_{int}, intrinsic clearance; C_{max}, peak plasma concentration; CYP, cytochrome P450; HIM, human intestine microsomes; HLM, human liver microsomes; k_{dep}, pseudo-first-order depletion rate constant; K_m, Michaelis–Menten constant; SNV, single nucleotide variation; t_{1/2}, elimination half-life; t_{max}, time at which peak plasma concentration occurs; UDPGA, uridine diphosphate glucuronic acid; UGT, UDP-glucuronosyltransferase.

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Abstract

The antiplatelet drug clopidogrel is metabolized to an acyl- β -D-glucuronide, which causes time-dependent inactivation of CYP2C8. Our aim was to characterize the UDP-glucuronosyltransferase (UGT) enzymes that are responsible for the formation of clopidogrel acyl- β -D-glucuronide. Kinetic analyses and targeted inhibition experiments were performed using pooled human liver and intestine microsomes (HLM and HIM, respectively) and selected human recombinant UGTs based on preliminary screening. The effects of relevant *UGT* polymorphisms on the pharmacokinetics of clopidogrel were evaluated in 106 healthy volunteers. UGT2B7 and UGT2B17 exhibited the highest clopidogrel carboxylic acid glucuronidation activities, with a $CL_{int,u}$ of 2.42 and 2.82 $\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$, respectively. Of other enzymes displaying activity (UGT1A3, UGT1A9, UGT1A10-H and UGT2B4), UGT2B4 ($CL_{int,u}$ 0.51 $\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) was estimated to contribute significantly to the hepatic clearance. Nonselective UGT2B inhibitors strongly inhibited clopidogrel acyl- β -D-glucuronide formation in HLM and HIM. The UGT2B17 inhibitor imatinib and the UGT2B7 and UGT1A9 inhibitor mefenamic acid inhibited clopidogrel carboxylic acid glucuronidation in HIM and HLM, respectively. Incubation of clopidogrel carboxylic acid in HLM with UDPGA and NADPH resulted in strong inhibition of CYP2C8 activity. In healthy volunteers, *UGT2B17*2* deletion allele was associated with a 10% decrease per copy in the plasma clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid AUC_{0-4} ratio ($P < 0.05$). To conclude, clopidogrel carboxylic acid is mainly metabolized by UGT2B7 and UGT2B4 in the liver, and by UGT2B17 in the small intestinal wall. The formation of clopidogrel acyl- β -D-glucuronide is impaired in carriers of the *UGT2B17* deletion. The findings may have implications regarding intracellular mechanisms leading to CYP2C8 inactivation by clopidogrel.

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Introduction

The platelet P2Y₁₂ adenosine diphosphate receptor inhibitor clopidogrel is widely used to reduce blood clotting in patients at high risk for vascular thrombotic events. Due to its complex pharmacokinetic profile, clopidogrel is susceptible to various drug-drug interactions and genetic polymorphisms resulting in variable drug response (Mega et al., 2009; Floyd et al., 2012; Frelinger et al., 2012; Tornio et al., 2014; Tarkiainen et al., 2015). Clopidogrel is a prodrug that is metabolized via two distinct routes. Approximately 10% of the absorbed dose undergoes a two-step oxidative metabolism pathway, producing the active *cis* 5-thiol metabolite, while the majority of the dose is metabolized by sequential hydrolysis and conjugation reactions into pharmacodynamically inactive clopidogrel carboxylic acid and clopidogrel acyl-β-D-glucuronide (Savi et al., 2000; Tang et al., 2006; Hagihara et al., 2009; Silvestro et al., 2011) (Figure 1).

The oxidative metabolism of clopidogrel is catalyzed by several cytochrome P450 (CYP) isoforms, including CYP2C19, CYP1A2, CYP2B6 and CYP3A4/5 (Clarke and Waskell, 2003; Hagihara et al., 2009; Kazui et al., 2010; Dansette et al., 2012). The *CYP2C19* loss-of-function alleles are associated with low plasma concentrations of the active metabolite and poor clopidogrel responsiveness (Hulot et al., 2006; Giusti et al., 2007; Sibbing et al., 2009; Simon et al., 2009; Hochholzer et al., 2010). Interestingly, clopidogrel is also a mechanism-based inhibitor of CYP2C19 and CYP2B6 (Richter et al., 2004; Nishiya et al., 2009). In healthy volunteers, pretreatment with clopidogrel significantly increased the area under the plasma concentration-time curve (AUC) of omeprazole in subjects with homozygous *CYP2C19* extensive metabolizer genotype and the AUC of the CYP2B6 substrate bupropion (Turpeinen et al., 2005; Chen et al., 2009). The tendency to form reactive metabolites that irreversibly inactivate CYP enzymes has recently been observed also for the secondary metabolite, clopidogrel acyl-β-D-glucuronide. This glucuronide metabolite was shown to be a strong mechanism-based inhibitor of CYP2C8, leading to

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a marked increase in the plasma concentrations of repaglinide and pioglitazone (Tornio et al., 2014; Itkonen et al., 2016).

The hydrolysis of clopidogrel into clopidogrel carboxylic acid is catalyzed by carboxylesterase 1 (CES1) (Tang et al., 2006), as demonstrated clinically by the increased antiplatelet effects of clopidogrel in carriers of a loss-of-function single nucleotide variant of *CES1* (Tarkiainen et al., 2015). What is not known, yet, is which UGT enzymes catalyse the glucuronidation of clopidogrel carboxylic acid into clopidogrel acyl- β -D-glucuronide. Identifying the UGT enzymes responsible for this activity is particularly interesting since the metabolic pathway leads to the formation of a bioactive glucuronide metabolite that inactivates CYP2C8. To this end, we carried out an *in vitro* study to elucidate the role of 13 human UGTs in the formation of clopidogrel acyl- β -D-glucuronide. Moreover, to gain insight into the clinical relevance of the findings, we investigated the effects of common variants in relevant *UGT* genes on the pharmacokinetics of clopidogrel carboxylic acid glucuronidation in a population comprising healthy volunteers from previous and ongoing pharmacokinetic studies.

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Materials and Methods

Materials

Pooled HLM (UltraPool), pooled HIM, and microsomes from baculovirus infected insect cells expressing recombinant human UGT isoforms (Supersomes) were purchased from Corning (Corning, NY, USA), except for His-tagged UGT1A10 (UGT1A10-H) which was prepared in Helsinki as previously described (Kuuranne et al., 2003). The rapid equilibrium dialysis device (RED®) was obtained from Thermo Scientific (Waltham, MA, USA). Aprepitant, S-(+)-clopidogrel hydrogen sulfate, (+/-)-clopidogrel carboxylic acid, (+/-)-clopidogrel-d4 carboxylic acid, gemfibrozil, N-desethylamodiaquine hydrochloride and N-desethylamodiaquine-d5 were purchased from Toronto Research Chemicals (North York, ON, Canada). Amodiaquine dihydrochloride dihydrate, (+/-)-ketamine hydrochloride, β -NADPH and UDPGA were from Sigma-Aldrich (St. Louis, MO, USA). Clopidogrel acyl- β -D-glucuronide was obtained from Santa Cruz Biotechnology (Dallas, TX, USA), fluconazole and mefenamic acid were from Pfizer (Sandwich, UK) and imatinib mesylate was from Sequoia Research Products, (Pangbourne, UK). Other solvents and reagents were of analytical grade.

In Vitro Study

UGT Incubation Conditions. Human recombinant UGTs (hrUGTs), pooled human liver microsomes (HLM) or pooled human intestinal microsomes (HIM) were diluted in 0.1 M sodium phosphate buffer (pH 7.4) containing MgCl_2 (10 mM). In addition, the pore forming peptide alamethicin ($10 \mu\text{g} \cdot \text{mL}^{-1}$) was added to incubation mixtures containing HLM or HIM. The protein concentrations in hrUGT, HLM and HIM incubations ranged from 0.2 to $0.8 \text{ mg} \cdot \text{mL}^{-1}$, as described for each assay below. Clopidogrel carboxylic acid concentration ranged from 0.5 to 3000

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μM , depending on the experiment. HLM and HIM incubation mixtures were kept on ice for 20 minutes before preincubation to allow pore formation by alamethicin. For consistency, the same treatment was applied to hrUGT incubations. The incubation mixtures were then moved to a heated shaker and preincubated for 5 minutes (at 37°C , 300 rpm), followed by addition of 5 mM uridine-diphosphate glucuronic acid (UDPGA) to initiate the reactions. Incubations were terminated after a designated time by mixing one part reaction mixture with three parts acetonitrile containing clopidogrel-d4 carboxylic acid as an internal standard. Samples were vortexed and cooled on ice for at least 10 minutes before centrifugation ($20\,800 \times g$ for 10 min). Finally, the supernatants were transferred to HPLC microvials for LC/MS/MS analysis. The non-specific binding of $0.5 \mu\text{M}$ clopidogrel carboxylic acid to HLM (0.4 and $0.8 \text{ mg} \cdot \text{mL}^{-1}$) and HIM ($0.2 \text{ mg} \cdot \text{mL}^{-1}$), 1 and $30 \mu\text{M}$ clopidogrel carboxylic acid to hrUGT2B17 ($0.2 \text{ mg} \cdot \text{mL}^{-1}$) and $100 \mu\text{M}$ clopidogrel to HLM and HIM ($0.8 \text{ mg} \cdot \text{mL}^{-1}$) was determined by equilibrium analysis using the rapid equilibrium dialysis method. The fraction unbound was at least 0.8 in all incubations. Substrate and inhibitor compounds were dissolved in acetonitrile and methanol, respectively. The final solvent concentrations in incubations were 1% or less. All incubations were performed in duplicates.

