Polymorphic organic anion transporting polypeptide 1B1 is a major determinant of repaglinide pharmacokinetics

Background and Objective: **A large interindividual variability exists in the plasma concentrations of repaglinide. Our aim was to investigate possible associations between the pharmacokinetics of repaglinide and single nucleotide polymorphisms (SNPs) in the genes encoding for the drug transporters organic anion transporting polypeptide 1B1 (OATP1B1) (*SLCO1B1*) and P-glycoprotein (*MDR1*, *ABCB1*) and the drug-metabolizing enzymes cytochrome P450 (CYP) 2C8 and CYP3A5.**

Methods: **A total of 56 healthy volunteers ingested a single 0.25-mg dose of repaglinide. Plasma repaglinide and blood glucose concentrations were measured for up to 7 hours. All subjects were genotyped for the −11187G>A and 521T>C SNPs in *SLCO1B1* and the 3435C>T and 2677G>T/A SNPs in *ABCB1*, as well as for the *CYP2C8*3* (416G>A, 1196A>G), *CYP2C8*4* (792C>G), and *CYP3A5*3* (6986A>G) alleles.**

Results: **The area under the plasma concentration–time curve from time 0 to infinity [AUC(0-∞)] and peak concentration in plasma (C_{max}) of repaglinide varied 16.9-fold and 10.7-fold, respectively, between individual subjects. Multiple regression analyses indicated that the *SLCO1B1* 521T>C SNP and the *CYP2C8*3* allele were independent predictors of the AUC(0-∞) and C_{max} of repaglinide (adjusted multiple R^2 = 45% and 36%, respectively). In subjects with the *SLCO1B1* 521CC genotype, the AUC(0-∞) of repaglinide was 107% and 188% higher, respectively, than in subjects with the *SLCO1B1* 521TC or 521TT (reference) genotype ($P < .0001$). In subjects with the *CYP2C8*1/*3* genotype, the AUC(0-∞) and C_{max} of repaglinide were 48% and 44% lower, respectively, than in those with the *CYP2C8*1/*1* genotype ($P < .05$). The pharmacokinetics of repaglinide was not associated with the studied *ABCB1* SNPs or the *CYP3A5*3* allele. The elimination half-life of repaglinide was not associated with any SNP. Only the *SLCO1B1* −11187GA genotype was significantly associated with an enhanced effect of repaglinide on blood glucose.**

Conclusions: **Genetic polymorphism in *SLCO1B1* is a major determinant of interindividual variability in the pharmacokinetics of repaglinide. The effect of *SLCO1B1* polymorphism on the pharmacokinetics of repaglinide may be clinically important. (Clin Pharmacol Ther 2005;77:468-78.)**

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A considerable interindividual variability is evident in the pharmacokinetics of repaglinide, a meglitinide analog antidiabetic drug.[1] The oral bioavailability of repaglinide is about 60%,[2] and it is eliminated completely by metabolism,[3] occurring presumably in the liver. In vitro studies have identified cytochrome P450 (CYP) 2C8 and CYP3A4 as the principal catalysts of the biotransformation of repaglinide.[4] In our previous investigation the *CYP2C8*3* allele was associated with

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reduced plasma concentrations of repaglinide, but this only partly explained the interindividual variability in repaglinide concentrations.[5]

Pharmacokinetic interaction studies in humans have shown that the CYP3A4 inhibitors itraconazole and clarithromycin modestly increase the area under the plasma concentration–time curve (AUC) of repaglinide, by about 40%.[6,7] In addition to inhibiting CYP3A4,[8,9] itraconazole and clarithromycin have been shown to inhibit the drug transporter P-glycoprotein.[10-12] The CYP2C8 inhibitor trimethoprim has been shown to increase the AUC of repaglinide by 61%.[13] Gemfibrozil showed a drastic effect on repaglinide pharmacokinetics, increasing the AUC of repaglinide by about 8-fold.[7] Gemfibrozil and especially its glucuronide conjugate inhibit CYP2C8[14,15] and also the uptake transporter organic anion transporting polypeptide 1B1 (OATP1B1, previously known as OATP-C, OATP2, and LST-1).[15] OATP1B1 expression is exclusive to the basolateral membrane of hepatocytes, and OATP1B1 is thought to mediate uptake of its substrates from blood into hepatocytes.[16] Therefore, in addition to CYP2C8 and CYP3A4, P-glycoprotein and OATP1B1 might play a role in the pharmacokinetics of repaglinide.

