SUPPLEMENTAL MATERIAL

Results

Patient characteristics and their effects on the antiplatelet response to clopidogrel and H4 exposure

Of 120 patients, three were excluded for failure of the VerifyNow test and two were excluded for deviation from the population, as shown by PCA (Figure S1). Thus, 115 patients passed QC and were used in the GWAS analysis.

Baseline clinical characteristics and their effects on the H4 concentration and PRU of 115 patients in stage I are shown in Table S1. Univariate linear regression analysis showed no association between the logarithm of the H4 concentration at 2 h after clopidogrel administration and any baseline clinical characteristic, except low-density lipoprotein cholesterol (LDLC) (estimate = −2.60; R^2 = 0.037; P = 0.0426). However, higher PRU was associated with increased age (estimate = −40.84; R^2 = 0.054; P = 0.0267), male sex (estimate = 1.72; R^2 = 0.043; P = 0.0120), high level of high-density lipoprotein cholesterol (estimate = 63.02; R^2 = 0.038; P = 0.0385), high level of LDLC (estimate = 18.15; R^2 = 0.034; P = 0.0495), and use of CCBs (estimate = 167.83; R^2 = 0.071; P = 0.0036). Concomitant use of CCBs may inhibit the antiplatelet activity of clopidogrel, because of the inhibition of *CYP3A4* and P-gp.(1-3) However, our results showed that the use of CCBs was not correlated with H4 but with PRU, thereby indicating that the role of CCB in interindividual variability in response to clopidogrel was more likely antiplatelet effects than PK.

Baseline clinical characteristics and their effects on PK of clopidogrel and H4 in 31 patients in stage IIa are shown in Table S2. A significant association was noted between hypertension and clopidogrel T_{max}, H4 T_{max}, and H4 C_{max} (P = 0.0106, 0.0435, and 0.0408, respectively), whereas negative correlations were detected between the use of proton pump inhibitors and clopidogrel AUC (P = 0.0475).

PK characteristics of clopidogrel and its metabolites H3 and H4

We first recruited 31 patients with CHD to obtain the plasma concentration–time profiles of clopidogrel and its metabolites H3 and H4. The plasma concentration–time profiles following the administration of 300 mg of clopidogrel in stage IIa are presented in Figure S3. The PK parameters calculated for clopidogrel and its active metabolites H3 and H4 are presented in Table S6. The absorption of clopidogrel from the gastrointestinal tract widely varied with T_{max} values of 0.5–4 h (mean ± SD: 1.96 ± 1.33 h) and C_{max} values of 15.90–530.00 ng/mL (mean ± SD: 163.18 ± 159.95 ng/mL), leading to a wide variation during H4 exposure, with AUC_{0-4h} of 11.58–262.70 ng·h/mL (mean ± SD: 93.75 ± 63.13 ng·h/mL).

The plasma concentrations of the biologically active H4 isomer at 2 and 4 h were two times lower than those of the inactive H3 following the administration of 300 mg of clopidogrel (Figure S3). AUC_{0-4h} of H4 was approximately three times lower than that of H3 in stage IIa in Chinese patients with CHD (Table S6). This result was very different from that in Caucasians. Karaźniewicz-Łada et al.(4) reported that the plasma concentrations of the biologically active H4 isomer following administration of 300 mg of clopidogrel were two times greater than those of H3. The difference in the H4 to H3 ratio was partly due to the differences in genetic variation in absorption and metabolism.

Because 8 of 31 (25.6%) Tmax for clopidogrel, 3 (9.7%) Tmax for H3, and 4 (12.9%) Tmax for H4 were up to 4 h, which was the last time point of blood drawn, simulating elimination phase for these patients would be difficult. Population PK estimation, including AUC and clearance, using nonlinear mixed-effects modeling may be biased. Actual measures with two sampling times at 2 and 4 h rather than estimated PK parameters were used for further analyses in another 120 patients with CHD after the administration of clopidogrel in stage I according to the literature.(4, 5)

Statistical analysis in stage IIa showed that AUC_{0-4h} of the active H4 isomer was significantly correlated with the H4 plasma concentrations at 0.25 (r = 0.6382, $P = 0.0001$), 0.5 (r = 0.6112, $P = 0.0003$), 1 (r = 0.9117, $P < 0.0001$), and 2 h (r = 0.8141, $P < 0.0001$) but not at 4 h (r = 0.1661, $P = 0.3717$) after treatment with 300 mg of clopidogrel. A significant correlation was found between AUC_{0-4h} of the active H4 isomer and C_{max} (r = 0.8894, $P < 0.0001$) (Figure S4). Accordingly, the measured concentrations of clopidogrel and its metabolite at 2 h were used as substitutes for AUC_{0-4h} in stage I of GWAS.

Relationship between antiplatelet effects and PK of clopidogrel treatment

The antiplatelet effects of clopidogrel at 4 h after treatment with 300 mg of clopidogrel widely varied. PRU values varied by more than threefold from the 10th to the 90th percentile, and coefficients of variation were large (41.7%) (mean ± SD: 196.0 ± 81.8) (Figure S2). Of the patients, 45% could be characterized as nonresponders, with PRU values > 208. By contrast, 10% exhibited low platelet aggregation, with PRU values < 98. Statistical analysis revealed that PRU was significantly associated with the logarithm of the H4 concentration at 2 h (r = −0.4147, $P < 0.0001$) and H3 concentration at 2 h (r = −0.2723, $P = 0.0032$). PRU was not correlated with the clopidogrel concentration at 2 h (r = −0.0788, $P = 0.4029$) nor the H4 and H3 concentrations at 4 h ($P > 0.05$) (Figure S6).

The correlation between the H4 concentration and PRU was similar to that in published data.(4, 6) Karaźniewicz-Łada et al.(4) reported that the goodness of fit of linear regression between C_{max} of the active H4 isomer and platelet aggregation was 0.439 in patients. Liang et al.(6) reported that the correlation between the active metabolites of clopidogrel on day 1 and PD measures of platelet inhibition was −0.387 to −0.562 in patients with CHD. In particular, an H4 concentration at 2 h different from that at 1 h may have compromised the correlation between the H4 concentration and PRU in the present study.

Effects of *CYP2C19*3* (rs4986893) on the antiplatelet effects and PK of clopidogrel treatment

*CYP2C19*3* didn't have a significant contribution to antiplatelet effects, PK, and activation of clopidogrel. In stage I, GWAS showed that *CYP2C19*3* was not associated with PRU at 4 h ($P_{PRU > 208}$ = 0.5003, P_{PRU} = 0.3513) and H4 concentration at 2h after loading dose of clopidogrel ($P_{H4\ C2h}$ = 0.1457). In stage IIa, *CYP2C19*3* was not associated with PK parameters either of clopidogrel and H4 ($P_{Clop\ Tmax}$ = 0.4654, $P_{Clop\ AUC0\text{-}4h}$ = 0.5537, $P_{H4\ Tmax}$ = 0.8844, $P_{H4\ AUC0\text{-}4h}$ = 0.5016). In stage IIb, *CYP2C19*3* decreased H4 formation to some extent in human liver S9 fractions (6.69 ± 6.02 ng/ml/mg protein for GG vs 4.18 ± 3.97 ng/ml/mg protein for GA), but the association didn't reach significance (P = 0.4263).

Methods

CHD patient recruitment and liver tissue collection

A group of 120 patients in stage I were sequentially enrolled in Guangdong General Hospital from October 2013 to January 2014. Thirty-one patients in stage IIa were sequentially enrolled in the same hospital from October 2012 to January 2013. All patients were unrelated Han Chinese male and female patients with CHD between 40 and 80 years old who clinically presented with stable angina, unstable angina, or acute myocardial infarction. CHD diagnosis was confirmed by coronary angiography with single or multiple-vessel stenosis (≥50% stenosis). Exclusion criteria included the presence of the following: advanced cancer, renal failure, hepatic failure, malignancies, concomitant use of oral coumarin derivatives or other anticoagulant treatment, any contraindication to aspirin or clopidogrel therapy, and pre-existing bleeding disorders.

In stage IIb, distant noncancerous liver tissues were obtained surgically or through liver resection for benign liver diseases at Sun Yat-Sen Memorial Hospital (Guangzhou,

Guangdong, China) between September 2012 and May 2015. In stage III, 91 CHD patients with MACE and 208 CHD patients without MACE were randomly selected from an independent study cohort during the 18-month follow-up period after PCI. Of these cases, 2411 CHD patients were sequentially enrolled in Guangdong General Hospital between January 2010 and October 2013. The clinical efficacy endpoint was the cumulative incidence of MACE, including cardiac death, non-fatal MI, stent thrombosis, and ischemic stroke.

This study was approved by the Medical Ethical Review Committee of Sun Yat-Sen Memorial Hospital (Guangzhou, China) and Guangdong General Hospital (Guangzhou, China). Written informed consent was obtained from each patient. A flow chart of the technological process is shown in Figure 1.

Sample processing

In stage I, patients received 300 mg of clopidogrel and 100 mg of aspirin. Blood samples were collected for determining the plasma concentration of clopidogrel, active metabolite isomer H4, and inactive metabolite isomer H3 at 2 and 4 h after the loading dose. On-treatment PRU was measured 4 h after the administration of the loading dose. Previous studies reported (7, 8) that maximum platelet inhibition was achieved at 2 h after a loading dose of clopidogrel. To confirm the association between significant SNPs with PK parameters of clopidogrel and H4, and further assess possible mechanisms between significant SNPs and PRU, a replication study in 31 patients with CHD was performed in stage IIa. Blood samples for the PK assessment of clopidogrel and its metabolites H3 and H4 were collected at 0, 0.25, 0.5, 1, 2, and 4 h following 300 mg of clopidogrel. Venous blood samples (4 mL) were collected into K_3EDTA tubes and then immediately added with 25 μL of 500 mM 3′-methoxyphenacyl bromide (MPBr) in acetonitrile to stabilize metabolites in the blood. Following centrifugation at 4°C within 60 min of collection, the plasma was collected and stored at −80°C.

Bioanalytical analysis

A high-performance LC-MS/MS assay was developed and validated for the simultaneous determination of clopidogrel, 3′-methoxyacetophenone derivatives [MP-H3 (SAR287550-1) and MP-H4 (SAR206251)] of free clopidogrel active metabolite stereoisomers (H3 and H4), and IS carbamazepine in human plasma and mixture of liver S9 fraction. Chromatography was performed using a Shim-pack XR-ODII column (2.0 mm I.D × 150 mm, 2.2 μm particle size, Shimadzu Corporation, Kyoto, Japan) at room temperature. The mobile phase, which consisted of acetonitrile and water (0.2% formic acid, 2 mM ammonium acetate; 60:40, V/V), was delivered at a flow rate of 0.25 mL/min. Mass spectrometric detection was performed using an API 4000 triple quadrupole instrument (Applied Biosystems, Foster City, CA, USA) in positive electrospray ionization mode. The precursor-to-product ion reactions monitored were m/z 322.0 to 213.6, 505.2 to 156.0, and 237.2 to 194.2 for clopidogrel, derivatized active metabolites, and carbamazepine, respectively. The representative MS/MS spectrum and LC-MS profiles are shown in Figure S5.

The PK analysis assay was validated over the linear range of 0.2–100 ng/mL for clopidogrel and its metabolites. The intra- and inter-assay precisions were ≤ 14.6% and ≤ 14.7%, respectively. The matrix effects of plasma were in the range of 103.6%–110.7% for clopidogrel, 101.0%–111.6% for metabolite H3, and 102.2%–111.8% for metabolite H4. This method was successfully applied in PK studies of clopidogrel in patients with CHD.

The PK parameters of clopidogrel, H3, and H4 were estimated using the NCA function in Phoenix WinNonlin version 6.3 software (Pharsight, Cary, NC, USA).

GWAS of the antiplatelet response to clopidogrel and H4 exposure in stage I

DNA was isolated from peripheral blood samples anticoagulated with K_3EDTA using the Gentra Puregene DNA Isolation Kit (Qiagen, Hilden, Germany). A total of 900,015 SNPs in a GWAS scan of 117 subjects were genotyped with the Illumina HumanOmniZhongHua-8

BeadChip according to the Infinium HD protocol from Illumina. The Illumina HumanOmniZhongHua-8 BeadChip covers 81% of common variants (MAF ≥ 5%; $r^2 > 0.8$) and 60% of rare variants (5% > MAF ≥ 2.5%; $r^2 > 0.8$) for individuals of Han Chinese descent.

Prior to association analysis, a systematic QC procedure was applied to the raw genotyping data to filter both unqualified SNPs and samples. We excluded SNPs from further analysis if they: (1) did not map to autosomal chromosomes; (2) had a low call rate in GWAS samples (< 95%); (3) had MAF < 0.05; and (4) deviated from the Hardy–Weinberg equilibrium ($P < 1.0 \times 10^{-5}$). We also removed individuals from further analysis if they (1) had an overall successful genotyping call rate < 98% or (2) showed sex discrepancies between records and genetically inferred data. We detected population outliers using a method based on PCA. After quality filtering and cleaning, 115 subjects with 703,143 SNPs were included in further analyses.