UGT Screening. To identify the specific UGTs responsible for clopidogrel carboxylic acid metabolism, glucuronidation was tested in human recombinant UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, His-tagged UGT1A10 (UGT1A10-H), UGT2B4, UGT2B7, UGT2B10, UGT2B15 and UGT2B17 at a final protein concentration of $0.4 \text{ mg} \cdot \text{mL}^{-1}$. In this experiment, clopidogrel carboxylic acid ($10 \mu\text{M}$) was incubated with each UGT enzyme for 30 and 90 minutes as described above.

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Kinetic Analyses. Based on the results of the preliminary screening, UGT1A3, UGT1A9, UGT1A10-H, UGT2B4, UGT2B7 and UGT2B17 were selected for further kinetic analyses. Incubations were conducted as previously described using a protein concentration of 0.2 mg· mL⁻¹ and a minimum of seven different substrate concentrations, ranging from 1 μM to 3000 μM for each hrUGT assay. Reactions were terminated after a 20-minute incubation.

Clopidogrel Carboxylic Acid Metabolism in HLM, HIM, hrUGT2B7 and hrUGT2B17. In order to determine the maximal linear range of glucuronidation, depletion of 0.5 μM clopidogrel carboxylic acid and formation of clopidogrel acyl-β-D-glucuronide were measured in HLM, HIM, and recombinant UGT2B7 and UGT2B17 (0.4 and 0.8 mg· mL⁻¹) for up to 90 minutes. This clopidogrel carboxylic acid concentration is within the clinically relevant range, since its average peak plasma concentration (C_{max}) in the clinical data was 35 and 72 μM, corresponding to unbound concentrations (C_{max,u}) of 0.7 and 1.4 μM for the 300 and 600 mg dose, respectively.

Inhibition Experiments. Clopidogrel carboxylic acid glucuronidation in HLM, HIM, UGT2B7 and UGT2B17 was investigated in the absence and presence of the potential UGT2B7 inhibitors aprepitant, fluconazole, gemfibrozil, ketamine and mefenamic acid, as well as the UGT2B17 inhibitor imatinib (Uchaipichat et al., 2006; Knights et al., 2009; Uchaipichat et al., 2011; House et al., 2015; Zhang et al., 2015). In addition, the selectivity of inhibition by gemfibrozil, mefenamic acid and imatinib was further investigated in UGT1A3, UGT1A9, UGT1A10-H and UGT2B4. Inhibition experiments were performed using clopidogrel carboxylic acid concentration of 0.5 μM for HLM and HIM incubations (0.4 mg· mL⁻¹) and 5 μM for hrUGT incubations (0.2 mg· mL⁻¹). Concentrations of aprepitant, fluconazole, gemfibrozil, ketamine, mefenamic acid and imatinib in the incubations were 10, 2500, 100, 500, 5 and 5 μM, respectively.

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Metabolism of Parent Clopidogrel in HLM and HIM. Clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide formation in HLM and HIM ($0.8 \text{ mg} \cdot \text{mL}^{-1}$) was investigated by incubating $10 \mu\text{M}$ or $100 \mu\text{M}$ clopidogrel for 90 minutes. Incubation mixtures were prepared as described for UGT incubation conditions, with the exception that UDPGA was added to the mixture before preincubation and the reaction was initiated by the addition of the substrate.

Clopidogrel Carboxylic Acid Metabolism and CYP2C8 Inhibition in HLM. The formation of clopidogrel acyl- β -D-glucuronide from clopidogrel carboxylic acid and the subsequent inactivation of CYP2C8 were studied in HLM using amodiaquine N-desethylation as a marker reaction for CYP2C8 activity. Briefly, incubation mixtures containing clopidogrel carboxylic acid ($50 \mu\text{M}$) in HLM ($0.5 \text{ mg} \cdot \text{mL}^{-1}$) were prepared as described previously. However, after preincubation for 5 minutes, a combined cofactor solution containing both 5 mM UDPGA (to initiate glucuronidation) and 1 mM NADPH (to initiate metabolism-dependent inactivation of CYP2C8 by clopidogrel acyl- β -D-glucuronide) was added to the mixtures. At determined time points, aliquots of $7.5 \mu\text{L}$ of the reaction mixture were transferred to other tubes containing amodiaquine ($10 \mu\text{M}$) and NADPH (1 mM) in buffer in a final incubation volume of $150 \mu\text{L}$ to measure CYP2C8 activity. Protein and inhibitor concentrations were thus diluted 20-fold and direct inhibition by clopidogrel acyl- β -D-glucuronide was minimized by using an amodiaquine concentration higher than its K_m for CYP2C8. The reaction mixture was incubated for 2 minutes (37°C , 300 rpm) and the reaction was stopped by mixing $50 \mu\text{L}$ sample with $150 \mu\text{L}$ of stop solution (0.3% formic acid in acetonitrile:methanol:water 90:5:5 v/v/v) containing deuterium-labeled N-desethylamodiaquine as an internal standard. Samples were vortexed, kept on ice for at least 10 minutes and centrifuged ($20\,800 \times g$ for 10 min). The supernatant was transferred to silanized glass tubes and evaporated to dryness under nitrogen. The

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residue was reconstituted with 50 μL of mobile phase A/ mobile phase B (95:5, v/v) (see section “Quantification of drug concentrations”). To confirm inhibition by clopidogrel acyl- β -D-glucuronide, controls without clopidogrel carboxylic acid and controls without either NADPH or UDPGA in the pre-dilution incubation mixture were prepared as above. Separate samples for clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide quantification were also collected directly after N-desethylamodiaquine sampling. The samples were treated and prepared as described for UGT incubation conditions.

Quantification of Drug Concentrations. The drug concentrations were measured with a Nexera X2 (Shimadzu, Kyoto, Japan) or an Agilent 1100 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to a 5500 Qtrap, API3000 or API2000 tandem mass spectrometer (AB Sciex, Toronto, Ontario, Canada). Clopidogrel carboxylic and clopidogrel acyl- β -D-glucuronide were separated on a Sun Fire C18 analytical column (150 \times 2.1 mm, 3 μm particle size; Waters, Millford, MA) using isocratic mobile phase conditions (2 mM ammonium acetate; pH 5.5:acetonitrile 58:44 v/v). The flow rate and the column oven temperature were set at 0.26 $\text{mL} \cdot \text{min}^{-1}$ and 30 $^{\circ}\text{C}$. The mass spectrometer was operated in positive turbo ion spray mode with the target ion transitions (m/z) of 308 to 198 and 484 to 308 for clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide, respectively. Deuterium-labeled clopidogrel carboxylic acid served as an internal standard for both analytes. The lower limits of quantitation of clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide were 0.0038 μM and 0.0023 μM . The chromatographic separation for N-desethylamodiaquine was performed on an Atlantis T3 column (100 \times 2.1 mm, 3 μm particle size; Waters Corporation, Milford, MA) using gradient elution as previously described (Filppula et al., 2012). The mobile phases consisted of 0.3% formic acid in 5 mM ammonium formate (mobile phase A) and 0.3% formic acid in 90/5/5 acetonitrile/methanol/water (mobile phase B), and the limit of quantification for N-

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desethylamodiaquine was 0.001 μM . The day-to-day coefficients of variation were below 15% at relevant concentrations for all analytes, except for the lowest quality control (3 nM) of N-desethylamodiaquine, whose coefficient of variation was 18%. None of the other drugs or drug metabolites interfered with the assays.