Functionally significant polymorphisms in *CYP3A4* appear to be relatively rare in white subjects.[17] Another member of the CYP3A subfamily, CYP3A5, is very similar to CYP3A4 with respect to its amino acid sequence and catalyzes the biotransformation of many CYP3A4 substrates.[18] Expression of CYP3A5 is polymorphic, which mainly results from a common single nucleotide polymorphism (SNP) (6986A>G) causing alternative splicing and protein truncation.[19] The 6986A>G SNP distinguishes the variant (CYP3A5 nonexpressor) allele *CYP3A5*3* from the reference (CYP3A5 expressor) *CYP3A5*1* allele.[19,20]

Several SNPs have been found in the *ABCB1* (*MDR1*) gene encoding for the P-glycoprotein.[21,22] The common synonymous *ABCB1* SNP, 3435C>T, has been associated in several, but not all, studies with reduced tissue expression of P-glycoprotein and increased plasma concentrations of P-glycoprotein substrates.[21,22] Another common *ABCB1* variant, 2677G>T (Ala893Ser), has also been variably associated with altered transport activity of P-glycoprotein.[21,22] Certain common SNPs in the *SLCO1B1* gene encoding for OATP1B1 are associated with a reduced transport activity of OATP1B1 in vitro (eg, 521T>C, Val174Ala)[23] and increased plasma concentrations of the OATP1B1 substrate pravastatin in healthy volunteers.[24-26]

Because genetic polymorphism in *CYP3A5*, *CYP2C8*, *ABCB1*, or *SLCO1B1* might contribute to the large interindividual variability in the pharmacokinetics of repaglinide, we have investigated possible associations between *CYP3A5*, *CYP2C8*, *ABCB1*, and *SLCO1B1* polymorphisms and the pharmacokinetics and effects of repaglinide in healthy volunteers.

METHODS

Subjects. A total of 56 healthy volunteers who had participated in our previous studies[7,13,27] or ongoing pharmacokinetic studies with repaglinide were included in this study. Eleven subjects were women and 45 were men. Their mean (±SD) age was 23 ± 2 years (range, 19-28 years); weight, 73 ± 12 kg (range, 46-100 kg); and height, 177 ± 9 cm (range, 155-197 cm). Three subjects were tobacco smokers, and none were taking any continuous medication. All subjects gave written informed consent.

Study design. The study protocols were approved by the Ethics Committee for Studies in Healthy Subjects of the Helsinki and Uusimaa Hospital District. After an overnight fast, the subjects ingested a single 0.25-mg dose of repaglinide (half of a 0.5-mg tablet of Novonorm; NovoNordisk, Bagsværd, Denmark) with 150 mL of water at 9 AM. The subjects received a standardized breakfast 15 minutes after the administration of repaglinide, a standardized snack after 1 and 2 hours, and a standardized warm meal after 3 hours, as described previously.[7,13,27] Plasma repaglinide concentrations were quantified in timed plasma samples for up to 7 hours by liquid chromatography–tandem mass spectrometry.[7,13,27] The intraday coefficient of variation was below 10% and the between-day coefficient of variation was below 15% at relevant concentrations. The limit of quantification was 0.1 ng/mL. Blood glucose concentrations were determined after each blood sampling by the glucose oxidase method with the Precision G Blood Glucose Testing System (Medisense, Bedford, Mass). For genetic analysis, a 10-mL ethylenediaminetetraacetic acid blood sample was drawn from each subject and stored at −20°C until deoxyribonucleic acid (DNA) extraction. DNA was extracted with standard methods (QIAamp DNA Blood Mini Kit; Qiagen, Hilden, Germany).

Pharmacokinetics. The pharmacokinetics of repaglinide was characterized by the peak concentration in plasma (C_{max}), time to C_{max} (t_{max}), elimination half-life ($t_{1/2}$), and AUC from time 0 to infinity [AUC(0-∞)]. The elimination rate constant (k_e) was determined by linear regression analysis of the log-linear part of the concentration-time curve. The $t_{1/2}$ was calculated by the equation $t_{1/2} = \ln 2/k_e$. AUC(0-∞) was calculated by a combination of the linear and log-linear trapezoidal

rules, with extrapolation to infinity by division of the last measured concentration by k_e.

Pharmacodynamics. The blood glucose response to repaglinide was characterized by the maximum increase and maximum decrease in blood glucose concentration, as well as by the mean change in blood glucose concentration from 0 to 7 hours. The mean change was calculated by dividing the net incremental AUC of blood glucose, calculated with the linear trapezoidal rule, by the corresponding time interval.

SLCO1B1 genotyping. All subjects were genotyped for the −11187G>A SNP in the promoter region and the 521T>C (Val174Ala) SNP in exon 5 of the *SLCO1B1* gene by allelic discrimination with *Taq*Man 5′-nuclease assays, by use of the ABI Prism 7700 Sequence Detection System (ABI, Weiterstadt, Germany). The primers used in 521T>C genotyping were 5′-GAAACACTCTCTTATCTACATAGGTTGTTTA-3′ (forward) and 5′-CCCCTATTCCACGAAGCAT-3′ (reverse). The *Taq*Man MGB probes were VIC-TACCCATGAACACATATA and FAM-TACCCATGAACGCATATA. The primers used for −11187G>A were 5′-CATATATGCATCCTCACATTACCACAT-3′ (forward) and 5′-AATAAAGTACAGACCCTTCTCTCACATAAA-3′ (reverse), and the *Taq*Man MGB probes were VIC-TGTATACAGGTAAAAGTG and FAM-TGTGTATACAAGTAAAAG. The polymerase chain reaction conditions for both assays were 1 cycle at 50°C for 2 minutes and at 95°C for 10 minutes, followed by 40 cycles at 92°C for 15 seconds and at 60°C for 1 minute, as recommended by the manufacturer. The validity of the method was confirmed by sequencing.