Clopidogrel, H3, and H4 concentrations were transformed by logarithms because of the non-normal distribution. Associations between SNP genotypes and PRU or log2-transformed clopidogrel, H3, or H4 concentration were assessed using a general linear model. Meanwhile, associations between SNP genotypes and resistance status to clopidogrel treatment (PRU > 208) were assessed using a logistic regression model that assumes an additive effect of allelic dosage using PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). In view of the limited number of subjects, a Bonferroni-corrected *P* value for the genome-wide significance threshold was too stringent. A suggestive significance and reporting threshold was set at *P* of 1.0×10^{-4}, corresponding approximately to the level at which *CYP2C19*2* was significantly associated with PRU. Solid evidence demonstrated that *CYP2C19*2* was associated with antiplatelet and PK response to clopidogrel treatment.(9-11)

Selected SNP genotyping for replication studies in stages II and III

To confirm the association between significant SNPs with PRU and PK parameters of clopidogrel and its active metabolite H4, as well as further assess the effects of SNPs on the risk of MACE in CHD after PCI, SNPs were selected based on the following criteria for further validation: (i) $P < 1.0 \times 10^{-4}$ for PRU or PRU > 208 for all SNPs or $P < 0.01$ for PRU or PRU > 208 for SNPs in the ADME genes in stage I; (ii) SNPs with $P < 0.05$ for the H4 concentration in stage I; and (iii) only the SNP with the lowest P value when multiple SNPs were observed but all SNPs in strong LD ($r^2 \geq 0.8$) in each gene. For *CYP2C19*, rs4244285 was selected. Eighteen SNPs satisfied the criteria for replication but those that had strong LD ($r^2 \geq 0.8$) with selected SNPs were not included in the replication.

Genotyping of 18 SNPs in stages II and III was performed using the iPLEX MassARRAY platform (Sequenom, Inc.). The concentration of clopidogrel and its metabolites in the plasma was analyzed using a validated LC-MS/MS assay. Considering that *3 (rs4986893) is one of important loss of function allele in *CYP2C19*, we genotyped the SNP by a TaqMan SNP assay using the ABI Vii7 Real-Time PCR system (Applied Biosystems, USA) additionally in stage II. The primers used were 5′-GATCAGCAATTTCTTAACTTGATGGA -3′ (forward) and 5′-ACTGTAAGTGGTTTCTCAGGAAGCA -3′ (reverse). The TaqMan MGB probes were HEX-ATTGTAAGCACCCCCTGGATCCAG-BHQ1 and FAM-TTGTAAGCACCCCCTGAATCCAGG-BHQ1.

Functional replication study in clopidogrel activation

In stage IIb, the function of these SNPs on clopidogrel activation was further investigated in 32 human liver tissues to identify a plausible mechanism for newly identified genetic variants. The formation of H4 in the liver S9 fraction was analyzed using a validated LC-MS/MS assay.

A GENMED liver S9 fraction isolation kit (Genmed Scientific Inc., USA) was used to prepare liver S9 fraction from tissue samples from distant noncancerous liver tissues obtained surgically or via liver resection for benign liver diseases. The incubation solution of clopidogrel in liver S9 fraction (200 μL) consisted of 100 mM phosphate buffer (pH 7.4), 3.3 mM $MgCl_2$, 1.3 M NaCl, 2 mM $CaCl_2$, 5 mM reduced glutathione, 2.5 mM $NADP^+$, 25 mM glucose-6-phosphoric acid monosodium salt, 2 U/mL glucose-6-phosphate dehydrogenase, 1 mg/mL liver S9 fraction, and 25 μg/mL clopidogrel. The system was incubated at 37°C for 5 min and started by adding clopidogrel.

After incubation for 30 min, 20 μL of carbamazepine (250 ng/mL) was added to the incubation mixture and mixed by a vortex for 3 s. Subsequently, a 0.8 mL aliquot of the extraction kit-acetonitrile (including the 6.25 mM derivatizing agent MPB) was added to the mixture, which was vortexed for 5 min and centrifuged for 20 min at 12,000 rpm. The organic layer was transferred to a new tube and evaporated to dryness at 40 °C in a vacuum drying chamber (Salvis Lab, VC20). The residue was reconstituted in a 100 μL mobile phase, vortex mixed for 2 min, and centrifuged for 15 min at 12,000 rpm. An 8 μL aliquot was injected into the LC-MS/MS system for analysis.

Statistics

Demographic and clinical characteristics were summarized using counts (percentages) for categorical variables and mean ± SD for continuous variables. The normality of distribution of continuous variables was checked by the Shapiro–Wilk normality test. As the ranges of the H4 concentration were skewed, logarithmic transformation was performed prior to analysis. Spearman correlation coefficients were calculated to describe the correlation between the clopidogrel/H3/H4 concentration at 2 h and PRU measured by VerifyNow in stage I. Similarly, the correlation between concentration at different times and AUC of clopidogrel, H3, or H4 was also determined. Linear (or logistic) regression analyses were applied to

evaluate the association of baseline clinical characteristics with PRU and H4 concentration in stage I, association of SNPs with PK parameters of clopidogrel and H4 in the replication study of stage IIa, and association of SNPs with the formation of H4 in stage IIb.

To develop a statistical model for predicting the variability of PRU and H4 concentration, forward stepwise linear regression analysis with calculation of the coefficient of determination (r^2) was used to determine the independent effects of clinical variables, genetic variables, and interacting drugs on PRU and H4 concentration. Variables with $P < 0.10$ were entered into the multivariable model, and only variables with $P < 0.05$ were retained in the model. Variables of variant alleles were coded as 0, 1, and 2 for zero, one, and two copies of the variant allele, respectively. Non-genetic variables included age, sex (female coded as 0, male coded as 1), medical history (coded as 0/1), biochemical measurements, and each interacting drug (coded as 0/1). $P < 0.05$ was considered significant. Logistic regression analysis was applied to evaluate the effects of genotypes on the risk to MACE and calculate ORs and 95% CIs in stage III. FDR control was used to correct for multiple comparisons using SAS with the FDR option. $P < 0.05$ was considered statistically significant while controlling the FDR at 0.05. Data analyses were performed in SAS 9.4 (SAS Inst, Cary, NC, USA).

Supplementary Figure S1- S6

Figure S1. Results of principal component analysis. PCA1, PCA2, and PCA3 indicate the first, second, and third principal component of study subjects, respectively. A. analysis of 117 patients (two highlighted in red were excluded for deviation from the population.); B. analysis of 115 patients

Figure S2. Distributions of PRU (A) and concentrations of clopidogrel (B), active metabolite isomer H4 (C), and inactive metabolite isomer H3 (D) at 2 h after administration of 300 mg of clopidogrel in 115 patients with CHD.

Figure S3. Average and individual concentration–time curve of clopidogrel (A, B) and its metabolites H4 (C, D) and H3 (E, F) in 31 patients with CHD.

Figure S4. Correlations of plasma concentrations of active metabolite isomer H4 at 0.25, 0.5, 1, 2, and 4 h and its maximal concentration with $AUC_{0\text{-}4h}$.

Figure S5. Representative MS/MS spectrum of derivative H4 metabolite (A) and clopidogrel (B) and representative LC-MS profiles of MP-H3 standard (C), MP-H4 standard (D), internal standard carbamazepine (E), clopidogrel (F), mixture of standards (G), and human sample (H). 1, 2, 3 and 4 represent MP-H3, MP-H4, clopidogrel and carbamazepine, respectively.

Figure S6. Association of PRU with concentrations of plasma clopidogrel and its metabolites H3 and H4 at 2 and 4 h.

Supplementary Tables S1–S9

Table S1. Baseline clinical characteristics and their effects on the H4 concentration and PRU of 115 patients.

Table S2. Baseline clinical characteristics and their effects on PK of clopidogrel and H4 in 31 patients.

Table S3. A total of 27 SNPs showing suggestive evidence of the relationship with the H4 concentration at 2 h ($P < 0.01$) among 125 SNPs associated with PRU or PRU > 208 ($P < 1 \times 10^{-4}$) in GWAS analysis.

Table S4. Association of SNPs in 295 candidate ADME genes from PharmaADME with PRU or PRU > 208 ($P < 0.01$) and H4 concentration ($P < 0.05$) in 115 patients with CHD.

Table S5. Differences in PRU and H4 concentration among different genotypes of 18 SNPs in 115 patients with CHD.

Table S6. Pharmacokinetic parameters of clopidogrel, active metabolite isomer H4, and inactive metabolite isomer H3 in 31 patients.

Table S7. Differences in pharmacokinetic parameters of clopidogrel and H4 among different genotypes of 18 SNPs after administration of 300 mg of clopidogrel in 31 patients with CHD.

Table S8. Differences in H4 formation among different genotypes of 18 SNPs in 32 human liver S9 fractions.

Table S9. Call rate of the SNPs examined in 299 patients with CHD.

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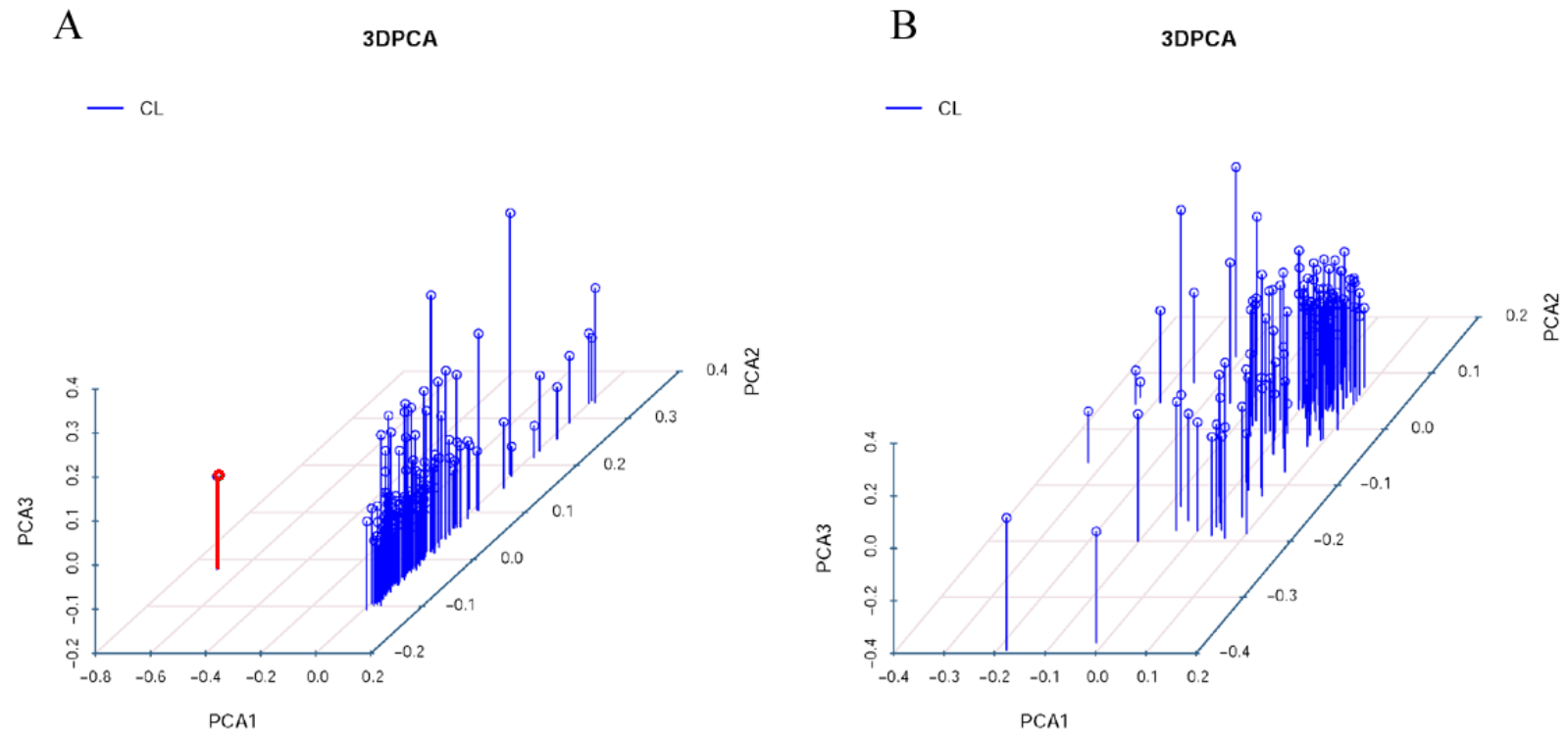
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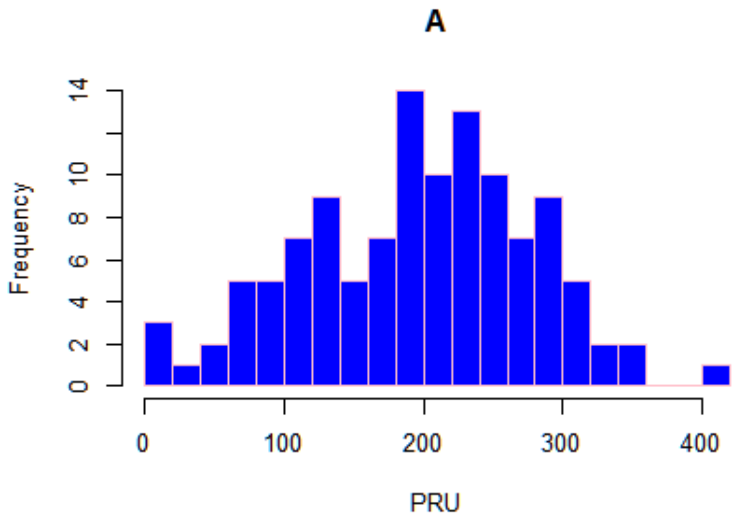
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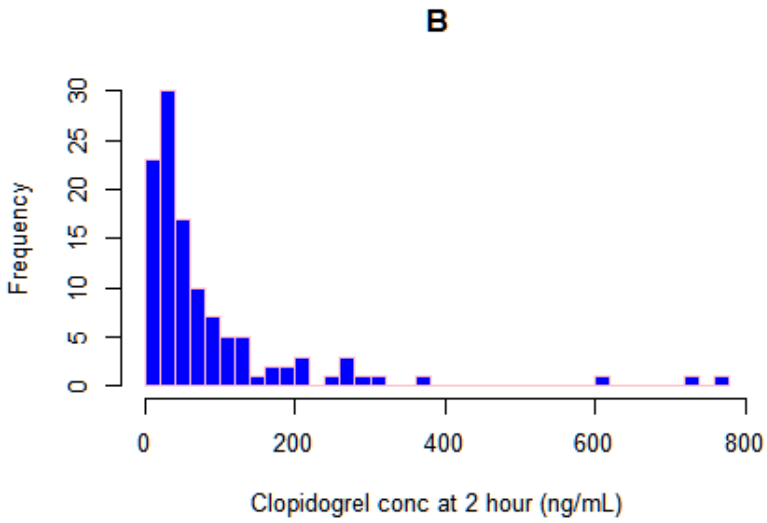
PRU