Data Analysis. The kinetics of clopidogrel acyl- β -D-glucuronide formation were analyzed using SigmaPlot, version 13.0 (Systat Software, San Jose, CA, USA). Selection of the best-fit enzyme model was based on the Akaike information criterion, the r^2 values, 95% confidence intervals (CI), and on the examination of Michaelis-Menten and Eadie-Hofstee plots. The data were analyzed with the following models:

Michaelis-Menten

$$v = \frac{V_{max} [S]}{K_m + [S]}$$

where v is the initial velocity of the enzyme reaction, V_{max} is the maximal reaction velocity, $[S]$ is the substrate concentration, and K_m is the Michaelis-Menten constant.

Substrate inhibition

$$v = \frac{V_{max}}{1 + \frac{K_m}{[S]} + \frac{[S]}{K_i}}$$

where K_i represents the dissociation constant for the inhibitory substrate-enzyme-substrate ternary complex.

Sigmoidal (Hill)

$$v = \frac{V_{max} [S]^n}{K_m^n + [S]^n}$$

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where n is the Hill coefficient.

Two binding sites

$$v = \frac{\frac{V_{max1}[S]}{K_{m1}} + \left(\frac{V_{max2}[S]^2}{K_{m1}K_{m2}}\right)}{1 + \frac{[S]}{K_{m1}} + \frac{[S]^2}{K_{m1}K_{m2}}}$$

where K_{m1} and K_{m2} represent the Michaelis-Menten constants for the binding of the substrate molecules to the first and second binding site, respectively (Korzekwa et al., 1998).

Intrinsic clearance for clopidogrel acyl- β -D-glucuronide formation (CL_{int}) was calculated according to $CL_{int} = V_{max}/K_m$. This equation can be used for determination of CL_{int} and reaction phenotyping when the substrate concentration is less than 10% of the K_m . In our clinical study, the average $C_{max,u}$ was less than 10% of the lowest K_m of the recombinant UGTs. Likewise, the substrate concentrations used for $CL_{int,u,dep}$ determination and the inhibition experiments in HLM and HIM were only 3% of the lowest K_m , corresponding to the clinically relevant concentration range. Pseudo-first-order depletion rate constants (k_{dep}) were determined for the depletion of $0.5 \mu\text{M}$ clopidogrel carboxylic acid in HLM and HIM using nonlinear regression analysis (SigmaPlot). The intrinsic clearance in depletion experiments was calculated as $CL_{int \times dep} = k_{dep} / [M]$, where $[M]$ is the microsomal protein concentration (Venkatakrisnan et al., 2003). The relative contributions of UGT1A3, UGT1A9, UGT2B4, UGT2B7, and UGT2B17 in the hepatic metabolism of clopidogrel carboxylic acid were estimated from the determined CL_{int} values using a conversion factor, which was determined from previously published peptide-based quantification studies by dividing the molar abundancy of each UGT isoform in human liver microsomes with the molar abundancy of the respective hrUGT isoform in baculovirus infected insect cells (Supersomes) (Fallon et al., 2013a; Fallon et al., 2013b; Sato et al., 2014). The relative contribution of each UGT isoform in

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clopidogrel carboxylic acid glucuronidation was calculated by multiplying recombinant CL_{int} values with the respective conversion factor. The $CL_{int, in vivo}$ value for the hepatic glucuronidation of clopidogrel carboxylic acid was calculated using the standard *in vitro-in vivo* scaling parameters of 40 mg of microsomal protein/g liver (Houston and Galetin, 2008) and 25.7 g of liver/kg body wt. (Davies and Morris, 1993).

Clopidogrel Pharmacokinetics and Pharmacogenetic Analysis in Healthy Volunteers

Subjects and Study Design. A total of 106 healthy Finnish volunteers from eight of our previous or ongoing pharmacokinetic studies (49 women and 57 men; mean \pm S.D.: age 24 ± 4 years, height 175 ± 9 cm, weight 71 ± 13 kg, and body mass index 23 ± 3 kg/m²), including six drug-drug interaction studies and two genotype panel studies, were available for pharmacogenetic analysis (Supplementary Table 1) (Holmberg et al., 2014; Tornio et al., 2014; Itkonen et al., 2015; Tarkiainen et al., 2015; Itkonen et al., 2016; Holmberg et al., unpublished data; Itkonen et al., unpublished data). No individuals with a non-Caucasian background or excess relatedness were included in the study. During these studies, each subject was given a single 300 mg (n=49) or 600 mg (n=57) dose of clopidogrel in the morning after an overnight fast. Timed EDTA blood samples, including a sample for DNA extraction, were drawn before and at designated times after clopidogrel ingestion. Plasma concentrations of clopidogrel and its metabolites were quantified using LC/MS/MS, as described in the respective studies. The health of the subjects was confirmed by their medical history, physical examination, and routine laboratory tests before entering the study. All subjects had normal blood platelet counts and haemoglobin values. None was a tobacco smoker and none used any continuous medication (e.g. oral contraceptives). A written informed consent was obtained from all study participants for participation in pharmacogenetic studies and the studies

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were approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District and the Finnish Medicines Agency Fimea.

Pharmacokinetics. For the present analysis, C_{\max} , time to C_{\max} (t_{\max}), the elimination half-life ($t_{1/2}$) and the area under the plasma concentration–time curve from 0 h to 4 h (AUC_{0-4}) of clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide were calculated for each of the 106 subjects using uniform noncompartmental methods with Phoenix WinNonlin, version 6.3 (Certara, St Louis, MO, USA). The clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid plasma AUC ratio was used as an index of the rate of clopidogrel carboxylic acid glucuronidation. The concentration-time points that were available from all the eight studies (0, 1, 1.5, 2, 3 and 4 hours postdose) were used in the pharmacokinetic calculations.

DNA Extraction, Genotyping and Copy Number Determination. Genomic DNA was extracted using the Maxwell 16 LEV Blood DNA Kit on a Maxwell 16 Research automated nucleic acid extraction system (Promega, Madison, WI). The samples were genotyped for the *UGT2B7**2 allele by genotyping the rs7668258 (c.-161T>C) single nucleotide variation (SNV), which is in a complete linkage disequilibrium ($r^2 = 1.00$) (1000 Genomes Project, www.internationalgenome.org) with the rs7439366 (c.802T>C, p.Y268H) SNV defining *2, with a TaqMan genotyping assay (C_27827970_40) on a QuantStudio™ 12K Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA). The *UGT2B17**2 deletion allele was genotyped with the TaqMan Copy Number assay Hs03185327_cn, targeting the *UGT2B17* intron 1 - exon 1 region (chr.4:68567750 on build GRCh38), with *RPPH1* (*Ribonuclease P RNA component H1*) as the reference gene, on the QuantStudio™ 12K Flex Real-Time PCR System.

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Statistical Analysis. The *in vitro* results are expressed as arithmetic means with S.D. The pharmacokinetic variables were logarithmically transformed before analysis and presented as geometric means with 95% CI. The comparisons between genotypes were carried out using forward stepwise linear regression with *UGT2B7* and *UGT2B17* genotypes, weight, body mass index, age, sex and dose as independent variables. Additive coding was employed for the genotypes. Differences were considered statistically significant when *P* was below 0.05. The data were analyzed using IBM SPSS Statistics, version 24 (Chicago, IL, USA).

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Results

UGT Screening and Kinetic Analyses. Among the 13 UGT enzymes screened, UGT2B17 and UGT2B7 displayed the highest activities at 10 μM clopidogrel carboxylic acid concentration, i.e. 39.3 and 16.9 $\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ protein at 90 minutes, respectively (Figure 2). UGT1A3, UGT1A9, UGT1A10-H and UGT2B4 also showed significant activities, between 5% and 20% of the UGT2B17 rates. The glucuronidation activities of UGT1A4, UGT1A6, UGT1A7 and UGT2B15 were very low or negligible and these enzymes were thus excluded from detailed kinetic analyses. UGT1A1, UGT1A8, UGT1A10 and UGT2B10 did not form any detectable clopidogrel acyl- β -D-glucuronide.

The kinetics of clopidogrel carboxylic acid glucuronidation by UGT1A3, UGT1A9, UGT2B7 and UGT2B17 followed the Michaelis-Menten model (Figure 3). For UGT1A10-H, several kinetic models gave a reasonable fit, and the Michaelis-Menten model was chosen based on confidence level in the parameter estimates. Clopidogrel carboxylic acid glucuronidation by UGT2B4 displayed non-hyperbolic kinetics and the best fit was obtained with the two binding sites model. The calculated kinetic parameters, including parameters that are not shown in Figure 3, are summarized in Table 1.