ABCB1 genotyping. All subjects were genotyped for the 3435C>T SNP in exon 26 and the 2677G>T/A SNP in exon 21 of the *ABCB1* gene by denaturing HPLC as described previously.[28] *ABCB1* haplotype analysis was done as described by Johne et al.[29]

CYP2C8 genotyping. Twenty-one of the subjects had been genotyped for the *CYP2C8*3* and *CYP2C8*4* alleles in a previous study.[5] The remaining 35 subjects were genotyped for the *CYP2C8*3* and *CYP2C8*4* alleles as described previously.[5]

CYP3A5 genotyping. All subjects were genotyped for the *CYP3A5*3* (6986A>G) allele by allelic discrimination with a *Taq*Man 5′-nuclease assay by use of the ABI Prism 7700 Sequence Detection System. The primers used were 5′-ATGGAGAGTGGCATAGGAGATACC-3′ (forward) and 5′-GGTAATGTGGTCCAAACAGGG-3′ (reverse). The *Taq*Man MGB probes were VIC-TGTCTTTCAGTATCTCTT and FAM-TGTCTTTCAATATCTCTT. The polymerase chain reaction conditions were 1 cycle at 50°C for 2 minutes and at 95°C for 10 minutes, followed by 40 cycles at 92°C for 15 seconds and at 60°C for 1 minute, as recommended by the manufacturer. The validity of the method was confirmed by sequencing.

Statistical analysis. Results are expressed as mean ± SD in the text and tables and, for clarity, as mean ± SEM in the figures. The contribution of different *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* SNPs and alleles to variability in the pharmacokinetic and pharmacodynamic variables of repaglinide was investigated by use of forward, stepwise multiple linear regression analysis. The different SNPs and alleles were put into the model as independent variables with each SNP and allele described by 2 binary variables (1:1 equals heterozygous carrier and 0 equals other; 2:1 equals homozygous carrier and 0 equals other). Statistical comparisons of all pharmacokinetic and pharmacodynamic variables between noncarriers and heterozygous and homozygous carriers of an SNP or allele were done by use of ANOVA, followed by a posteriori testing with the Tukey test. Data for t_{max} were analyzed by the Mann-Whitney *U* test or the Kruskal-Wallis test with a posteriori testing with the Dunn test. Relationships between the pharmacokinetic and pharmacodynamic variables of repaglinide were investigated with the Pearson correlation coefficient. The data were analyzed with the statistical programs Systat for Windows, version 6.0.1, and SPSS 11.0 for Windows (SPSS Inc, Chicago, Ill). Differences were considered statistically significant at $P < .05$.

RESULTS

Genotypes. The *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* genotypes and allele frequencies of the subjects are shown in Table I. All observed genotype frequencies were in Hardy-Weinberg equilibrium.

Regression analyses. The AUC(0-∞) and C_{max} of repaglinide varied 16.9-fold and 10.7-fold, respectively, and the $t_{1/2}$ varied 4.2-fold between individual subjects. In a stepwise, forward multiple regression analysis, heterozygosity ($P = .0432$) and homozygosity ($P < .0001$) for the *SLCO1B1* 521T>C SNP and heterozygosity for the *CYP2C8*3* allele ($P = .0269$) independently predicted the AUC(0-∞) of repaglinide (adjusted multiple $R^2 = 45\%$). The C_{max} of repaglinide was predicted by homozygosity for the *SLCO1B1* 521T>C SNP ($P < .0001$) and heterozygosity for the *CYP2C8*3* allele ($P = .0253$) (adjusted multiple $R^2 = 36\%$), but the $t_{1/2}$ of repaglinide was not predicted by any of these SNPs.

Transporter genotypes and repaglinide pharmacokinetics. In subjects with the *SLCO1B1* 521CC genotype, the AUC(0-∞) of repaglinide was 107% and

Table I. Genotype and allele frequencies of *SLCO1B1*, *ABCB1*, *CYP2C8*, and *CYP3A5* variants in 56 Finnish healthy volunteers