B
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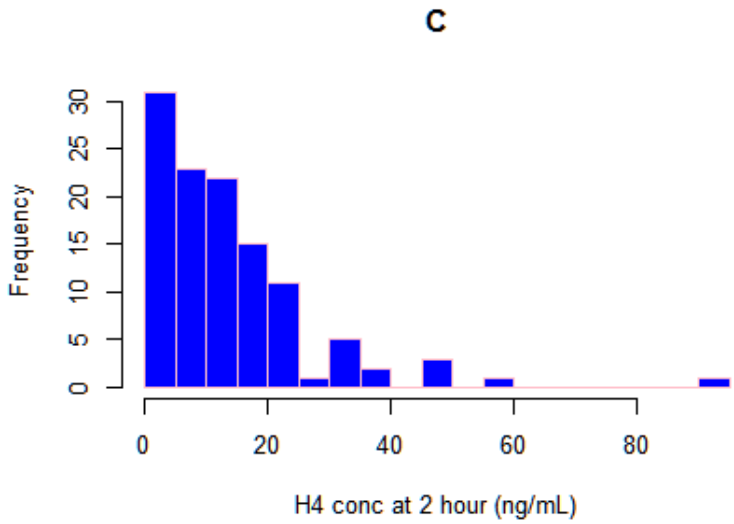
400
600
800
Clopidogrel conc at 2 hour (ng/mL)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Frequency
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20
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30
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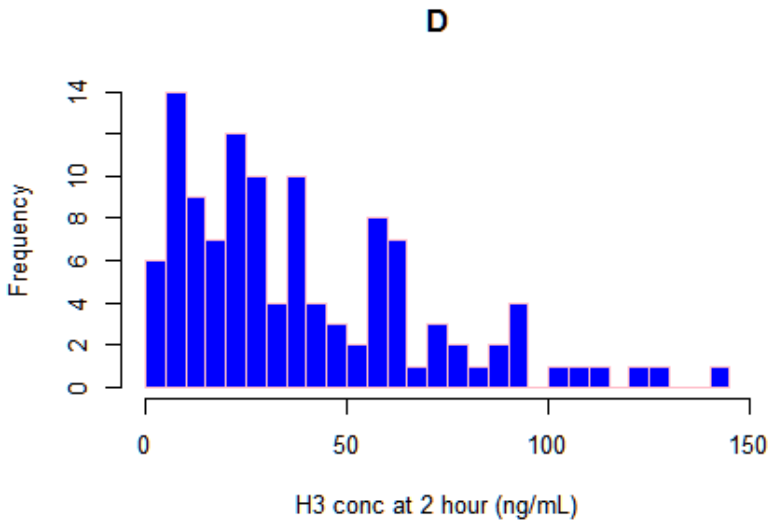
20
40
60
80
H4 conc at 2 hour (ng/mL)


D
Frequency
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6
8
10
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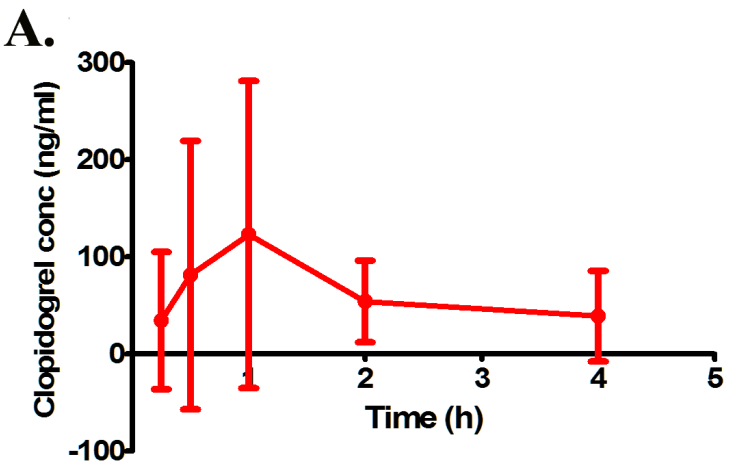
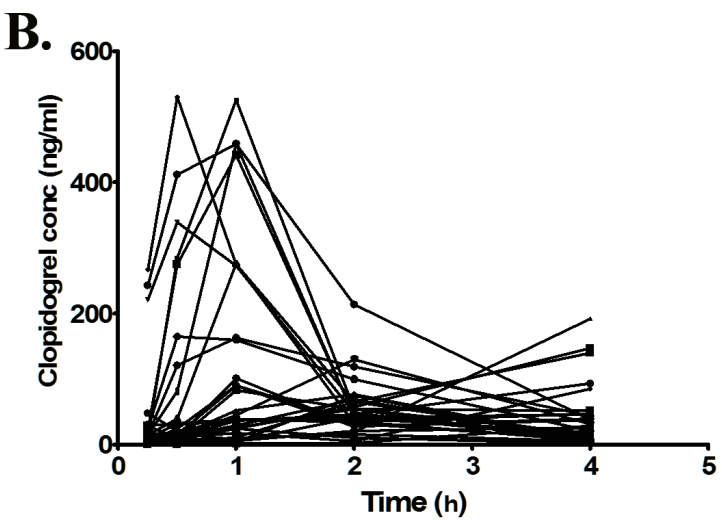
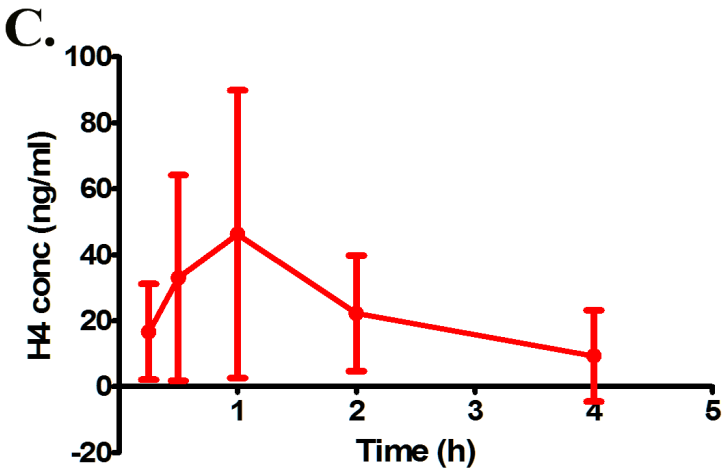
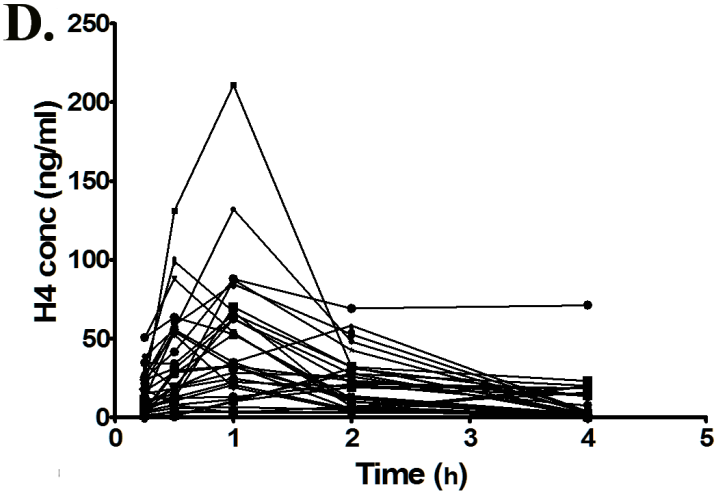
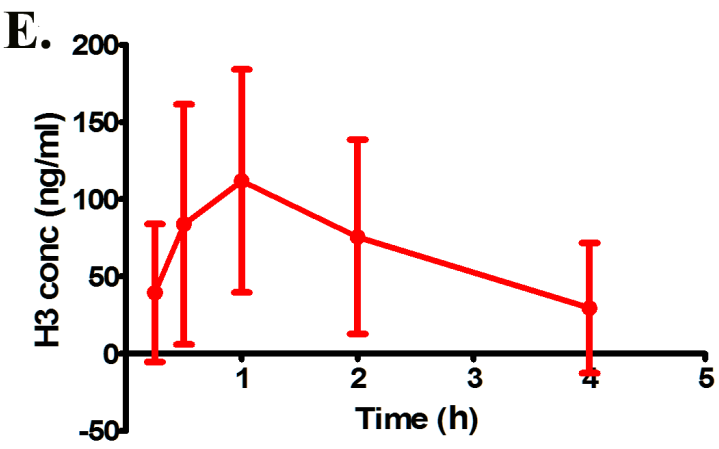
0
50
100
150
H3 conc at 2 hour (ng/mL)