To allow estimation of the clinical relevance of each UGT in the metabolism of clopidogrel, we scaled the *in vitro* $\text{CL}_{\text{int,u}}$ data to the average hepatic and intestinal microsomal intrinsic clearance on the basis of published protein expression data. Despite its weaknesses, this scaling method could be used to approximate the contributions of most UGTs identified in this study. UGT2B17 and UGT2B7 exhibited the highest $\text{CL}_{\text{int,u}}$ values of clopidogrel carboxylic acid glucuronidation (Table 1). After abundance-based scaling, UGT2B7 was estimated to have the highest unbound intrinsic liver microsomal clearance ($\text{CL}_{\text{int,u,HLM}}$), accounting for about 55-60% of the total (combined) intrinsic clearance ($\text{CL}_{\text{int,u,HLM,tot}}$). UGT2B7 was followed by UGT1A9, UGT2B4 and UGT2B17,

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whose estimated combined contribution to the metabolism of clopidogrel carboxylic acid was approximately 40% of the $CL_{int,u,HLM,tot}$. However, the estimated contribution of UGT1A3 was only minor. In the intestine, the scaled unbound intrinsic clearance ($CL_{int,u,HIM}$) of UGT2B17 accounted for 87% of the total unbound intrinsic clearance ($CL_{int,u,HIM,tot}$), while the contribution of UGT2B7 to $CL_{int,u,HIM,tot}$ was only about 10%, and those of UGT1A10-H and UGT1A3 were <1%.

Clopidogrel Carboxylic Acid Depletion in HLM, HIM, UGT2B7 and UGT2B17. In a substrate depletion experiment, approximately 15-30% of the initial clopidogrel carboxylic acid concentration (0.5 μ M) was consumed during a 90 minute incubation with UDPGA in HLM or HIM. In recombinant UGT2B7 and UGT2B17, 5-10% of the initial substrate concentration was consumed during similar incubations. In all experiments, depletion was log-linear until at least 30 minutes, allowing for estimation of the intrinsic clearance from the results (Table 2).

Inhibition of Clopidogrel Carboxylic Acid Glucuronidation by Chemical Inhibitors of UGTs. In preliminary experiments in hrUGT2B7 and hrUGT2B17, aprepitant (10 μ M), fluconazole (2.5 mM) and ketamine (500 μ M) inhibited both UGT2B7 and UGT2B17-mediated clopidogrel carboxylic acid glucuronidation by >90% (Figure 4A). Gemfibrozil (100 μ M) showed very strong UGT2B7 inhibition (100%), but also moderate inhibition (30-70%) of other UGTs, including UGT1A9, UGT1A10-H, UGT2B4 and UGT2B17 (Figure 4B). Mefenamic acid (5 μ M) had a strong inhibitory effect on both UGT2B7 (95%) and UGT1A9 (98%), but no inhibitory effect on UGT1A3, UGT2B4 and UGT2B17. In contrast, 5 μ M imatinib strongly and selectively inhibited UGT2B17 activity (100% inhibition), with only a small inhibitory effect on UGT2B7 (16% inhibition) and a moderate effect on UGT1A10-H (59%).

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The UGT2B7 and UGT1A9 inhibitor mefenamic acid inhibited clopidogrel acyl- β -D-glucuronide formation in HLM by 38% but only slightly in HIM (12%), whereas gemfibrozil inhibited clopidogrel glucuronidation both in HLM and HIM (34-43%) (Figure 4C). The preferential UGT2B7 inhibitor imatinib inhibited clopidogrel carboxylic acid glucuronidation only in HIM (by 85%). The nonselective UGT2B inhibitors aprepitant, fluconazole and ketamine substantially inhibited clopidogrel acyl- β -D-glucuronide formation both in HLM and HIM (by 60-90%).

Metabolism of Parent Clopidogrel in HLM and HIM. During incubation of 10 μ M parent clopidogrel in HLM, clopidogrel carboxylic acid concentration rose to 8.9 μ M rapidly, in 15 minutes, followed by a gradual decrease (Figure 5A). At the same time, clopidogrel carboxylic acid was further metabolized to clopidogrel acyl- β -D-glucuronide, whose concentration reached 19% of that of clopidogrel carboxylic acid at 90 minutes (Figure 5B). In HIM, the formation of clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide from 10 μ M clopidogrel was substantially slower than in HLM. At 90 minutes, clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide concentrations were 1.0 μ M and 0.06 μ M, respectively (Figure 5A, 5B). After incubating 100 μ M clopidogrel in HIM for 90 minutes, clopidogrel carboxylic acid concentration rose to 4.1 μ M, whereas the concentration of clopidogrel acyl- β -D-glucuronide rose to the same level (0.06 μ M) only as in the experiment with 10 μ M clopidogrel (Figure 5C, 5D). Coincubation with 5 μ M imatinib in HIM did not affect clopidogrel carboxylic acid formation but markedly reduced clopidogrel acyl- β -D-glucuronide formation (by 62% at 90 minutes) (Figure 5D).

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UGT-mediated Bioactivation of Clopidogrel Carboxylic Acid and Subsequent Inactivation of CYP2C8. Preincubation of 50 μ M clopidogrel carboxylic acid for 90 minutes in HLM in the presence of UDPGA or a combined cofactor solution containing both UDPGA and NADPH, produced approximately 19 μ M clopidogrel acyl- β -D-glucuronide. Following preincubation of clopidogrel carboxylic acid with both UDPGA and NADPH for 90 minutes, amodiaquine N-desethylation (CYP2C8 marker reaction) was inhibited by 53% (Figure 6). No inhibition of amodiaquine N-desethylation could be observed when amodiaquine was incubated with a corresponding sample from preincubation mixtures containing only either one of the two cofactors.

Clopidogrel Pharmacokinetic and Pharmacogenetic Analysis in Healthy Volunteers. Linear regression analysis of the pharmacokinetic data obtained in 106 healthy volunteers revealed that of the tested genetic covariates, the *UGT2B17**2 deletion allele was independently associated with pharmacokinetic variables of clopidogrel carboxylic acid and its acyl- β -D-glucuronide. In addition, clopidogrel dose was independently associated with the AUC and AUC-ratio values of these clopidogrel metabolites ($P < 0.05$). Each *UGT2B17**2 deletion allele reduced the clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid plasma AUC_{0.4} ratio by 10.1% (95% CI, 1.7% - 17.8%; $P = 0.021$) (Figure 7A). This AUC-ratio was 47% higher with the 600 mg dose than with the 300 mg dose of clopidogrel (95% CI, 28% - 68%; $P < 0.001$). Moreover, *UGT2B17**2 had a significant association with the $t_{1/2}$ of clopidogrel carboxylic acid and clopidogrel acyl- β -D-glucuronide and the AUC_{0.4} of clopidogrel acyl- β -D-glucuronide (Figure 7B). Each *UGT2B17**2 allele prolonged the $t_{1/2}$ of clopidogrel carboxylic acid by 8.7% (95% CI, 2.2% - 15.7%; $P = 0.008$) and the $t_{1/2}$ of clopidogrel acyl- β -D-glucuronide by 6.5% (95% CI, 0.34% - 13.0%; $P = 0.039$) and decreased the AUC_{0.4} of clopidogrel acyl- β -D-glucuronide by 11.9% (95% CI, 1.1% - 21.5%; $P = 0.032$). On the other hand, the *2 polymorphism in the *UGT2B7* gene did not have a significant association with the AUC_{0.4} or $t_{1/2}$ values of clopidogrel, the active metabolite, clopidogrel

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carboxylic acid and clopidogrel acyl- β -D-glucuronide or with the clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid plasma AUC ratio.

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Discussion

In the present study, we investigated the glucuronidation of clopidogrel carboxylic acid *in vitro*, and assessed the relative contributions of the different UGT enzymes to clopidogrel acyl- β -D-glucuronide formation by using two *in vitro* approaches. Both an approach with chemical UGT inhibitors in HLMs and a scaling approach of recombinant UGT mediated clearances using peptide-based quantification data from previous publications (Fallon et al., 2013a; Fallon et al., 2013b; Sato et al., 2014) were consistent with an important role of UGT2B7 and UGT2B17 in the glucuronidation of clopidogrel carboxylic acid. In addition, UGT1A3, UGT1A9, UGT1A10-H and particularly UGT2B4 were estimated to participate in clopidogrel carboxylic acid glucuronidation. The role of UGT2B17 could also be verified in a clinical pharmacogenetic study.