Gene and variant	*Genotype*	*Frequency (N = 56)*	*Allele*	*Frequency (N = 112)*
SLCO1B1 −11187G>A	GG	48 (85.7%)	G	104 (92.9%)
	GA	8 (14.3%)	A	8 (7.1%)
SLCO1B1 521T>C	TT	36 (64.3%)	T	88 (78.6%)
	TC	16 (28.6%)	C	24 (21.4%)
	CC	4 (7.1%)		
ABCB1 2677G>T/A	GG	14 (25.0%)	G	57 (50.9%)
	GT	27 (48.2%)	T	53 (47.3%)
	TT	13 (23.2%)	A	2 (1.8%)
	GA	2 (3.6%)		
ABCB1 3435C>T	CC	12 (21.4%)	C	43 (38.4%)
	CT	19 (33.9%)	T	69 (61.6%)
	TT	25 (44.6%)		
CYP2C8	**1/*1*	41 (73.2%)	**1*	97 (86.6%)
**3* (416G>A, 1196A>G)	**1/*3*	10 (17.9%)	**3*	10 (8.9%)
**4* (792C>G)	**1/*4*	5 (8.9%)	**4*	5 (4.5%)
CYP3A5	**1/*1*	8 (14.3%)	**1*	8 (7.1%)
**3* (6986G>A)	**1/*3*	48 (85.7%)	**3*	104 (92.9%)

188% higher, respectively, than in subjects with the *SLCO1B1* 521TC and 521TT (reference) genotypes ($P < .0001$) (Fig 1 and Table II). The mean C_{max} of repaglinide was 102% and 152% higher, respectively, in subjects with the *SLCO1B1* 521CC genotype than in subjects with the *SLCO1B1* 521TC and 521TT genotypes ($P = .0003$ and $P < .0001$). In subjects with the 521TC genotype, the AUC(0-∞) of repaglinide was 39% higher than in those with the 521TT genotype ($P = .0707$). There was also a tendency toward a higher AUC(0-∞) of repaglinide in subjects heterozygous for the *SLCO1B1* −11187G>A SNP, possibly because of partial linkage between the −11187G>A and 521T>C SNPs.[26] The pharmacokinetics of repaglinide was not associated with *ABCB1* SNPs (Table III) or haplotypes (data not shown).

CYP genotypes and repaglinide pharmacokinetics. In subjects with the *CYP2C8*1/*3* genotype, the AUC(0-∞) and C_{max} of repaglinide were 48% and 44% lower, respectively, than in those with the *CYP2C8*1/*1* genotype ($P = .0319$ and $P = .0394$) (Fig 1 and Table IV). Because the *SLCO1B1* 521T>C SNP had a major effect on the pharmacokinetics of repaglinide, the effect of the *CYP2C8*3* allele on repaglinide pharmacokinetics was also investigated in relation to the 521T>C genotypes. In subjects with the 521TT (reference) genotype, the AUC(0-∞) of repaglinide was 27% lower in heterozygous carriers of *CYP2C8*3* (n = 8) than in noncarriers of *CYP2C8*3* (n = 28) ($P = .049$). In subjects with the 521TC genotype, the AUC(0-∞) and C_{max} of repaglinide were 66% and 69% lower, respectively, in heterozygous carriers of *CYP2C8*3* (n = 2) than in noncarriers (n = 14) ($P < .001$ and $P = .035$). Similarly, among noncarriers of *CYP2C8*3*, the AUC(0-∞) of repaglinide was 90% or 171% higher in subjects with the *SLCO1B1* 521CC genotype (n = 4) than in subjects with the 521TC (n = 14) or 521TT (n = 28) (reference) genotypes, respectively ($P = .0005$ and $P < .0001$). In noncarriers of *CYP2C8*3*, the AUC(0-∞) of repaglinide was 43% higher in subjects with the 521TC genotype than in those with the 521TT genotype ($P = .0576$). The C_{max} of repaglinide was 139% higher in *CYP2C8*3* noncarriers with the 521CC genotype than in those with the 521TT genotype ($P < .0001$). The pharmacokinetics of repaglinide was not associated with the *CYP3A5*1* allele (Fig 1 and Table IV).

Pharmacodynamics of repaglinide. In a stepwise, forward multiple regression analysis, only the *SLCO1B1* −11187G>A SNP independently predicted the maximum decrease in blood glucose concentration ($R^2 = 11\%$, $P = .0126$) and the mean change in blood glucose concentration ($R^2 = 6.6\%$, $P = .0561$). None of the other SNPs was significantly associated with the blood glucose–lowering effect of repaglinide (Fig 2). Blood glucose variables are presented in relation to the *SLCO1B1* SNPs and *CYP2C8* alleles in Table V. The blood glucose response to repaglinide was greatest in 1 subject with the *SLCO1B1* −11187GA/521CC genotype, who also had the largest AUC(0-∞) of repaglinide. The C_{max} and AUC(0-∞) of repaglinide correlated