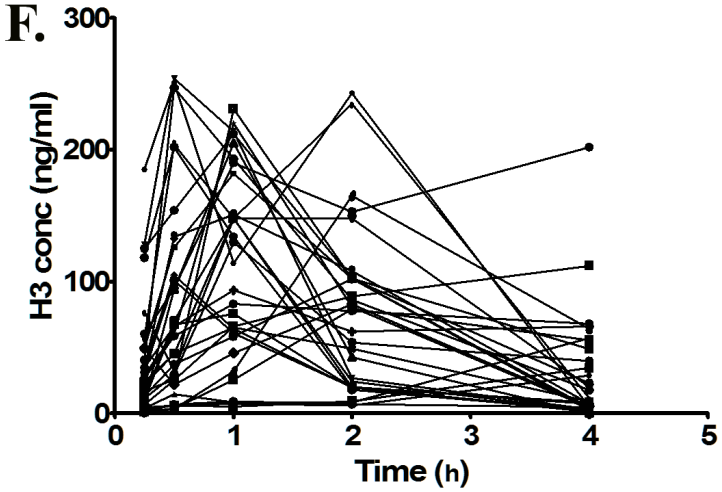

A.
Clopidogrel conc (ng/ml)
300
200
100
0
-100
1
2
3
4
5
Time (h)

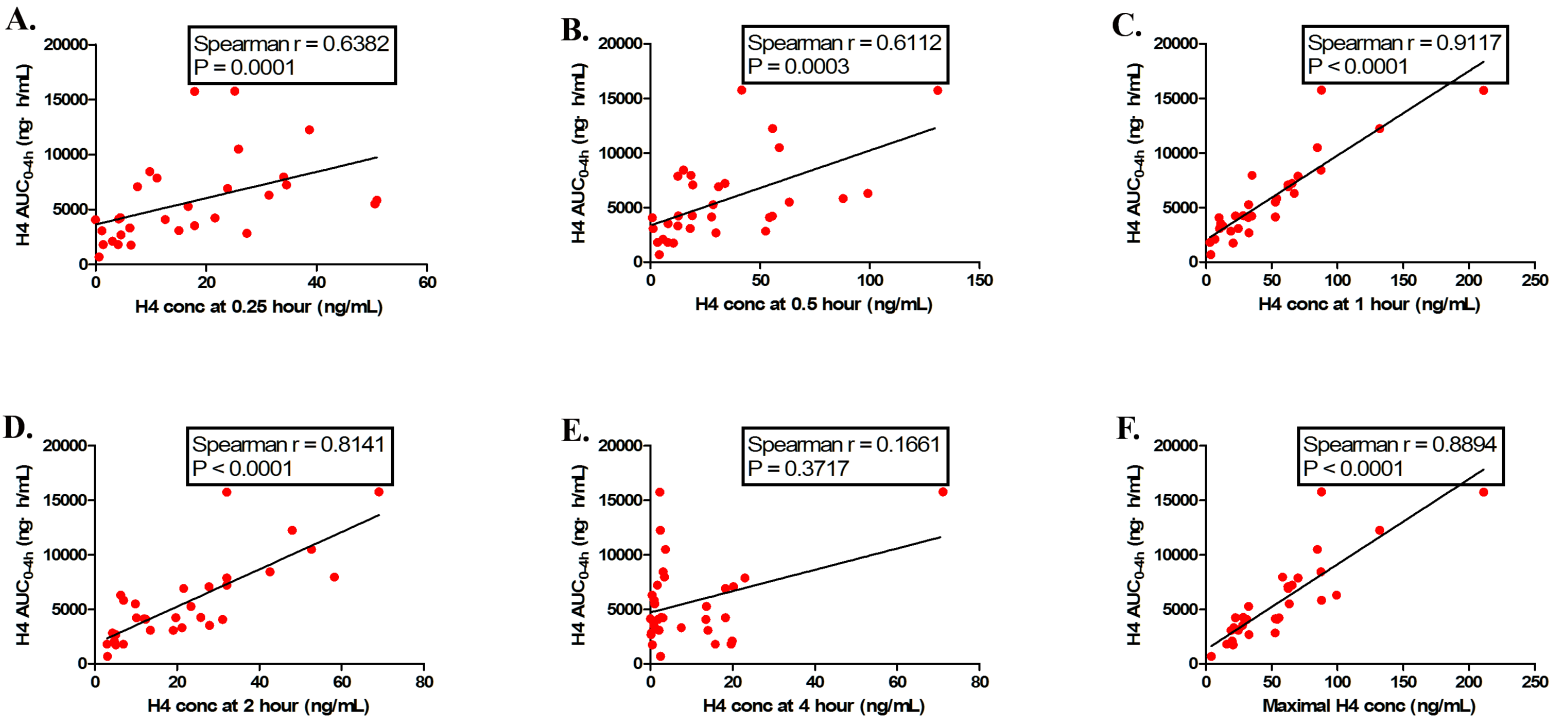

B.
Clopidogrel conc (ng/ml)
600
400
200
0
0
1
2
3
4
5
Time (h)


C.
H4 conc (ng/ml)
100
80
60
40
20
0
-20
1
2
3
4
5
Time (h)

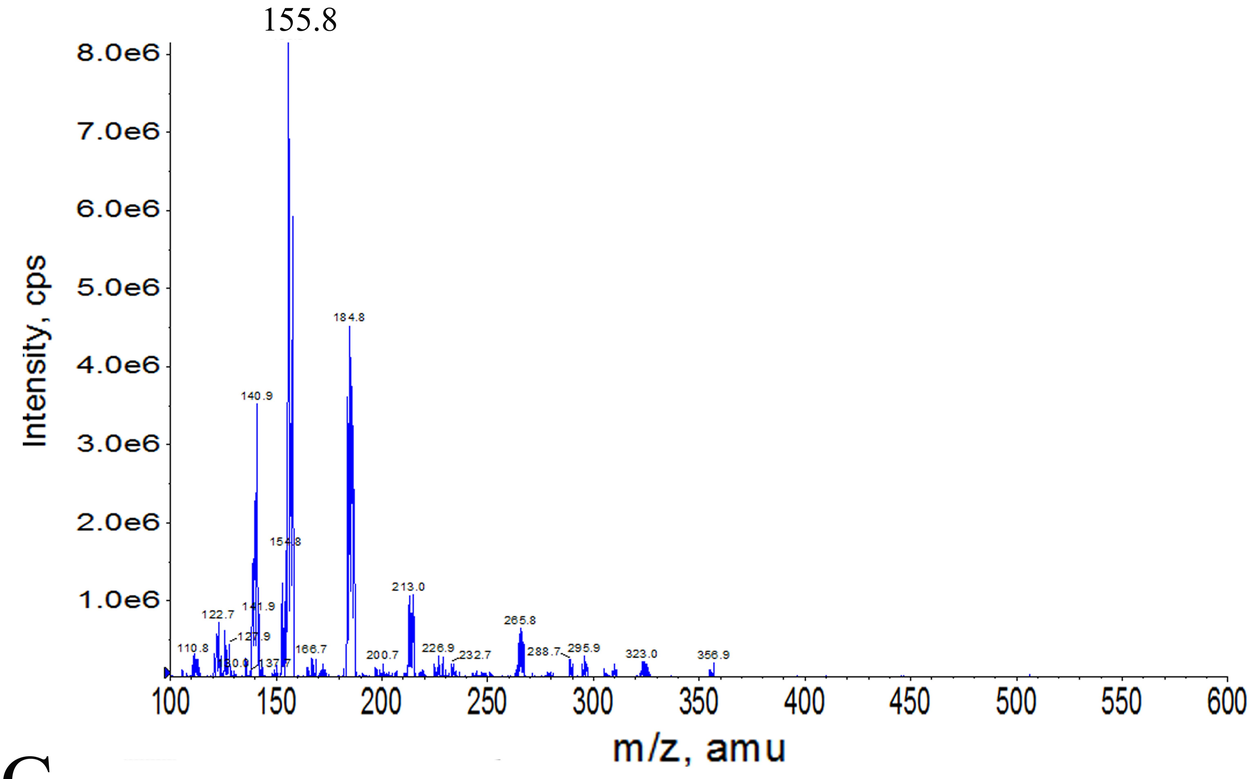

D.
H4 conc (ng/ml)
250
200
150
100
50
0
0
1
2
3
4
5
Time (h)


E.
H3 conc (ng/ml)
200
150
100
50
0
-50
1
2
3
4
5
Time (h)

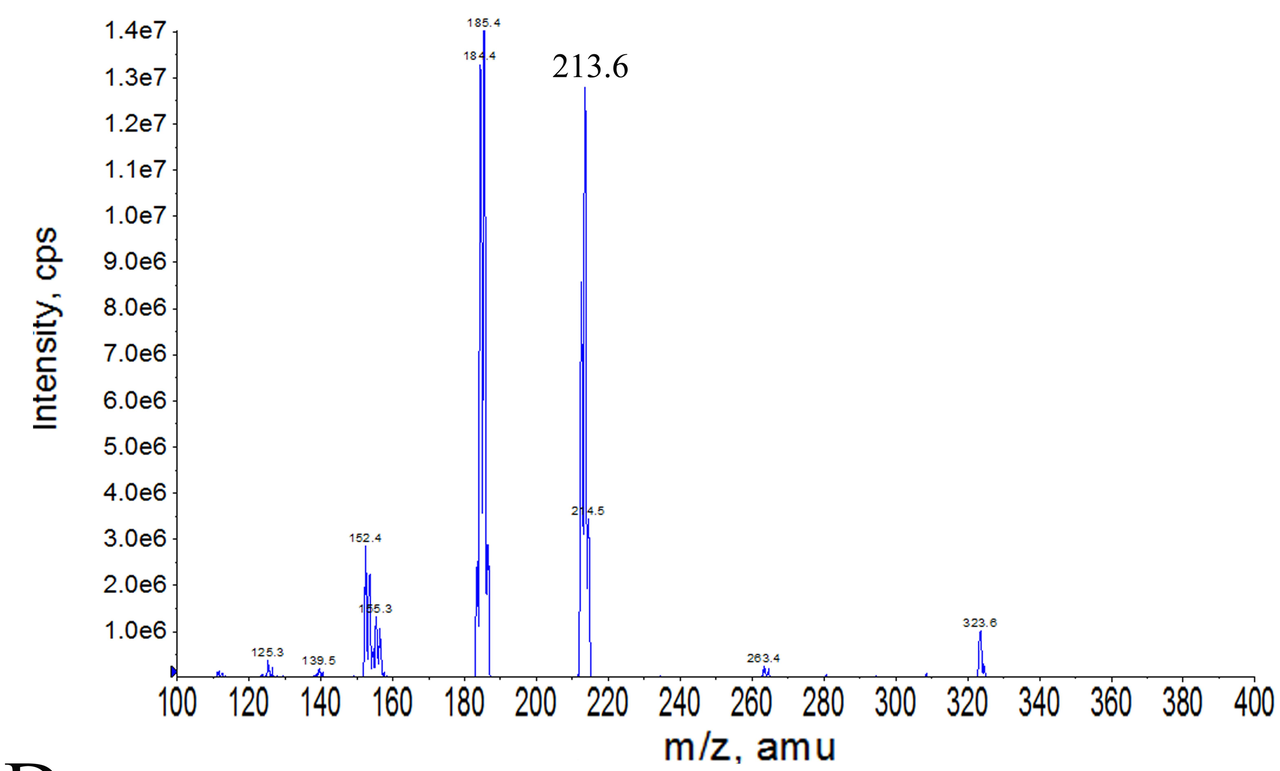

F.
H3 conc (ng/ml)
300
200
100
0
0
1
2
3
4
5
Time (h)


A.
Spearman r = 0.6382
P = 0.0001
H4 AUC0-4h (ng· h/mL)
H4 conc at 0.25 hour (ng/mL)
B.
Spearman r = 0.6112
P = 0.0003
H4 AUC0-4h (ng· h/mL)
H4 conc at 0.5 hour (ng/mL)
C.
Spearman r = 0.9117
P < 0.0001
H4 AUC0-4h (ng· h/mL)
H4 conc at 1 hour (ng/mL)
D.
Spearman r = 0.8141
P < 0.0001
H4 AUC0-4h (ng· h/mL)
H4 conc at 2 hour (ng/mL)
E.
Spearman r = 0.1661
P = 0.3717
H4 AUC0-4h (ng· h/mL)
H4 conc at 4 hour (ng/mL)
F.
Spearman r = 0.8894
P < 0.0001
H4 AUC0-4h (ng· h/mL)
Maximal H4 conc (ng/mL)

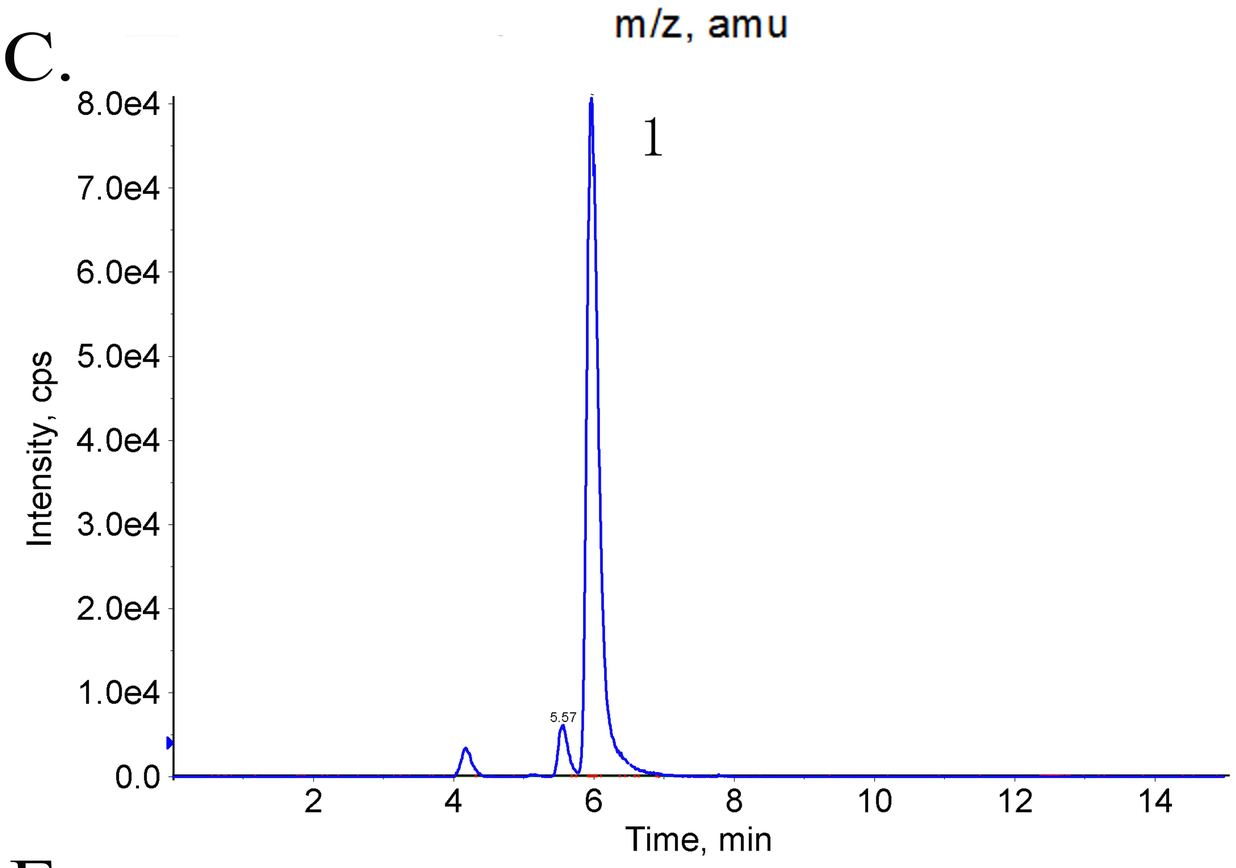
A.


155.8
Intensity, cps
m/z, amu

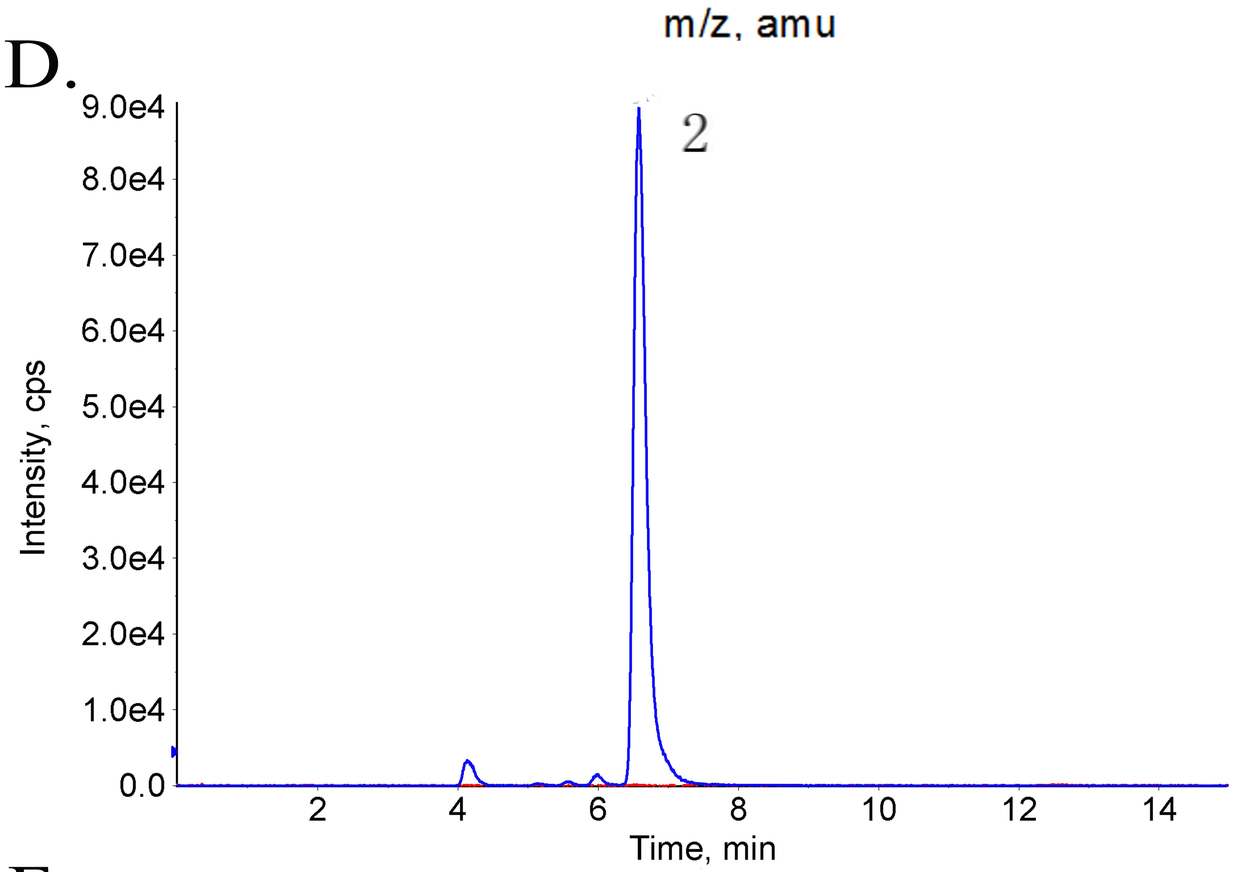
B.