Fluconazole and ketamine strongly inhibit UGT2B4, UGT2B7 and UGT2B17, with only minor effects on other relevant UGTs (Uchaipichat et al., 2006; Raungrut et al., 2010; Uchaipichat et al., 2011; House et al., 2015). In HLM, they reduced clopidogrel carboxylic acid glucuronidation by up to 90%, suggesting that collectively, UGT2B4, UGT2B7 and UGT2B17 are responsible for the majority of clopidogrel acyl- β -D-glucuronide formation in the liver. Furthermore, the nonselective UGT2B7 inhibitors mefenamic acid and gemfibrozil inhibited clopidogrel carboxylic acid glucuronidation in HLM by about 40%. Accordingly, UGT2B7 is likely to play a large role in the formation of clopidogrel acyl- β -D-glucuronide in the liver. On the other hand, the UGT2B17 inhibitor imatinib had practically no effect on clopidogrel carboxylic acid glucuronidation, suggesting a very small role for UGT2B17 in the liver.

In the liver, UGT2B7, UGT2B4 and UGT1A9 are relatively abundant, whereas the expression of UGT2B17 is low (Oda et al., 2012; Achour et al., 2014; Sato et al., 2014). Consequently, the abundancy-based scaling suggested that UGT2B7 accounts for almost 60% of the total unbound hepatic microsomal intrinsic clearance of clopidogrel carboxylic acid, while particularly UGT2B4,

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but also UGT2B17 and UGT1A9 account for the remaining $CL_{int,u,HLM,tot}$. This result is in agreement with the inhibition results. Yet, considering the effects of the various UGT2B inhibitors, this scaling probably slightly overestimated the contributions of UGT2B7 and UGT1A9 and underestimated the contribution of UGT2B4.

UGT2B17, UGT2B7 and UGT1A10 are expressed in the intestinal wall, but most other relevant UGTs are virtually absent (Sato et al., 2014). The strong (85%) inhibition of clopidogrel acyl- β -D-glucuronide formation by the UGT2B17 inhibitor imatinib in HIM indicates that UGT2B17 is mostly responsible for clopidogrel carboxylic acid metabolism in the intestinal wall. Since UGT1A9 is only minimally expressed in enterocytes (Oda et al., 2012), the minor (12%) inhibition by the UGT2B7 and UGT1A9 inhibitor mefenamic acid in HIM suggests that also UGT2B7 has a small role. In excellent agreement, the scaling approach estimated a major role for UGT2B17 in the unbound intestinal intrinsic clearance, UGT2B7 having a less significant role. In addition to these UGT2B enzymes, UGT1A10 may slightly contribute to the metabolism of clopidogrel carboxylic acid in the intestinal wall, since imatinib weakly inhibits UGT1A10 and the scaling suggested a minimal role for UGT1A10 in the $CL_{int,u,HIM,tot}$.

Analysis of clinical data provided the final approach to investigate the contributions of UGT2B7 and UGT2B17 to the glucuronidation of clopidogrel carboxylic acid. No statistically significant associations between *UGT2B7* variants and clopidogrel pharmacokinetics; were observed. However, the common *UGT2B7**2 allele has rarely had any effect on the glucuronidation kinetics of UGT2B7 substrates and is thought to have little impact on enzyme activity (Bhasker et al., 2000; Peterkin et al., 2007; Parmar et al., 2011; Rae et al., 2012). Therefore, the clinical data do not allow direct estimation of the contribution of UGT2B7 to clopidogrel metabolism.

In contrast to *UGT2B7*, there is a common deletion in the *UGT2B17* gene, which leads to complete UGT2B17 deficiency (Murata et al., 2003). The absence of UGT2B17 due to homozygous

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deletion of the *UGT2B17* gene is common in Caucasian and African populations (~10-40%) and very frequent in East Asia (up to ~90%) (Wilson et al., 2004; Jakobsson et al., 2006; Xue et al., 2008; Chen et al., 2010; Giroux et al., 2012). As expected, the *UGT2B17* deletion was common in our Finnish population, with an allele frequency of 45%. Moreover, clopidogrel acyl- β -D-glucuronide formation was impaired in carriers of the deletion, leading to a 10% or 19% lower clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid plasma AUC_{0.4} ratio in heterozygous and homozygous carriers, respectively, than in individuals carrying two functional copies of *UGT2B17*. Given that homozygous carriers of the deletion lack any metabolic activity by *UGT2B17*, the results suggest that the combined contribution of intestinal and hepatic *UGT2B17* to clopidogrel carboxylic acid glucuronidation is about 10% in heterozygous carriers and 20% in noncarriers, leaving 90% or 80% to other UGTs, respectively. As the *in vitro* findings indicate that *UGT2B17* is largely responsible for clopidogrel carboxylic acid glucuronidation in the intestinal wall, with about 10% contribution in the liver, it could be estimated that up to about 10% of clopidogrel acyl- β -D-glucuronide is formed in the intestine, depending on *UGT2B17* genotype. Moreover, as both clopidogrel carboxylic acid and acyl- β -D-glucuronide were formed from clopidogrel in HIM, there seems to be an interplay of *CES1* and *UGT2B17* in the intestinal wall.

CYPs and *UGTs* are located intracellularly on opposite sides of the endoplasmic membranes and there is evidence for a protein-protein interplay between certain *UGT* and *CYP* enzymes (Takeda et al., 2005; Ishii et al., 2010; Miyauchi et al., 2015). This interplay and the close proximity of the enzymes could theoretically facilitate interaction of glucuronide metabolites with *CYP* enzymes. In the present study, we demonstrated UDPGA-dependent formation of clopidogrel acyl- β -D-glucuronide (19 μ M in 90 min) and simultaneous NADPH-dependent 60% inhibition of *CYP2C8* activity in HLM. This *CYP2C8* inhibitory effect was slightly stronger than what we expected on the basis of the obtained clopidogrel acyl- β -D-glucuronide concentrations, suggesting that the proximity of *UGTs* and *CYP2C8* may facilitate the access of the acyl- β -D-glucuronide to

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CYP2C8. Such a mechanism might also partially explain why the clopidogrel-repaglinide interaction was best predicted, when the unbound concentration of clopidogrel acyl- β -D-glucuronide was assumed to be almost 20 times higher in hepatocytes than in plasma (Tornio et al., 2014).

In conclusion, the present study indicates that UGT2B7 is the main enzyme involved in clopidogrel carboxylic acid glucuronidation in the liver, while at least UGT2B4, and possibly also UGT1A9 and UGT2B17 play a smaller role. On the other hand, UGT2B17 has a major role in the intestinal metabolism of clopidogrel. Consequently, the formation of clopidogrel acyl- β -D-glucuronide is impaired in carriers of the *UGT2B17* gene deletion. The findings may have implications with regard to drug-drug interactions caused by inactivation of CYP2C8 by clopidogrel acyl- β -D-glucuronide.

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Authorship Contributions

Conceived and designed the experiments: Kahma, Filppula and Backman

Conducted the laboratory work: Kahma, Filppula, Neuvonen M. and Tarkiainen

Conducted the clinical studies: Tarkiainen, Tornio, Holmberg, Itkonen, Neuvonen P.J., Niemi and Backman

Performed the data analysis: Kahma, Filppula, Tarkiainen and Niemi

Contributed new reagents: Finel

Involved in analysis and interpretation of the data: Finel, Niemi and Backman

Wrote or contributed to the writing of the manuscript: all authors

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Footnotes

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Figure Legends

Figure 1. Metabolic pathways of clopidogrel.

Figure 2. Glucuronidation of 10 μM clopidogrel carboxylic acid in human recombinant UGTs.

Each column represents the mean \pm S.D. of duplicate incubations.

Figure 3. Enzyme kinetics of clopidogrel carboxylic acid glucuronidation by human recombinant UGT1A3 (A), UGT1A9 (B), UGT1A10-H (C), UGT2B4 (D), UGT2B7 (E) and UGT2B17 (F).

Eadie–Hofstee plots are shown as insets. Each point represents the mean \pm S.D. of at least duplicate incubations.

Figure 4. Effects of chemical inhibitors on clopidogrel carboxylic acid glucuronidation *in vitro*.

Aprepitant (10 μM), fluconazole (2.5 mM) and ketamine (500 μM) (A) and gemfibrozil (100 μM), mefenamic acid (5 μM) and imatinib (5 μM) (B) were incubated with 5 μM clopidogrel carboxylic acid in human recombinant UGTs (0.2 mg \cdot mL⁻¹) or 0.5 μM clopidogrel carboxylic acid in HLM and HIM (0.4 mg \cdot mL⁻¹) (C) for 15 minutes. Complete inhibition of UGT2B7 was caused by gemfibrozil (100 μM) and of UGT2B17 by imatinib (5 μM). Each column represents the mean \pm S.D. of duplicate incubations.