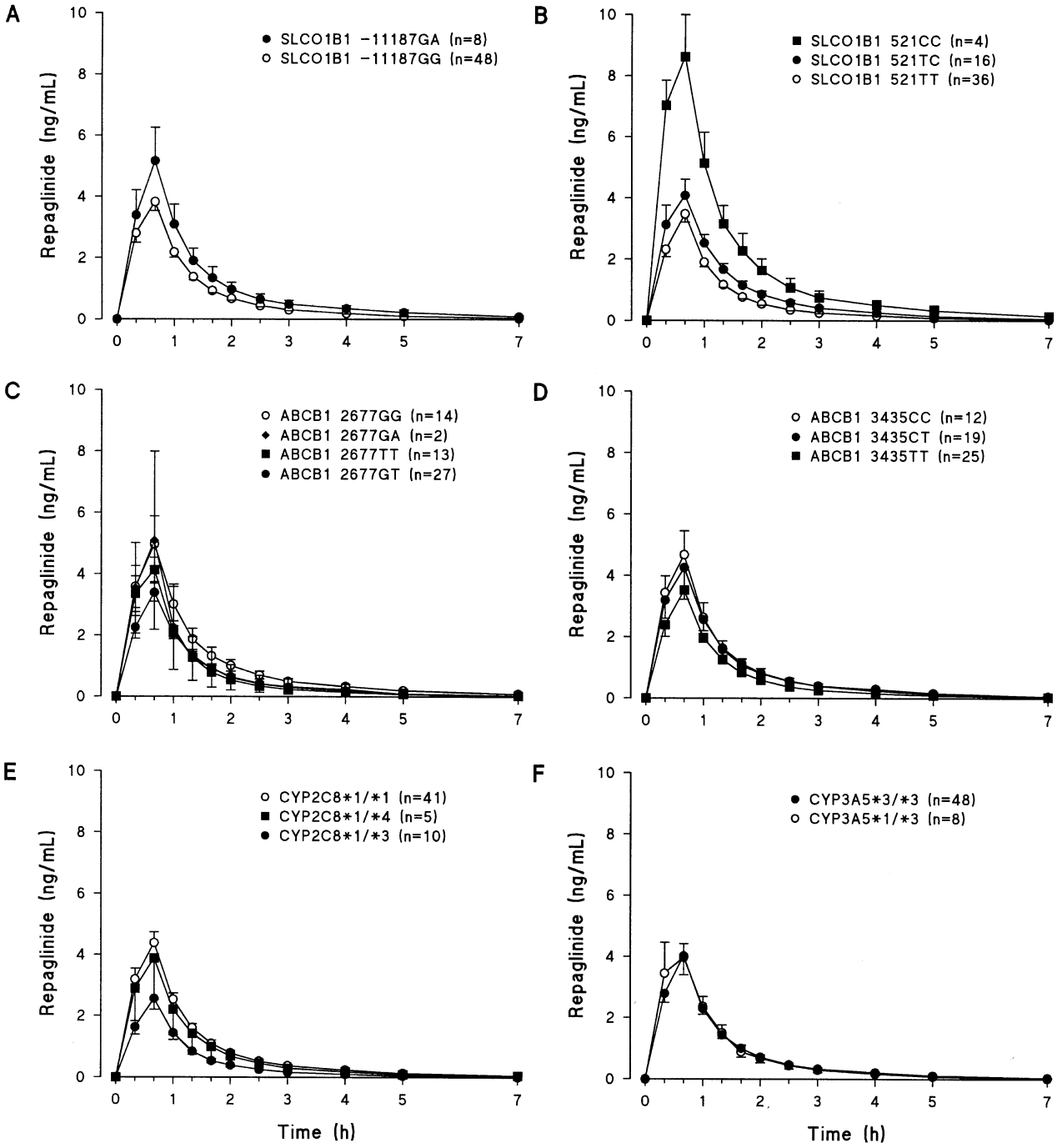

Fig 1. Mean (±SEM) plasma concentration of repaglinide in 56 healthy volunteers in relation to *SLCO1B1* −11187G>A (**A**), *SLCO1B1* 521T>C (**B**), *ABCB1* 2677G>T/A (**C**), and *ABCB1* 3435C>T (**D**) single-nucleotide polymorphisms (SNPs) and *CYP2C8* (**E**) and *CYP3A5* (**F**) genotypes. A single oral dose of 0.25 mg repaglinide was given after an overnight fast.

with the mean change in blood glucose from 0 to 7 hours ($r = -0.28$ [$P = .0356$] and $r = -0.37$ [$P = .0053$], respectively).