185.4
184.4
213.6
Intensity, cps
m/z, amu

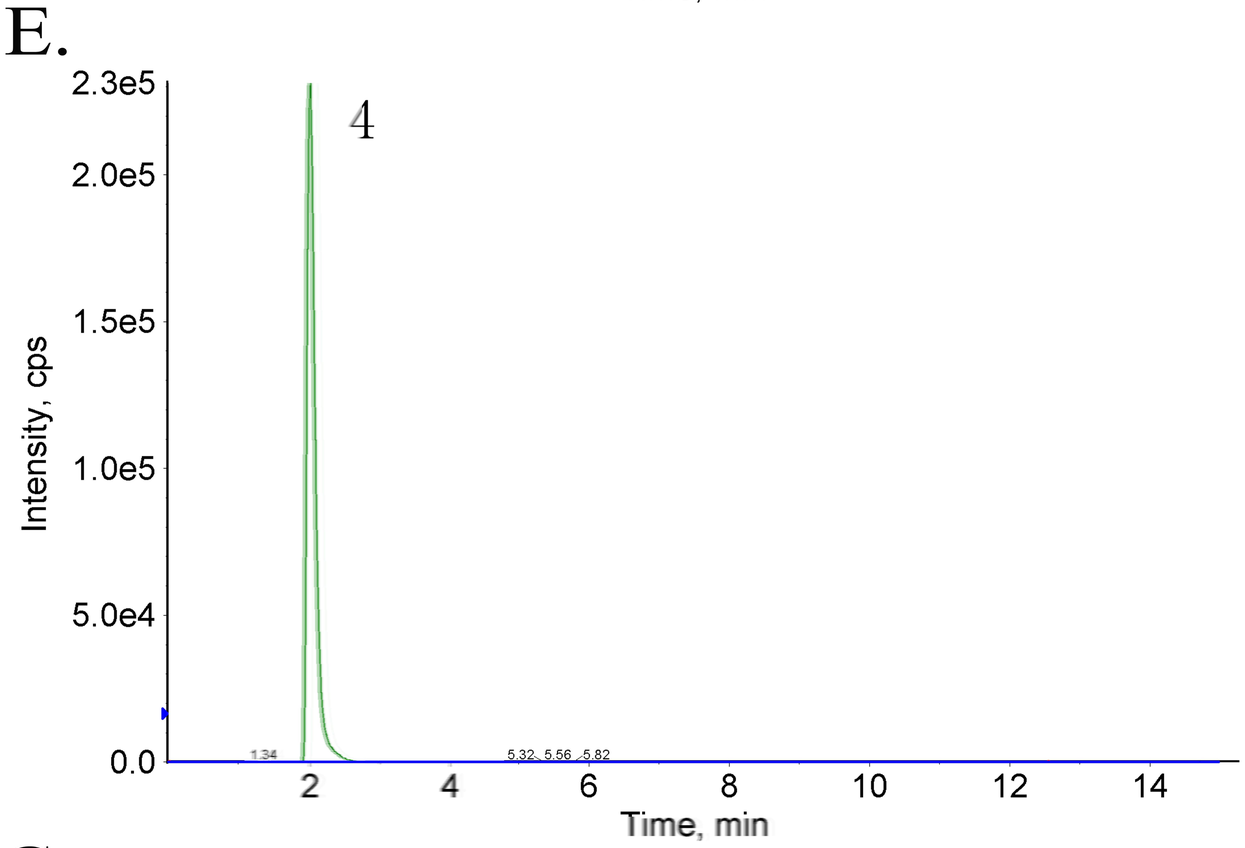
C.


1
5.57
Intensity, cps
Time, min

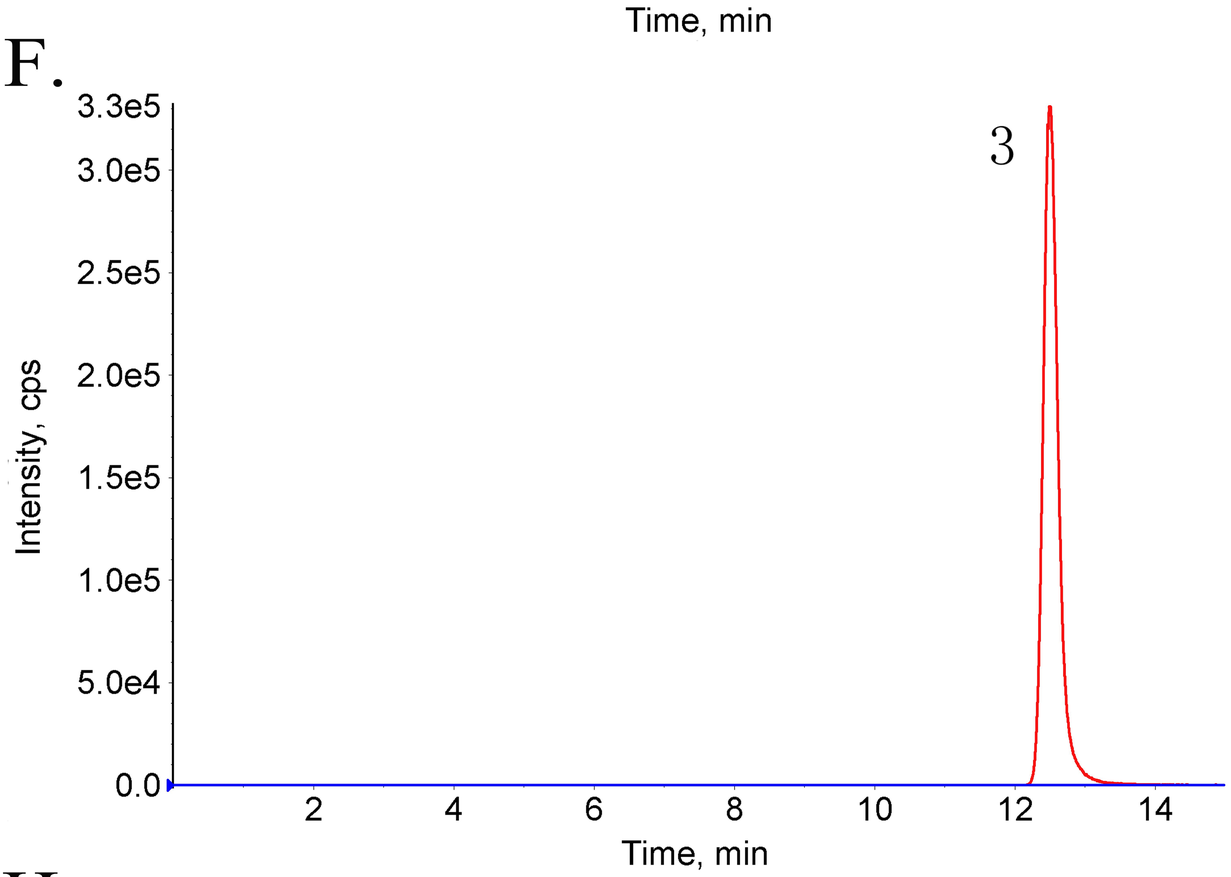
D.


2
Intensity, cps
Time, min

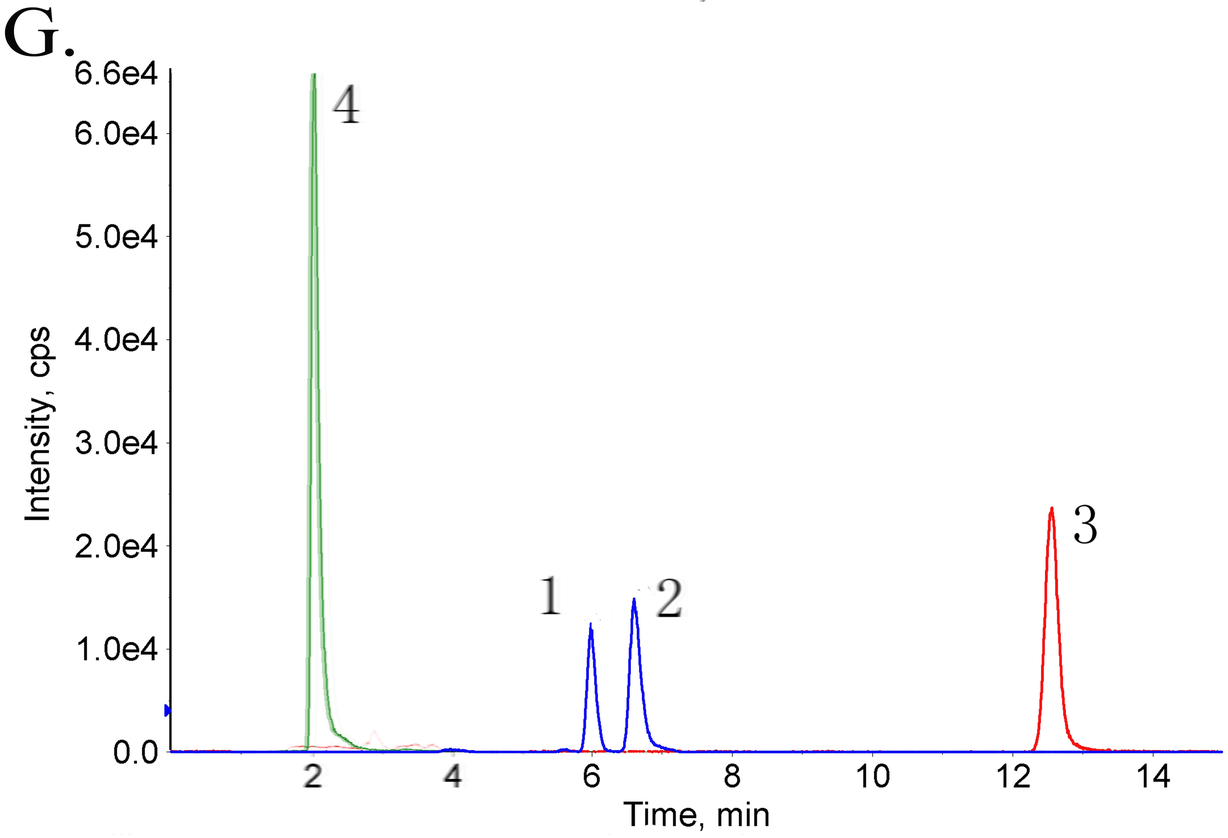
E.


4
1.34
5.32
5.56
5.82
Intensity, cps
Time, min

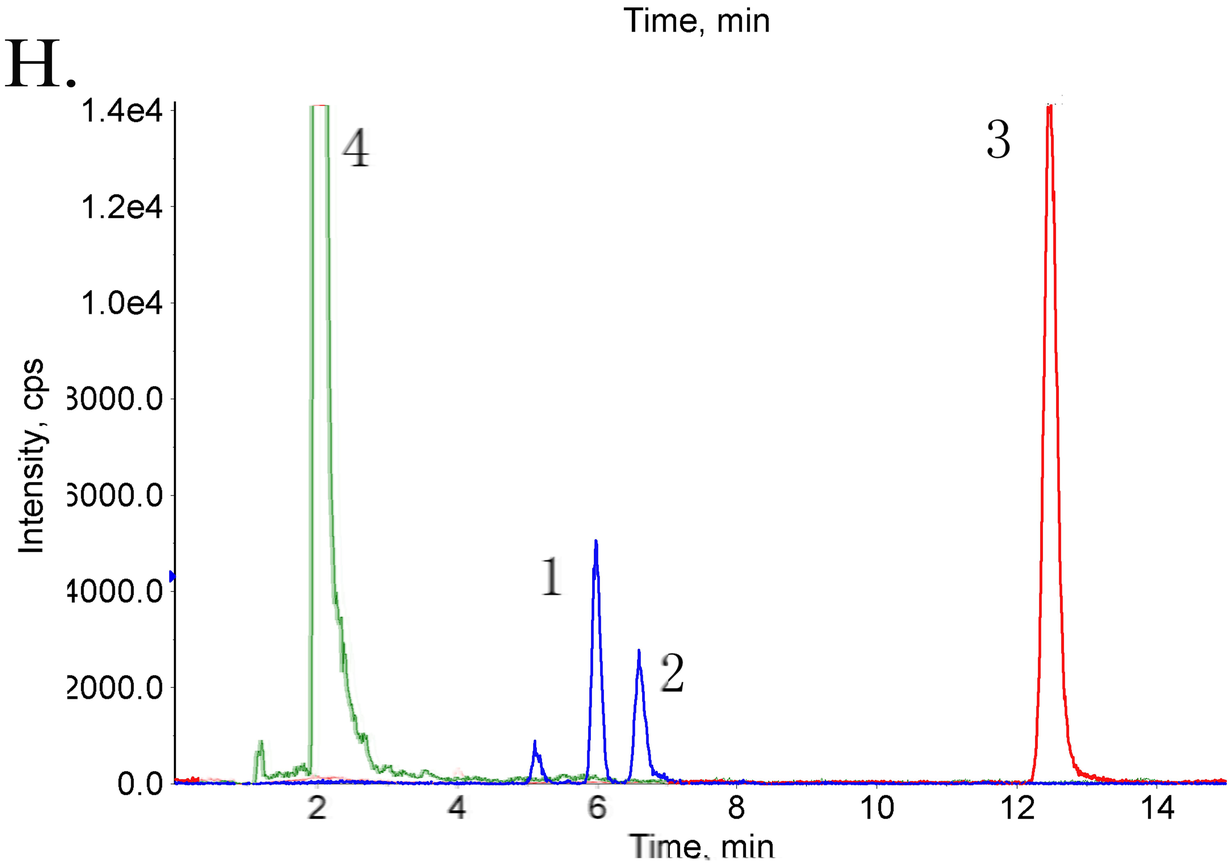
F.