Figure 5. Metabolism of 10 μM clopidogrel in HLM and HIM (A, B) and 100 μM clopidogrel in HIM (C, D) (0.8 mg \cdot mL⁻¹). Each point represents the mean \pm S.D. of duplicate incubations.

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Figure 6. The effect of a preincubation containing UDPGA, NADPH and clopidogrel carboxylic acid ($50 \mu\text{M}$) on the metabolism of amodiaquine ($10 \mu\text{M}$) by CYP2C8. Each column represents the mean \pm S.D. of duplicate incubations.

Figure 7. Effect of *UGT2B17* copy number variation on the dose-adjusted clopidogrel acyl- β -D-glucuronide to clopidogrel carboxylic acid plasma AUC_{0-4} ratio (A) and the dose-adjusted clopidogrel acyl- β -D-glucuronide AUC_{0-4} (B). Central line shows the geometric mean and the bars show the 95% confidence intervals. Dose adjustments to the 300 mg dose were carried out on the basis of the corresponding regression coefficients.

Tables

Table 1. Kinetic parameters (mean \pm S.D.) of clopidogrel carboxylic acid glucuronidation by UGT1A3, UGT1A9, UGT1A10-H, UGT2B4, UGT2B7 and UGT2B17 and scaling of their contributions to the total microsomal intrinsic clearance in the liver and intestine.

	UGT1A3	UGT1A9	UGT1A10-H	UGT2B4	UGT2B7	UGT2B17
Model	MM	MM	MM	Two binding sites	MM	MM
V_{\max} ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	595 \pm 28	61.5 \pm 2.8	28.7 \pm 1.4	8.57 \pm 1.77 ($V_{\max 1}$) 131 \pm 5.97 ($V_{\max 2}$)	50.5 \pm 1.7	511 \pm 11
K_m (μM)	1980 \pm 150	74.8 \pm 14.5	307 \pm 38	19.0 \pm 8.50 (K_{d1}) 2340 \pm 274 (K_{d2})	20.9 \pm 3.2	181 \pm 12
$CL_{\text{int,u}}$ ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	0.301	0.822	0.0936	0.451 ($CL_{\text{int,u}}$) 0.0560 ($CL_{\text{int,u}}$)	2.42	2.82
Liver						
$CL_{\text{int,u,HLM}}$ Sato ^a ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg HLM}^{-1}$)	0.00380 (1.3%)	0.0257 (9.1%)	N/A	0.0577 (20.3%)	0.157 (55.3%)	0.0394 (13.9%)
$CL_{\text{int,u,HLM}}$ Fallon ^b ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg HLM}^{-1}$)	0.00528 (1.6%)	0.0395 (12.3%)	N/A	0.0528 (16.5%)	0.194 (60.4%)	0.0293 (9.1%)
Intestine						
$CL_{\text{int,u,HIM}}$ Sato ^a ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg HIM}^{-1}$)	0.000380 (0.4%)	N/A	0.000740 (0.7%)	N/A	0.0126 (12.0%)	0.0912 (87.0%)

^a Scaling of intrinsic clearance values was calculated using UGT abundancies in the liver and intestine published by Sato et al. (2014).

^b Scaling of intrinsic clearance values was calculated using UGT abundancies in the liver published by Fallon et al. (2013a; 2013b)

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Table 2. Kinetic parameters of clopidogrel carboxylic acid ($0.5 \mu\text{M}$) depletion in HLM, HIM and human recombinant UGT2B7 and UGT2B17 at protein concentration of $0.4 \text{ mg} \cdot \text{mL}^{-1}$.

	HLM	HIM	UGT2B7	UGT2B17
k_{dep} ($\text{l} \cdot \text{min}^{-1}$)	0.00215	0.00151	0.000405	0.000718
$\text{CL}_{\text{int,u,dep}}$ ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	5.38	3.77	3.35	1.80
$\text{CL}_{\text{int,in vivo}}$ ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$)	5.53	N/A	N/A	N/A

Figures

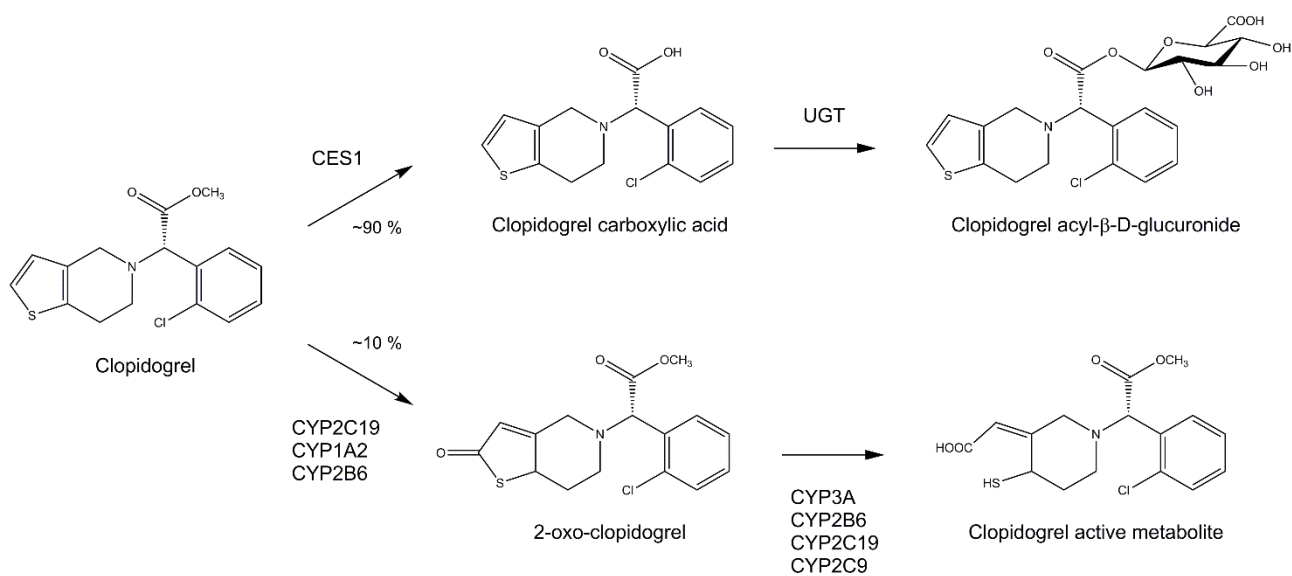


Figure 1.