DISCUSSION

This study shows that the *SLCO1B1* 521T>C SNP and the *CYP2C8*3* allele are significant and independent pre-

Table II. Pharmacokinetic variables of single 0.25-mg oral dose of repaglinide in relation to *SLCO1B1* −11187G>A and 521T>C (Val174Ala) single nucleotide polymorphisms

SLCO1B1 variant	C_{max} *(ng/mL)*	t_{max} *(min)*	$t_{1/2}$ *(h)*	*AUC(0-∞) (ng · h/mL)*
−11187GG genotype (n = 48)	4.0 ± 2.2	40 (20-60)	1.3 ± 0.4	4.8 ± 2.7
−11187GA genotype (n = 8)	5.2 ± 3.1	40 (40-40)	1.5 ± 0.4	7.0 ± 4.8
P value	.1855	.2596	.1250	.0665
521TT genotype (n = 36)	3.5 ± 1.6	40 (20-40)	1.2 ± 0.3	4.1 ± 2.0
521TC genotype (n = 16)	4.4 ± 2.4	40 (20-60)	1.4 ± 0.5	5.8 ± 2.5
P value	.2957	NS	.2465	.0707
521CC genotype (n = 4)	8.9 ± 2.6	40 (20-40)	1.4 ± 0.6	11.9 ± 5.0
P value, versus TT genotype	< .0001	NS	.5931	< .0001
ANOVA *P* value	< .0001	.8587	.2195	< .0001

Data are given as mean ± SD, except for t_{max}, which is given as median and range.
C_{max}, Peak concentration in plasma; t_{max}, time to peak concentration in plasma; $t_{1/2}$, elimination half-life; AUC(0-∞), area under plasma concentration–time curve from time 0 to infinity; NS, not significant.

Table III. Pharmacokinetic variables of single 0.25-mg oral dose of repaglinide in relation to *ABCB1* 2677G>T/A and 3435C>T single nucleotide polymorphisms

ABCB1 variant	C_{max} *(ng/mL)*	t_{max} *(min)*	$t_{1/2}$ *(h)*	*AUC(0-∞) (ng · h/mL)*
2677GG genotype (n = 14)	5.0 ± 3.4	40 (20-40)	1.4 ± 0.5	6.9 ± 4.1
2677GT genotype (n = 27)	3.6 ± 1.8	40 (20-60)	1.2 ± 0.3	4.4 ± 2.0
P value	.2375	NS	.3064	.0651
2677TT genotype (n = 13)	4.2 ± 1.6	40 (20-40)	1.3 ± 0.4	4.7 ± 1.8
P value, versus GG genotype	.8140	NS	.7250	.2483
2677GA genotype (n = 2)	5.1 ± 4.1	40 (40-40)	1.3 ± 0.0	5.4 ± 4.5
P value, versus GG genotype	1.0000	NS	.9476	.9122
ANOVA *P* value	.2703	.3572	.3876	.0924
3435CC genotype (n = 12)	4.8 ± 2.6	40 (20-40)	1.3 ± 0.3	6.0 ± 3.5
3435CT genotype (n = 19)	4.5 ± 2.9	40 (20-60)	1.2 ± 0.4	5.7 ± 4.0
P value	.9568	NS	.7609	.9714
3435TT genotype (n = 25)	3.6 ± 1.6	40 (20-40)	1.3 ± 0.5	4.3 ± 1.8
P value, versus CC genotype	.3333	NS	.9999	.2582
ANOVA *P* value	.2636	.4965	.6487	.1744

Data are given as mean ± SD, except for t_{max}, which is given as median and range.

dictors of the pharmacokinetics of repaglinide. The AUC of repaglinide was nearly 3-fold higher in subjects homozygous for the *SLCO1B1* 521T>C SNP than in subjects with the 521TT (reference) genotype. The *CYP2C8*3* allele had an opposite effect on plasma repaglinide concentrations: subjects with the *CYP2C8*1/*3* genotype had a 30% to 50% lower AUC of repaglinide than subjects with the reference genotype. Analysis of data stratified by the *SLCO1B1* 521T>C SNP or the *CYP2C8*3* allele and stepwise multiple linear regression analyses indicated that the 521T>C SNP and the *CYP2C8*3* allele had independent effects on the pharmacokinetics of repaglinide and confirmed that the differences noted in the analysis of single SNPs were not a result of unequal distribution of the other significant SNP. The *SLCO1B1* 521T>C SNP and the *CYP2C8*3* allele were not significantly associated with changes in the blood glucose–lowering effect of repaglinide, whereas the *SLCO1B1* −11187G>A SNP was associated with an increased glucose-lowering effect. Repeated food intake and the use of a subtherapeutic dose of repaglinide probably masked differences in blood glucose concentrations between subjects with different genotypes. Nevertheless, the blood glucose–lowering effect of repaglinide correlated with the AUC of repaglinide. This is the first study to show that *SLCO1B1* polymorphism may significantly affect the disposition of a drug that is completely metabolized by CYP enzymes.