3
Intensity, cps
Time, min

G.


4
1
2
3
Intensity, cps
Time, min

H.


4
1
2
3
Intensity, cps
Time, min

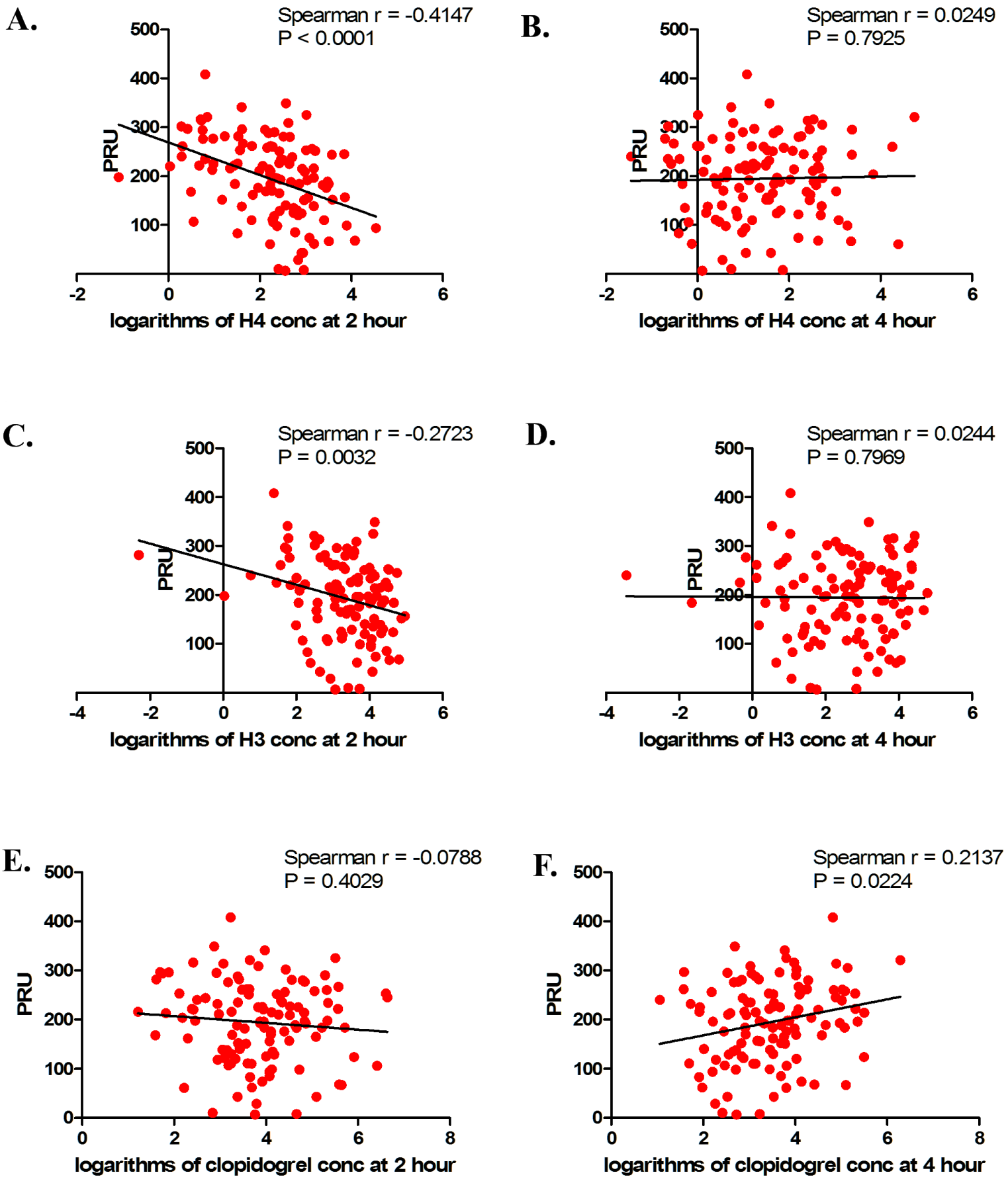

A.
Spearman r = -0.4147
P < 0.0001
PRU
500
400
300
200
100
-2
0
2
4
6
logarithms of H4 conc at 2 hour
B.
Spearman r = 0.0249
P = 0.7925
PRU
500
400
300
200
100
-2
0
2
4
6
logarithms of H4 conc at 4 hour
C.
Spearman r = -0.2723
P = 0.0032
PRU
500
400
300
200
100
-4
-2
0
2
4
6
logarithms of H3 conc at 2 hour
D.
Spearman r = 0.0244
P = 0.7969
PRU
500
400
300
200
100
-4
-2
0
2
4
6
logarithms of H3 conc at 4 hour
E.
Spearman r = -0.0788
P = 0.4029
PRU
500
400
300
200
100
0
0
2
4
6
8
logarithms of clopidogrel conc at 2 hour
F.
Spearman r = 0.2137
P = 0.0224
PRU
500
400
300
200
100
0
0
2
4
6
8
logarithms of clopidogrel conc at 4 hour

Table S1. Baseline clinical characteristics and their effects on the H4 concentration and PRU of 115 patients.

Characteristics	N(%) or Mean ± SD	H4			PRU		
		Estimate	R^2	*P*	Estimate	R^2	*P*
Demographic data							
Age	64.74 ± 9.9	-3.16	0.017	0.2842	-40.84	0.054	0.0267
Male	81 (70.43)	0.12	0.010	0.1691	1.72	0.043	0.0120
Medical history							
Diabetes mellitus	30 (26.09)	-2.11	0.007	0.3825	32.81	0.031	0.0577
Hypertension	62 (53.91)	1.63	0.005	0.4419	20.75	0.016	0.1717
Biochemical measurements							
ALT,U/L	64.74 ± 9.9	-0.04	0.006	0.4269	-0.65	0.025	0.0899
AST,U/L	28.02 ± 20.09	-0.05	0.009	0.3313	0.21	0.003	0.5644
LDLC, mmol/L	30.8 ± 21.15	-2.60	0.037	0.0426	18.15	0.034	0.0495
HDLC, mmol/L	2.74 ± 0.85	2.68	0.004	0.5263	63.02	0.038	0.0385
Triglycerides, mmol/L	1.06 ± 0.26	-1.20	0.009	0.3191	-11.31	0.015	0.1955
Total cholesterol	1.51 ± 0.9	-1.63	0.023	0.1118	13.92	0.032	0.0578
CREA,umol/L	4.52 ± 1.07	-0.01	0.001	0.7237	0.05	0.000	0.8488
Medication							
Statins	7 (6.09)	-4.41	0.009	0.3199	18.35	0.003	0.5671
β-blockers	7 (5.98)	4.25	0.008	0.3374	-19.03	0.003	0.5528
ACE inhibitors	6 (5.98)	-0.20	0.000	0.9686	16.13	0.002	0.6400
CCBs	3 (2.61)	-11.66	0.018	0.1494	167.83	0.071	0.0036
PPIs	4(3.49)	-7.91	0.017	0.1707	28.70	0.004	0.4928

ALT Alanine aminotranferease, AST aspartate aminotransferase, LDLC low-density lipoprotein cholesterol, HDLC high-density lipoprotein cholesterol, CREA Creatinine, CCBs Calcium channel blockers, CREA Creatinine, PPIs proton pump inhibitors.

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Table S2. Baseline clinical characteristics and their effects on PK of clopidogrel and H4 in 31 patients.

Characteristics	N(%) or Mean ± SD	*P* value of clopidogrel			*P* value of H4		
		T_{max}	C_{max}	AUC	T_{max}	C_{max}	AUC
Demographic data							
Age (years)	58.76 ± 14.18	0.4155	0.9666	0.7446	0.9443	0.9747	0.8224
Male	26 (83.87)	0.3512	0.1603	0.1326	0.2687	0.571	0.653
Medical history							
Diabetes mellitus	9 (29.03)	0.5099	0.1839	0.2621	0.5757	0.7623	0.9952
Hypertension	16 (51.61)	0.0106	0.9583	0.9508	0.0435	0.0408	0.0656
Biochemical measurements							
ALT,U/L	26.65 ± 9.95	0.8438	0.6276	0.7670	0.6416	0.8436	0.532
AST,U/L	23.08 ± 8	0.6761	0.4834	0.5457	0.7625	0.7526	0.9817
LDLc, mmol/L	3.15 ± 0.97	0.4056	0.5635	0.3346	0.1354	0.1019	0.0528
HDLc, mmol/L	1.1 ± 0.25	0.9645	0.2959	0.486	0.8271	0.6911	0.727
Triglycerides, mmol/L	1.81 ± 1.03	0.6589	0.3782	0.0558	0.1452	0.8332	0.2331
Total cholesterol	4.8 ± 1.24	0.3343	0.1553	0.3214	0.1522	0.2193	0.649
CREA,umol/L	81.74 ± 21.51	0.7112	0.3204	0.767	0.5477	0.5882	0.532
Medication							
Statins	3 (9.68)	0.3126	0.4724	0.62	0.1349	0.3914	0.5226
β-blockers	1 (3.23)						
ACE inhibitors	0						
CCBs	0						
PPIs	6 (19.35)	0.6219	0.0593	0.0475	0.2706	0.4773	0.1252

ALT Alanine aminotranferease, AST aspartate aminotransferase, LDLc low-density lipoprotein cholesterol, HDLc high-density lipoprotein cholesterol, CCBs Calcium channel blockers, CREA Creatinine, PPIs proton pump inhibitors.

Table S3. A total of 27 SNPs showing suggestive evidence of the relationship with the H4 concentration at 2 h ($P < 0.01$) among 125 SNPs associated with PRU or PRU > 208 ($P < 1 \times 10^{-4}$) in GWAS analysis.

SNP	CHR	BP	Gene symble	*P* value		
				PRU > 208	PRU	H4
rs2860838	10	96453039	*CYP2C18*	2.38E-05	7.79E-05	1.51E-04
rs2296680	10	96466938	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs1926712	10	96477854	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs1326832	10	96485584	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs932809	10	96488469	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs2281890	10	96493328	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs1042194	10	96495484	*CYP2C18*	1.66E-05	6.01E-05	2.20E-04
rs4532967	10	96519197	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs4986894	10	96522365	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs4244285	10	96531606	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs6583954	10	96534263	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs12571421	10	96541982	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs35390752	10	96543823	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs12767583	10	96547463	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs12772672	10	96566889	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs4641393	10	96567386	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs1322179	10	96575242	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs35709381	10	96614725	*CYP2C19*	1.66E-05	6.01E-05	2.20E-04
rs11598738	10	96140822	Intergenic	2.55E-05	6.26E-05	3.89E-04
rs1048196	10	96361776	*HELLS*	1.45E-05	4.05E-05	4.20E-04
rs28399513	10	96602398	*CYP2C19*	1.38E-05	1.98E-05	5.05E-04
rs4741806	9	3099009	Intergenic	4.04E-04	6.28E-05	2.40E-03
rs1926711	10	96484777	*CYP2C18*	8.24E-06	3.51E-05	6.61E-03
rs12768009	10	96525865	*CYP2C19*	1.23E-05	4.37E-05	7.84E-03
rs12456693	18	42914264	*SLC14A2*	6.83E-05	1.07E-02	8.52E-03
rs7292279	22	19629837	Intergenic	7.39E-04	4.34E-05	9.08E-03
rs2254638	21	30256283	*N6AMT1*	1.87E-03	5.37E-05	6.29E-03

Table S4. Association of SNPs in 295 candidate ADME genes from PharmaADME with PRU or PRU > 208 ($P < 0.01$) and H4 concentration ($P < 0.05$) in 115 patients with CHD.