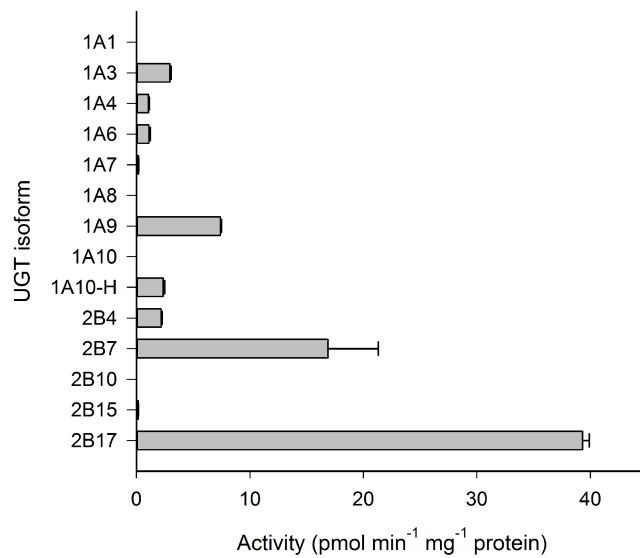


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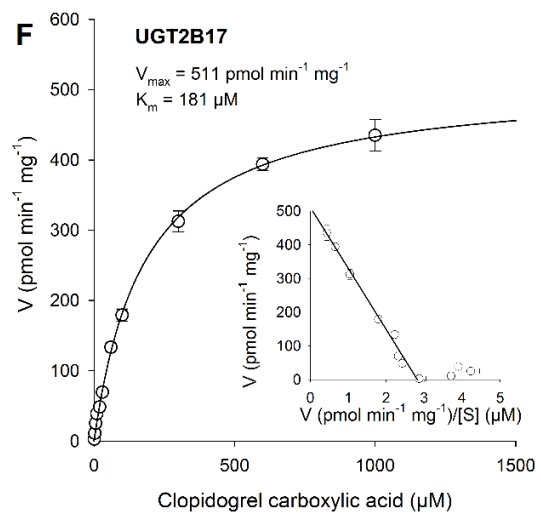
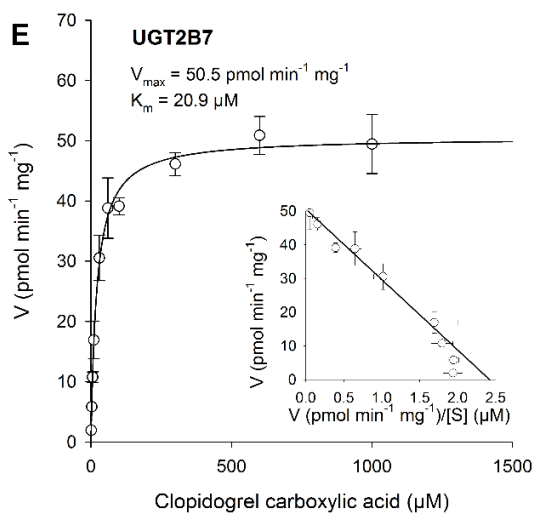
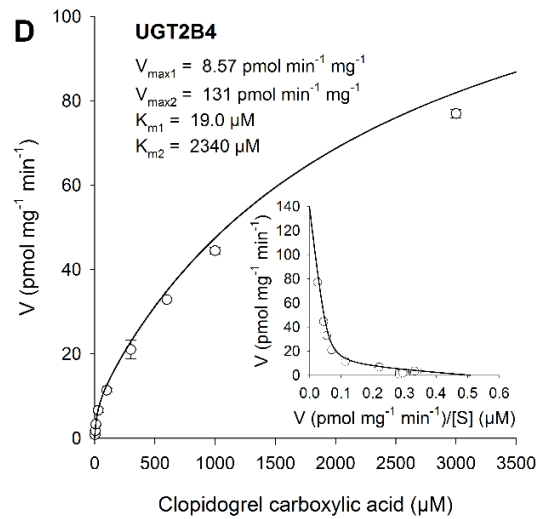
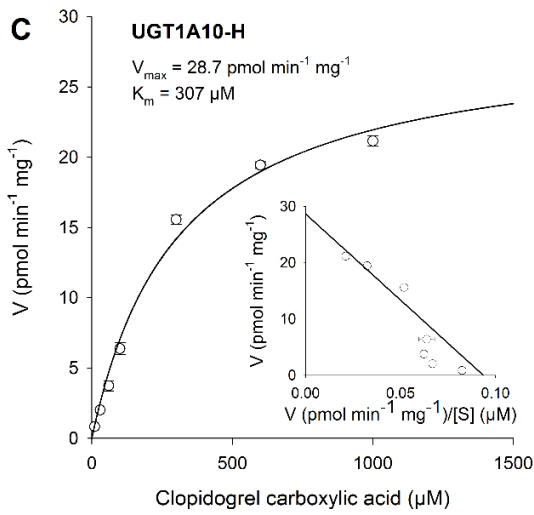
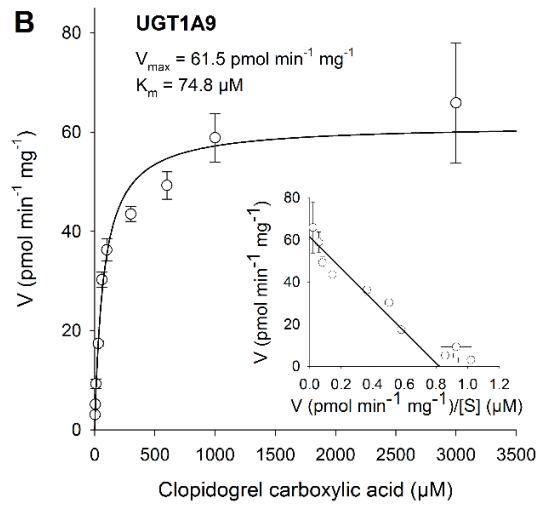
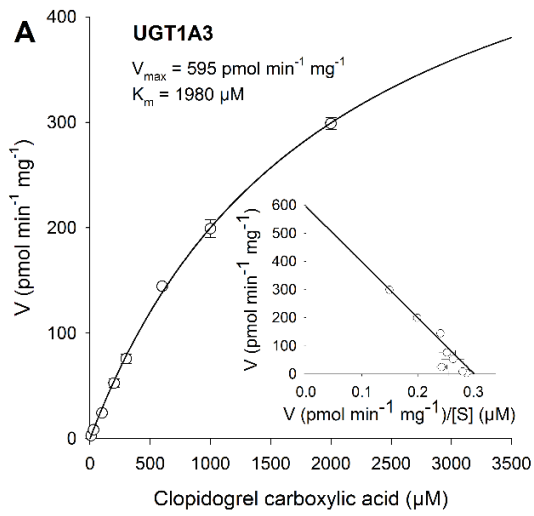


Figure 3.

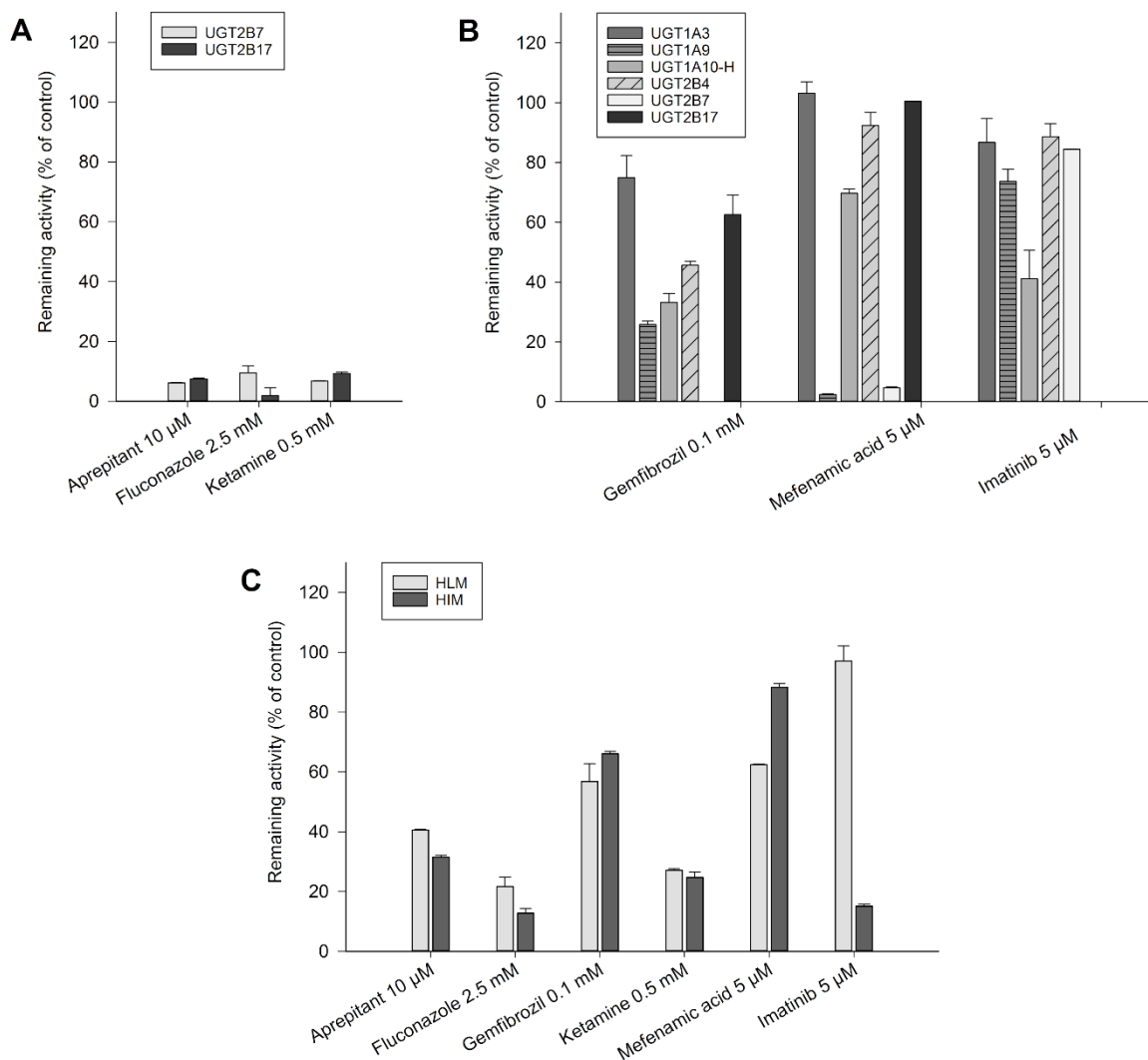
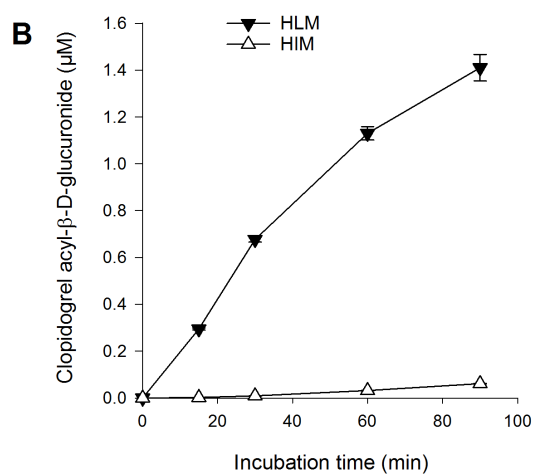
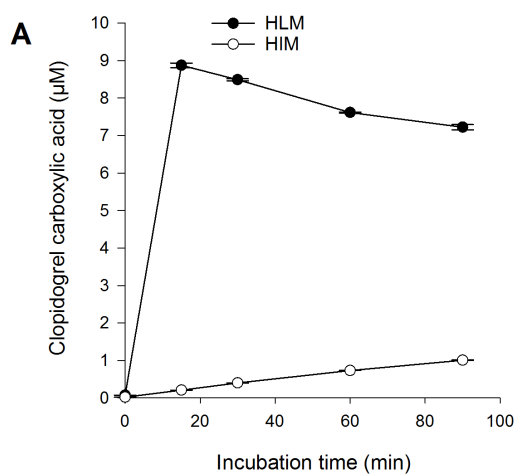