Table IV. Pharmacokinetic variables of single 0.25-mg oral dose of repaglinide in relation to *CYP2C8* and *CYP3A5* genotypes

Genotype	C_{max} *(ng/mL)*	t_{max} *(min)*	$t_{1/2}$ *(h)*	*AUC(0-∞) (ng · h/mL)*
*CYP2C8*1/*1* (n = 41)	4.6 ± 2.3	40 (20-60)	1.3 ± 0.4	5.7 ± 3.1
*CYP2C8*1/*3* (n = 10)	2.6 ± 1.1	40 (40-40)	1.3 ± 0.5	3.0 ± 1.2
P value	.0394	NS	.9914	.0319
*CYP2C8*1/*4* (n = 5)	4.0 ± 3.0	40 (20-40)	1.4 ± 0.2	5.0 ± 4.1
P value, versus **1/*1* genotype	.8591	NS	.6799	.8660
ANOVA *P* value	.0502	.4324	.6827	.0413
*CYP3A5*1/*3* (n = 8)	4.6 ± 2.2	40 (20-60)	1.2 ± 0.4	5.2 ± 2.1
*CYP3A5*3/*3* (n = 48)	4.1 ± 2.4	40 (20-40)	1.2 ± 0.4	5.1 ± 3.3
P value	.5815	.9859	.7574	.9922

Data are given as mean ± SD, except for t_{max}, which is given as median and range.

Repaglinide has an oral bioavailability of about 60%.[2] It is eliminated completely by metabolism,[3] and CYP2C8 and CYP3A4 are the principal enzymes catalyzing its biotransformation.[4] That the plasma concentrations of repaglinide were markedly increased in subjects with the *SLCO1B1* 521CC genotype suggests that OATP1B1-mediated (OATP1B1 is encoded by *SLCO1B1*) hepatic uptake of repaglinide is important for its elimination by CYP-catalyzed biotransformation. The AUC and C_{max} of repaglinide were greatly increased, whereas the $t_{1/2}$ remained unchanged by the *SLCO1B1* polymorphism, which suggests that OATP1B1 affects the pharmacokinetics of repaglinide mainly during the absorption phase. This might be explained by the much higher concentrations of repaglinide in the portal vein during the absorption phase than in the hepatic circulation during the elimination phase. In addition to OATP1B1, some other transporters may participate in the hepatic uptake of repaglinide, with OATP1B1 playing a major role at higher concentrations and another transporter at lower concentrations. In vitro studies are required to characterize the kinetics of OATP1B1-mediated uptake of repaglinide.

The *SLCO1B1* polymorphism has been associated with the pharmacokinetics of pravastatin.[24-26] In addition, cyclosporine (INN, ciclosporin), an inhibitor of OATP1B1,[30] greatly increases the AUC and C_{max} of pravastatin (nearly 10-fold) without any effect on its $t_{1/2}$.[31] Pravastatin and repaglinide differ considerably in their pharmacokinetic characteristics. Most important, the hydrophilic pravastatin is not significantly metabolized by CYP enzymes and a considerable fraction of absorbed pravastatin is excreted unchanged, whereas repaglinide is completely biotransformed via hepatic CYP enzymes.[1,32] It is possible that *SLCO1B1* polymorphism also affects the pharmacokinetics of other drugs that are metabolized in the liver. Drug substrates for OATP1B1 include, for example, rosuvastatin and cerivastatin,[30,33] but its contribution to the hepatic uptake of other drugs is largely unknown.

This study substantiates our earlier finding that the *CYP2C8*3* allele is associated with reduced plasma concentrations of repaglinide.[5] Comprehensive analyses showed that the observed effect was not caused by uneven distribution of *SLCO1B1* SNPs. On the basis of previous in vitro studies with other CYP2C8 substrates,[34-36] one would have expected higher plasma concentrations of repaglinide in carriers of *CYP2C8*3* compared with subjects with the reference genotype. This apparent discrepancy might be explained, for example, by substrate specificity of the *CYP2C8*3* variant, by increased expression of CYP enzymes as a result of *CYP2C8*3*, by linkage of the *CYP2C8*3* variant to a hypothetic causative variant, or through effects of *CYP2C8*3* on physiologic mediators, as discussed previously.[5] The plasma concentrations of the CYP2C8 and CYP2C9 substrate ibuprofen were increased in subjects carrying the *CYP2C8*3* variant.[37]

The allelic frequency of the *SLCO1B1* 521C variant was about 21% in this population, which is similar to that reported in other white populations.[23,25] Tirona et al[23] reported an allelic frequency of 14% for this variant in a sample of 49 European Americans. Between 2% and 5% of the white population can be estimated to be homozygous for this variant. Therefore the *SLCO1B1* 521T>C SNP is likely to play a major role in interindividual variability of the pharmacokinetics of drugs such as pravastatin and repaglinide.