Gene symbol	SNP	CHR	BP	stand	A1	A2	*P* value		
							PRU	PRU > 208	H4 conc
ABCA1	rs2487032	9	107703934	-	A	G	3.75E-02	3.46E-03	5.34E-03
ABCA1	rs2472519	9	107715878	-	G	A	7.83E-02	3.85E-03	4.16E-02
ABCA4	rs4147823	1	94561272	-	C	A	1.78E-03	2.86E-03	1.01E-02
ABCA4	rs4147820	1	94562084	-	A	G	8.42E-04	1.74E-03	7.00E-03
ABCB5	rs6461515	7	20778646	+	G	A	5.59E-03	1.16E-02	1.40E-02
CHST2	rs1056360	3	142850990	+	A	G	8.60E-03	8.19E-03	3.77E-02
CHST3	rs4148920	10	73731651	+	A	G	5.27E-02	8.84E-03	4.27E-02
CHST3	rs4148923	10	73735864	+	A	G	4.19E-02	7.25E-03	4.82E-02
CHST3	rs16929534	10	73744566	+	G	A	5.27E-02	8.84E-03	4.27E-02
CYP11B1	kgp3262150	8	143946223	-	C	A	1.27E-03	2.39E-02	1.09E-02
CYP11B1	rs11781082	8	143999901	-	A	G	6.06E-03	3.43E-02	1.44E-02
CYP1B1	rs151096	2	38316571	-	C	A	1.03E-03	8.35E-04	3.39E-02
CYP2C18	rs2104543	10	96429971	+	A	G	1.07E-03	7.72E-05	8.63E-04
CYP2C18	rs2860838	10	96453039	+	C	G	1.40E-04	3.85E-05	1.62E-04
CYP2C18	rs2901783	10	96453099	+	G	A	4.73E-04	3.39E-05	1.18E-02
CYP2C18	rs2296680	10	96466938	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs1926712	10	96477854	+	G	A	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs1926711	10	96484777	+	A	G	6.22E-05	1.33E-05	6.72E-03
CYP2C18	rs1326832	10	96485584	+	G	A	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs932809	10	96488469	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs2281890	10	96493328	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs2281889	10	96493358	+	G	A	4.14E-04	3.74E-05	4.02E-02
CYP2C18	rs1042194	10	96495484	+	A	C	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs1326830	10	96495793	+	A	C	1.50E-02	4.42E-03	4.74E-04
CYP2C18	rs1926707	10	96500106	+	A	G	4.14E-04	3.74E-05	4.02E-02
CYP2C18	rs4532967	10	96519197	+	A	C	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs4986894	10	96522365	+	G	A	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs12768009	10	96525865	+	A	G	7.72E-05	1.98E-05	7.95E-03
CYP2C18	rs4244285	10	96531606	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs6583954	10	96534263	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C18	rs7916649	10	96534584	+	A	G	4.14E-04	3.74E-05	4.02E-02
CYP2C18	rs12571421	10	96541982	+	G	A	1.08E-04	2.69E-05	2.37E-04

CYP2C18	rs35390752	10	96543823	+	C	A	1.08E-04	2.69E-05	2.37E-04
CYP2C19	rs12767583	10	96547463	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C19	rs12772672	10	96566889	+	G	A	1.08E-04	2.69E-05	2.37E-04
CYP2C19	rs4641393	10	96567386	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C19	rs1853205	10	96575069	+	C	G	4.04E-04	3.05E-05	6.36E-04
CYP2C19	rs1322179	10	96575242	+	A	G	1.08E-04	2.69E-05	2.37E-04
CYP2C19	rs28399513	10	96602398	+	A	T	3.73E-05	2.26E-05	5.43E-04
CYP2C19	rs35709381	10	96614725	+	A	C	1.08E-04	2.69E-05	2.37E-04
CYP2C9	rs12772675	10	96706409	+	G	C	1.31E-03	2.82E-04	4.59E-02
CYP2C9	rs10509679	10	96708226	+	A	G	3.28E-04	7.48E-04	5.28E-03
CYP2C9	rs4918766	10	96711884	+	A	G	1.31E-03	2.82E-04	4.59E-02
CYP2C9	rs12569850	10	96727160	+	G	A	5.90E-04	1.36E-03	8.35E-03
CYP2C9	rs2298037	10	96746078	+	A	G	3.48E-04	1.00E-03	4.62E-03
MGST1	rs2417568	12	16545189	+	G	A	2.43E-03	6.08E-04	1.48E-02
MGST1	rs10846357	12	16551609	+	C	A	2.14E-03	3.61E-04	1.02E-02
MGST1	kgp12102026	12	16553361	+	A	G	3.57E-03	3.43E-04	9.18E-04
MGST1	rs12369968	12	16562428	+	G	A	3.57E-03	3.43E-04	9.18E-04
NOS1	kgp9005557	12	117795503	-	A	G	8.29E-03	1.67E-03	3.16E-02
NOS1	kgp8427406	12	117802208	-	A	G	1.13E-02	1.67E-03	1.31E-02
SLC13A1	rs10500089	7	122745653	-	G	A	4.99E-03	5.08E-03	2.64E-02
SLC13A1	rs12706485	7	122753122	-	G	A	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs12706486	7	122758190	-	C	A	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs10255262	7	122762539	-	A	G	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs12706487	7	122763787	-	G	A	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs13229376	7	122769628	-	A	G	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs13244086	7	122770085	-	G	A	7.55E-03	1.15E-02	3.60E-02
SLC13A1	rs12706494	7	122773032	-	G	A	6.76E-03	1.87E-02	3.80E-02
SLC13A1	rs10236058	7	122783013	-	A	C	6.63E-03	1.73E-02	3.90E-02
SLC13A1	rs12666250	7	122785918	-	A	G	6.63E-03	1.73E-02	3.90E-02
SLC13A1	rs2470976	7	122788305	-	T	A	6.62E-03	1.50E-02	3.56E-02
SLC13A1	rs10258700	7	122790109	-	G	A	6.63E-03	1.73E-02	3.90E-02
SLC13A1	rs13245959	7	122791856	-	C	A	6.63E-03	1.73E-02	3.90E-02
SLC13A1	rs1343905	7	122807680	-	G	A	3.57E-03	3.02E-02	2.59E-02
SLC13A1	rs2140516	7	122809234	-	G	A	4.81E-03	3.28E-02	4.85E-02
SLC14A2	rs12455102	18	42883959	+	C	A	6.05E-02	1.74E-03	6.97E-03
SLC14A2	rs7235709	18	42899939	+	G	A	4.23E-02	6.41E-03	5.97E-03
SLC14A2	kgp9050051	18	42902001	+	A	G	1.33E-02	5.14E-04	2.49E-02
SLC14A2	rs12456693	18	42914264	+	A	G	1.07E-02	6.83E-05	8.52E-03

SLC14A2	rs17743443	18	42920373	+	G	A	4.55E-02	5.99E-04	3.36E-03
SLC22A2	rs9378798	6	3452974	-	A	G	4.91E-03	6.77E-03	3.96E-02
SLC2A5	kgp15569204	1	9135330	-	A	G	2.70E-02	6.24E-03	2.32E-02
SLC6A6	rs2102916	3	14569733	+	A	G	2.77E-02	7.93E-03	2.27E-02
UGT2B11	kgp11722914	4	70087412	-	A	G	4.16E-03	1.04E-02	4.20E-02
UGT2B11	rs13123057	4	70095992	-	A	C	8.94E-03	8.69E-03	8.69E-04
XDH	kgp4036053	2	31567632	-	C	A	3.07E-02	8.48E-03	3.44E-02

Red highlights indicate associations of SNPs in 295 candidate ADME genes with PRU or PRU > 208 and H4 concentration at 2 h at the significance level of $P < 0.01$.

Table S5. Differences in PRU and H4 concentration among different genotypes of 18 SNPs in 115 patients with CHD.

SNP	HGVS Names	Gene symble	CHR	BP	Genotype	N	PRU > 208	*P*	PRU	*P*	H4 conc (ng/mL)	*P*
rs74460025	c.442-926T>C	*GABRB2*	5	160722361	TT	95	54 (56.84)	2.61E-05	205.33±81.75	5.08E-03	12.29±10.53	5.19E-03
					TC	15	3 (20)		154.73±76.99		17.8±12.8	
					CC	6	0 (0)		138±26.08		23.69±14.56	
rs17145154	c.2519-864T>G	*DNAH11*	7	21777455	TT	63	43 (68.25)	4.72E-05	221.68±74.79	6.36E-04	12.25±11.21	0.024
					TG	46	13 (28.26)		165.83±83.05		14.7±11.34	
					GG	8	2 (25)		167.75±64.14		18.34±11.45	
rs4741806	g.3089009A>C	Intergenic	9	3099009	AA	84	33 (39.29)	4.04E-04	178.08±80.04	6.28E-05	15.54±11.63	2.40E-03
					AC	31	22 (74.19)		238.48±68.92		9.2±9.11	
					CC	2	2 (100)		292±14.14		2.98±2.33	
rs1048196	c.*397C>T	*HELLS*	10	96361776	CC	57	19 (33.33)	1.45E-05	171.72±75.25	4.05E-05	17.24±12.71	4.20E-04
					CT	48	27 (56.25)		204.98±84.47		11.39±8.79	
					TT	12	12 (100)		275.75±29.41		4.85±3.94	
rs1926711	c.1149+487G>A	*CYP2C18*	10	96484777	GG	50	15 (30)	8.24E-06	167.46±77.84	3.51E-05	17.43±12.31	6.61E-03
					GA	50	28 (56)		203.32±82.38		11.47±8.96	
					AA	17	15 (88.24)		258.65±47.61		8.19±11.2	
rs4244285	*2, c.681G>A	*CYP2C19*	10	96531606	GG	54	17 (31.48)	1.66E-05	170.44±76.22	6.01E-05	17.76±12.73	2.20E-04
					GA	52	30 (57.69)		206.15±83.04		10.98±8.78	
					AA	11	11 (100)		273.82±30.03		5.15±3.98	
rs2852213	c.1476+13091C>T	*GRIK4*	11	120789293	CC	34	10 (29.41)	8.77E-05	167±77.22	5.12E-03	17.81±12.72	0.0162
					CT	49	23 (46.94)		198.2±90.4		11.82±9.47	
					TT	34	25 (73.53)		221.94±63.97		12.06±11.57	
rs774392	.58+34448C>A	*GRIP1*	12	67428382	CC	2	2 (100)	2.49E-05	295±26.87	3.18E-04	2.1±0.04	6.72E-03
					CA	19	17 (89.47)		247.11±72.46		11.25±13.59	
					AA	96	39 (40.63)		183.86±79.29		14.33±10.8	
rs12913988	c.740+2730C>T	*ATP10A*	15	25978473	CC	18	14 (77.78)	1.57E-03	244.44±62.84	9.69E-06	9.21±8.86	0.0147
					CT	61	32 (52.46)		206.66±70.47		11.84±9.98	
					TT	38	12 (31.58)		156.05±90.18		18.36±12.82	

rs12456693	c.-125+121108C>T	*SLC14A2*	18	42914264	CC	83	51 (61.45)	6.83E-05	210.04±80.77	0.0107	11.59±9.62	8.52E-03
					CT	29	6 (20.69)		158.28±79.09		18.41±13.27	
					TT	5	1 (20)		182.6±42.46		18.4±16.96	
rs1571678	g.30242208A>G	*N6AMT1*	21	30242208	AA	22	8 (36.36)	1.21E-03	160.05±86.82	2.65E-05	15.97±12.04	6.85E-03
					AG	54	21 (38.89)		179.48±80.52		15.77±11.5	
					GG	41	29 (70.73)		237.15±63.5		9.44±9.63	
rs2254638	c.135-890T>C	*N6AMT1*	21	30256283	TT	22	8 (36.36)	1.87E-03	160.05±86.82	5.37E-05	15.97±12.04	6.29E-03
					TC	53	21 (39.62)		180.64±80.83		15.88±11.58	
					CC	42	29 (69.05)		234.31±65.36		9.46±9.51	
rs17209532	g.19622523G>A	Intergenic	22	19622523	GG	49	31 (63.27)	2.46E-03	225.04±72.75	1.92E-05	10.93±11.59	0.0308
					GA	51	23 (45.1)		190.47±73.88		14.87±10.01	
					AA	17	4 (23.53)		129.12±90.12		17.45±12.97	
rs7292279	g.19629837G>A	Intergenic	22	19629837	GG	46	31 (67.39)	7.39E-04	227±74.31	4.34E-05	10.76±11.35	9.08E-03
					GA	50	21 (42)		190.04±76.85		14.52±10.42	
					AA	21	6 (28.57)		142.48±81.18		17.58±12.2	
rs13123057	g.70061403G>A	*UGT2B11*	4	70095992	GG	58	33 (56.9)	8.69E-03	210.93±86.11	8.94E-03	10.69±10.87	8.69E-04
					GA	47	24 (51.06)		191.15±75.12		15.2±11.31	
					AA	12	1 (8.33)		143.17±64.73		20.98±9.31	
rs12369968	g.16562428A>G	*MGST1*	12	16562428	AA	41	12 (29.27)	3.43E-04	163.22±86.68	3.57E-03	18.73±12.04	9.18E-04
					AG	51	29 (56.86)		214.33±72.98		11.23±9.83	
					GG	25	17 (68)		212.52±76.64		10.12±10.38	
rs2487032	g.107703934G>A	*ABCA1*	9	107703934	GG	31	22 (70.97)	3.46E-03	223.61±78.03	0.0375	10.32±8.18	5.34E-03
					GA	57	27 (47.37)		190.14±78.65		14.13±12.29	
					AA	27	9 (33.33)		179.41±89.56		16.35±12.15	
rs4147820	c.768+2266G>A	*ABCA4*	1	94562084	GG	68	2 (100)	1.74E-03	211.12±77.39	8.42E-04	12.01±10.82	7.00E-03
					GA	41	23 (74.19)		189.39±79.32		14.79±11.63	
					AA	8	33 (39.29)		101.88±70.36		20.7±11.61	

PRU > 208 was summarized using n (%), PRU and H4 concentration using mean ± SD.

Table S6. Pharmacokinetic parameters of clopidogrel, active metabolite isomer H4, and inactive metabolite isomer H3 in 31 patients.

Compound	Clopidogrel	H4	H3
C_{max} (ng/mL)	163.18 ± 159.95	55.07 ± 41.67	142.44 ± 72.76
T_{max} (h)	1.96 ± 1.33	1.34 ± 1.00	1.56 ± 1.20
AUC_{0-4h} (ng·h/mL)	251.20 ± 201.40	93.75 ± 63.13	268.12 ± 149.22

Pharmacokinetic parameters were summarized using mean ± SD.