Figure 4.

10 μM Clopidogrel



100 μM Clopidogrel

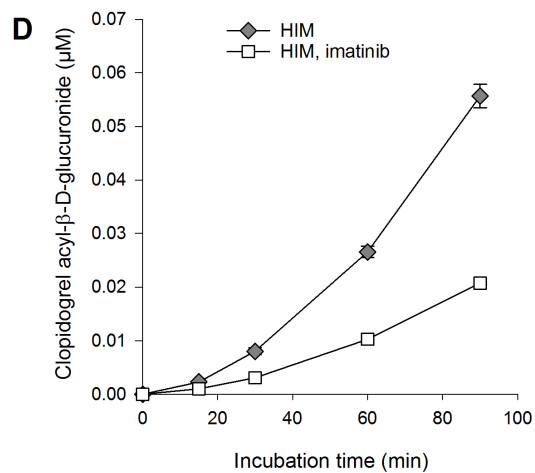
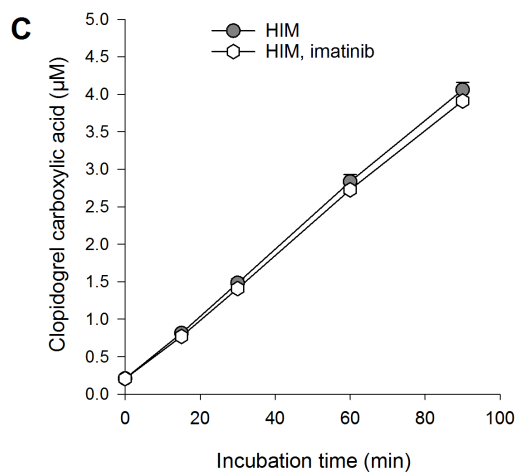


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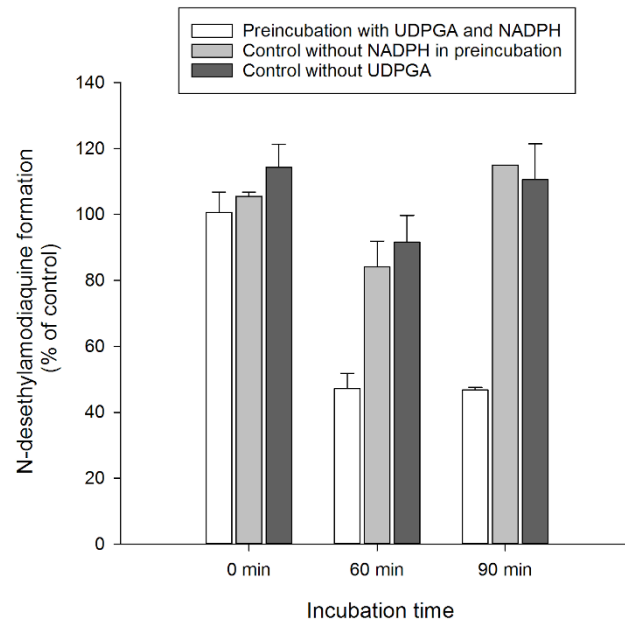


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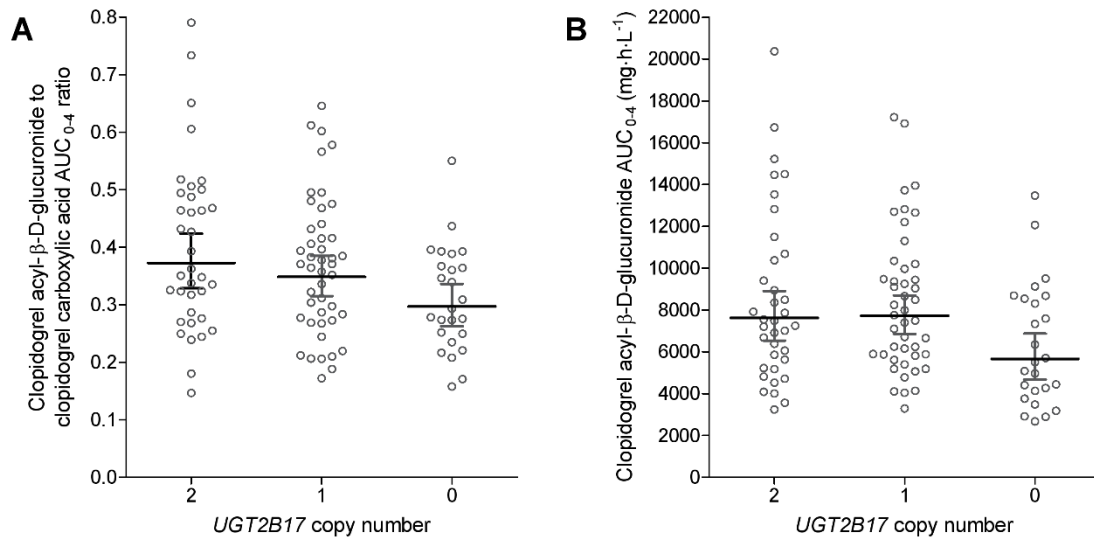


Figure 7.

Supplemental Data to:

Clopidogrel carboxylic acid glucuronidation is mediated mainly by UGT2B7, UGT2B4 and UGT2B17: Implications for pharmacogenetics and drug-drug interactions

Helinä Kahma, Anne M. Filppula, Mikko Neuvonen, E. Katriina Tarkiainen, Aleksi Tornio, Mikko T. Holmberg, Matti K. Itkonen, Moshe Finel, Pertti J. Neuvonen, Mikko Niemi and Janne T. Backman

Drug Metabolism and Disposition

Supplementary Table 1. Characteristics of the eight clinical studies that were included in the analysis of the possible associations between UGT genotypes and the pharmacokinetic variables of clopidogrel carboxylic acid.

Reference	Primary study type	Role of clopidogrel	Dose (mg)	Sex (f/m)	Age (years)	Weight (kg)	BMI (kg/m ²)
Holmberg et al., 2014	Crossover DDI study	Victim	600	6/7	22 ± 1	68 ± 10	22 ± 2
Itkonen et al., 2015	Crossover DDI study	Perpetrator	300	3/8	22 ± 2	74 ± 9	23 ± 2
Itkonen et al., 2016	Crossover DDI study	Perpetrator	300	4/4	25 ± 6	68 ± 11	22 ± 2
Tarkiainen et al., 2015	Genotype panel study	Substrate	600	8/13	25 ± 4	72 ± 14	23 ± 3
Tornio et al., 2014	Crossover DDI study	Perpetrator	300	4/3	24 ± 3	63 ± 9	21 ± 2
Holmberg et al., unpublished data	Genotype panel study	Substrate	600	11/12	25 ± 5	72 ± 13	23 ± 3
Itkonen et al., unpublished data I	Crossover DDI study	Perpetrator	300	6/5	23 ± 2	71 ± 12	23 ± 2
Itkonen et al., unpublished data II	Crossover DDI study	Perpetrator	300	7/5	25 ± 4	74 ± 17	24 ± 3
Total				49/57	24 ± 4	71 ± 13	23 ± 3

Data are presented as mean ± S.D., unless otherwise indicated. BMI, body mass index; DDI, drug-drug interaction; f, females; m, males; UGT, UDP-glucuronosyltransferase.

Supplemental References

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