Table S7. Differences in pharmacokinetic parameters of clopidogrel and H4 among different genotypes of 18 SNPs after administration of 300 mg of clopidogrel in 31 patients with CHD.

SNP	Gene symbol	CHR	Genotype	N	Clopidogrel						H4					
					Tmax (h)	*P*	Cmax (ng/mL)	*P*	AUC (ng·h/mL)	*P*	Tmax (h)	*P*	Cmax (ng/mL)	*P*	AUC_{0-4h} (ng·h/mL)	*P*
rs74460025	*GABRB2*	5	TT	28	2.05±1.34	0.679	160.62±155.56	0.973	236.93±163.81	0.672	1.36±1.04	0.76	54.83±42.96	0.74	93.51±65.51	0.668
			TC	3	2.33±1.53		187.07±236.88		384.43±465.53		1.17±0.76		57.33±33.51		96±42.84	
rs17145154	*DNAH11*	7	TT	20	2.03±1.26	0.672	141.66±140.31	0.557	248.07±202.88	0.799	1.08±0.77	0.0513	50.46±27.48	0.95	93.4±57.17	0.794
			TG	10	2.2±1.6		215.09±197.02		270.03±214.81		1.8±1.3		67.01±62.49		97.97±78.91	
			GG	1	2.00		74.4		125.67		2.000		27.9		58.71	
rs4741806	Intergenic	9	AA	24	2.1±1.32	0.972	152.72±149.08	0.483	243.28±203.08	0.544	1.27±0.94	0.495	57.54±45.68	0.918	99.26±67.55	0.541
			AC	7	2±1.5		199.04±201.94		278.37±208.83		1.57±1.24		46.61±23.97		74.86±43.51	
rs1048196	*HELLS*	10	CC	15	1.9±1.2	0.978	135.19±125.15	0.402	223.38±143.24	0.587	1.27±0.86	0.742	64.23±45.69	0.248	117.33±69.38	0.0273
			CT	11	2.5±1.5		141.33±178.9		240.22±271.45		1.41±1.04		43.97±37.79		76.32±54.54	
			TT	5	1.7±1.4		295.24±176.04		358.83±174.55		1.4±1.47		52.02±38.47		61.39±37.07	
rs1926711	*CYP2C18*	10	GG	11	1.95±1.39	0.667	116.08±71.02	0.153	211.35±110.06	0.291	1.18±0.96	0.773	59.19±31.97	0.517	110.93±68.15	0.0843
			GA	14	2.43±1.28		126.82±158.95		220.35±244.13		1.5±0.92		53.11±52.37		95.13±66.18	
			AA	6	1.5±1.34		334.36±184.33		396.25±181.04		1.25±1.37		52.1±34.41		59.06±33.64	
rs4244285	*CYP2C19*	10	GG	16	1.84±1.18	0.955	128.26±124.04	0.125	211.93±145.77	0.205	1.25±0.84	0.842	68.47±47.28	0.172	122.74±70.44	0.00861
			GA	9	2.89±1.36		111.13±135.61		224.32±268.53		1.56±1.1		33.24±26.03		65.35±37.97	
			AA	6	1.5±1.34		334.36±184.33		396.25±181.04		1.25±1.37		52.1±34.41		59.06±33.64	
rs2852213	*GRIK4*	11	CC	10	2.35±1.49	0.593	136.42±170.17	0.147	191.64±194.43	0.0794	1.75±1.27	0.121	75.05±61.48	0.679	129.07±90.95	0.495
			CT	15	1.9±1.2		152.95±136.1		269.65±218.19		1.2±0.9		40.25±20.57		71.02±35.82	
			TT	6	2.08±1.56		233.36±204.58		304.36±174.71		1±0.55		58.84±30.91		91.72±37.14	
rs774392	*GRIP1*	12	CA	6	2.42±1.74	0.432	193.98±159.98	0.444	329.75±307.9	0.369	1.67±1.33	0.382	40.38±18.77	0.611	63.77±20.25	0.387
			AA	25	2±1.25		155.79±162.34		232.35±170.41		1.26±0.93		58.6±45.06		100.95±67.95	
rs12913988	*ATP10A*	15	CC	5	3±1.41	0.307	102±59.59	0.831	217.5±134.62	0.691	1.7±1.4	0.895	41.34±30.36	0.538	97.07±94.09	0.617
			CT	18	1.86±1.29		196.11±183.33		287.68±234.12		1.11±0.85		60.5±50.17		89.94±62.02	
			TT	8	2±1.31		127.32±138.05		190.19±147.92		1.63±1.06		51.43±23.97		100.25±50.83	

rs12456693	*SLC14A2*	18	CC	22	2.36±1.33	0.00485	132.73±131.75	0.262	203.33±137.83	0.172	1.57±1.11	0.0444	47.44±44.19	0.0245	83.13±57	0.129
			CT	9	1.39±1.14		237.6±203.94		368.22±283.79		0.78±0.26		73.73±28.87		119.71±73.11	
rs1571678	*N6AMT1*	21	AA	11	1.27±0.61	0.0249	217.59±198.96	0.969	293.92±198.69	0.958	0.86±0.45	0.0551	70±53.11	0.286	100.29±69.09	0. 3578
			AG	14	2.54±1.39		99.93±78.72		181.14±109.12		1.54±1.12		50.49±36.72		99.64±68.89	
			GG	6	2.5±1.64		211±194.32		336.35±327.11		1.75±1.26		38.41±19.69		68.03±32.21	
rs2254638	*N6AMT1*	21	TT	11	1.27±0.61	0.0249	217.59±198.96	0.969	293.92±198.69	0.958	0.86±0.45	0.0551	70±53.11	0.286	100.29±69.09	0.358
			TC	14	2.54±1.39		99.93±78.72		181.14±109.12		1.54±1.12		50.49±36.72		99.64±68.89	
			CC	6	2.5±1.64		211±194.32		336.35±327.11		1.75±1.26		38.41±19.69		68.03±32.21	
rs17209532	Intergenic	22	GG	10	2.15±1.38	0.218	145.51±157.56	0.266	211.47±170.79	0.167	1.65±1.29	0.067	66.75±56.72	0.52	117.44±90.3	0.306
			GA	14	2.54±1.39		127.52±123.38		231.09±225.39		1.43±0.92		47.37±34.64		85.96±44.51	
			AA	7	1.07±0.45		259.74±208.16		348.19±185.57		0.71±0.27		53.79±29.78		75.5±44.01	
rs7292279	Intergenic	22	GG	9	1.78±1.37	0.73	169.39±153.91	0.689	289.42±272.12	0.863	1.28±1.12	0.385	52.57±26.67	0.944	91.38±70.42	0.86
			GA	14	2.68±1.44		153.82±165.61		222.89±172.39		1.68±1.1		52.71±54.78		91.95±67.49	
			AA	8	1.38±0.52		172.59±176.94		257.76±174.62		0.81±0.26		62.02±31.31		99.58±53.85	
rs13123057	*UGT2B11*	4	GG	11	1.73±1.25	0.334	181.25±178.81	0.984	266.38±192.67	0.847	1.59±1.3	0.332	61.68±53.95	0.396	96.58±71.86	0.85
			GA	16	2.19±1.33		144.3±164.45		226.5±229.04		1.22±0.86		54.44±34.96		97.12±65.7	
			AA	4	2.63±1.7		189±100.24		308.26±105.59		1.13±0.63		39.43±32.54		72.5±19	
rs12369968	*MGST1*	12	AA	15	1.9±1.4	0.459	180.37±164.68	0.44	269.34±175.37	0.448	1.2±0.9	0.131	63.46±45.76	0.0904	99.88±55.15	0.135
			AG	15	2.13±1.25		144.07±164.11		234±235.54		1.3±0.9		49.05±37.71		91.86±71.87	
			GG	1	4.00		192		237.23		4.000		19.6		30.28	
rs2487032	*ABCA1*	9	GG	9	2.06±1.24	0.243	198.76±182.95	0.0211	321.16±265.11	0.0059	0.94±0.46	0.0189	37.2±20.3	0.382	67.92±35.84	0.329
			GA	15	1.73±1.27		190.25±164.42		277.61±170.77		1.2±0.9		66.54±48.11		106.77±70.91	
			AA	7	2.86±1.46		59.43±62.59		104.67±83.21		2.14±1.35		53.49±43.82		99.07±70.86	
rs4147820	*ABCA4*	1	GG	17	2.21±1.43	0.498	167.85 ± 174.6	0.59	238.01±172.59	0.838	1.24±0.87	0.572	60.56 ± 50.96	0.572	100.48±76.01	0.551
			GA	12	2.08±1.29		139.4 ± 131.26		257.01±243.46		1.54±1.25		51.69 ± 27.75		91.83±44.83	
			AA	2	1±0		266.15 ± 248.69		328.43±270.13		1±0		28.7 ± 5.66		48.11±4.74	

Pharmacokinetic parameters were summarized using mean ± SD.

Red highlights indicate differences in pharmacokinetic parameters of clopidogrel and its metabolites among different genotypes are significant.

Table S8. Differences in H4 formation among different genotypes of 18 SNPs in 32 human liver S9 fractions.

SNP	HGVS Names	Gene symble	CHR	BP	Genotype	N	Mean ± SD (ng/ml/mg protein)	*P* value
rs74460025	c.442-926T>C	*GABRB2*	5	160722361	TT	24	6.57 ± 6.16	0.9402
					TC	8	6.39 ± 4.8	
rs17145154	c.2519-864T>G	*DNAH11*	7	21777455	TT	20	8.01 ± 5.07	0.4269
					TG	11	2.44 ± 2.96	
					GG	1	21.7	
rs4741806	g.3089009A>C	Intergenic	9	3099009	AA	20	6.66 ± 5.73	0.9502
					AC	10	6.12 ± 6.44	
					CC	2	7.21 ± 5.9	
rs1048196	c.*397C>T	*HELLS*	10	96361776	CC	11	9.46 ± 6.6	0.0135
					CT	17	5.73 ± 4.99	
					TT	4	1.84 ± 1.57	
rs1926711	c.1149+487G>A	*CYP2C18*	10	96484777	GG	12	9.35 ± 6.54	0.0153
					GA	14	5.7 ± 5.01	
					AA	6	2.81 ± 3.15	
rs4244285	*2, c.681G>A	*CYP2C19*	10	96531606	GG	14	8.53 ± 6.46	0.0334
					GA	14	5.86 ± 5.08	
					AA	4	1.84 ± 1.57	
rs2852213	c.1476+13091C>T	*GRIK4*	11	120789293	CC	15	6.13 ± 5.83	0.9842
					CT	14	7.34 ± 6.26	
					TT	3	4.7 ± 3.67	
rs774392	.58+34448C>A	*GRIP1*	12	67428382	CA	7	6.62 ± 8.27	0.961
					AA	25	6.5 ± 5.1	
rs12913988	c.740+2730C>T	*ATP10A*	15	25978473	CC	8	6.63 ± 3.43	0.9948
					CT	14	6.41 ± 6.39	
					TT	10	6.59 ± 6.84	
rs12456693	c.-125+121108C>T	*SLC14A2*	18	42914264	CC	20	7.18 ± 5.28	0.4131
					CT	12	5.43 ± 6.62	
rs1571678	g.30242208A>G	*N6AMT1*	21	30242208	AA	4	9.21 ± 3.68	0.0643
					AG	17	7.56 ± 6.66	
					GG	11	3.95 ± 4.06	
rs2254638	c.135-890T>C	*N6AMT1*	21	30256283	TT	4	9.21 ± 3.68	0.0386
					TC	18	7.69 ± 6.48	
					CC	10	3.36 ± 3.75	
rs17209532	g.19622523G>A	Intergenic	22	19622523	GG	17	6.77 ± 5.28	0.7581
					GA	14	6.33 ± 6.7	
					AA	1	5	
rs7292279	g.19629837G>A	Intergenic	22	19629837	GG	14	4.96 ± 3.9	0.1483
					GA	16	7.49 ± 6.93	
					AA	2	9.8 ± 6.79	

rs13123057	g.70061403G>A	*UGT2B11*	4	70095992	GG	10	6.77 ± 7.06	0.7324
					GA	17	6.68 ± 5.59	
					AA	5	5.5 ± 4.43	
rs12369968	g.16562428A>G	*MGST1*	12	16562428	AA	10	6.75 ± 6.67	0.9919
					AG	15	6.24 ± 6.14	
					GG	7	6.81 ± 4.14	
rs2487032	g.107703934G>A	*ABCA1*	9	107703934	GG	4	6.48 ± 5.79	0.8812
					GA	22	6.41 ± 5.48	
					AA	6	6.96 ± 7.76	
rs4147820	c.768+2266G>A	*ABCA4*	1	94562084	GG	16	6.15 ± 6.05	0.662
					GA	15	6.78 ± 5.84	
					AA	1	8.68	

Red highlights indicate differences in H4 formation among different genotypes are significant.

Table S9. Call rate of the SNPs examined in 299 patients with CHD.

Polymorphisms	Alleles	Call Rate(%)
rs74460025	T/C	98.98%
rs17145154	T/G	96.33%
rs4741806	A/C	98.78%
rs1048196	C/T	97.56%
rs1926711	G/A	97.76%
rs4244285	G/A	98.37%
rs2852213	C/T	97.96%
rs774392	C/A	99.19%
rs12913988	C/T	98.98%
rs12456693	C/T	99.59%
rs2254638	T/C	97.15%
rs17209532	G/A	97.56%
rs7292279	G/A	99.80%
rs13123057	G/A	99.39%
rs12369968	A/G	99.80%
rs2487032	G/A	98.78%
rs4147820	G/A	99.80%