The *ABCB1* 3435T variant showed a relatively high allelic frequency (62%) in this population (Finns). This is a much higher frequency than that found previously in a West African population (Ghanians, 10%) and also some-

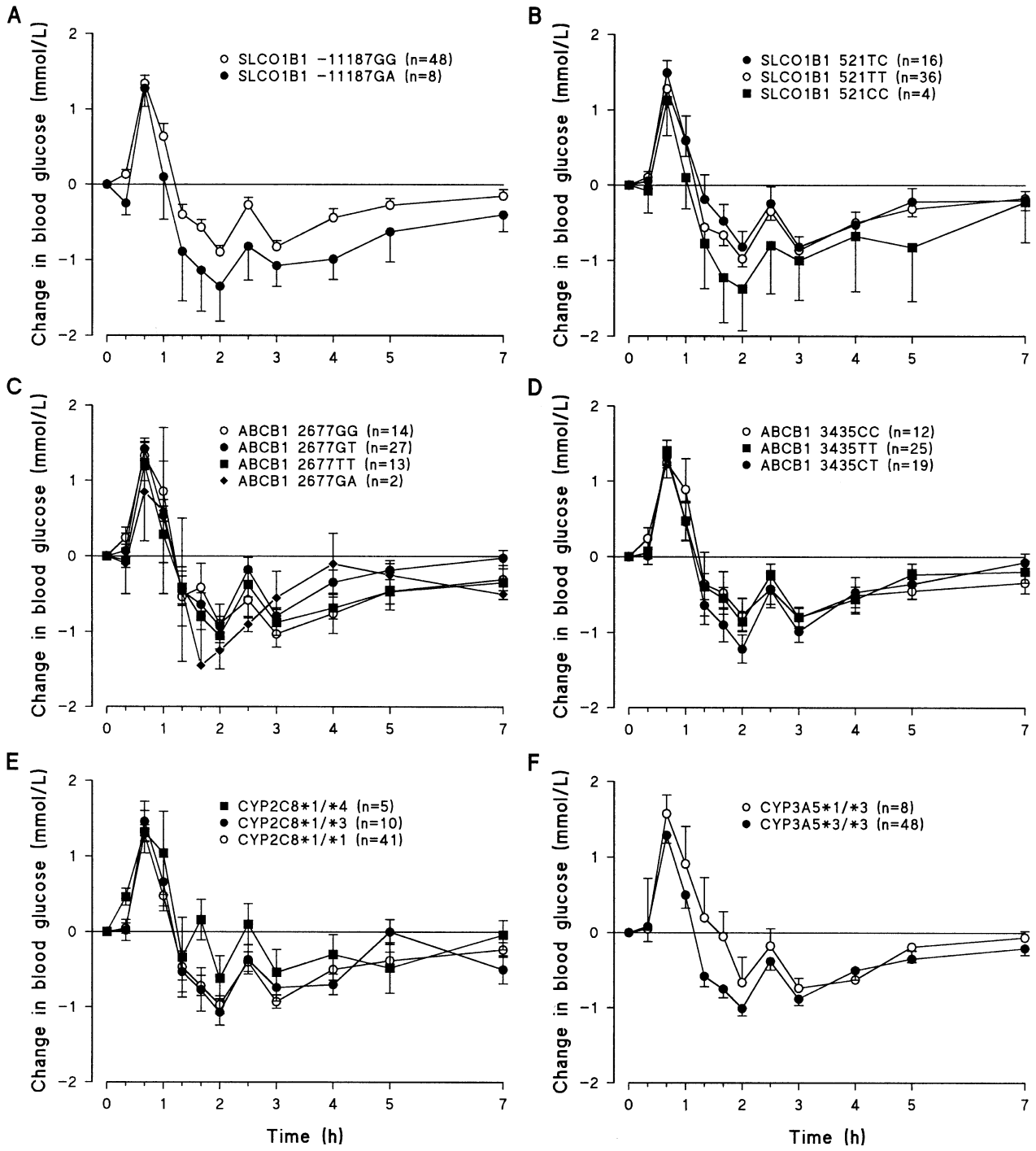

Fig 2. Mean (±SEM) change in blood glucose concentration after single oral dose of 0.25 mg repaglinide in 56 healthy volunteers in relation to *SLCO1B1* −11187G>A (**A**), *SLCO1B1* 521T>C (**B**), *ABCB1* 2677G>T/A (**C**), and *ABCB1* 3435C>T (**D**) SNPs and *CYP2C8* (**E**) and *CYP3A5* (**F**) genotypes. A standardized breakfast was given 15 minutes after repaglinide, a standardized snack was given after 1 and 2 hours, and a standardized warm meal was given after 3 hours.

Table V. Blood glucose variables of single 0.25-mg oral dose of repaglinide in relation to *SLCO1B1* −11187G>A and 521T>C (Val174Ala) single nucleotide polymorphisms and *CYP2C8* genotypes

Variant	*Maximum increase (mmol/L)*	*Maximum decrease (mmol/L)*	*Mean change from 0 to 7 h (mmol/L)*
−11187GG genotype (n = 48)	1.5 ± 0.8	1.2 ± 0.5	−0.2 ± 0.4
−11187GA genotype (n = 8)	1.4 ± 1.0	1.8 ± 0.9	−0.6 ± 0.8
P value	.8644	.0126	.0561
521TT genotype (n = 36)	1.5 ± 0.8	1.3 ± 0.6	−0.3 ± 0.5
521TC genotype (n = 16)	1.6 ± 0.8	1.3 ± 0.5	−0.2 ± 0.5
P value	.9279	.9376	.9004
521CC genotype (n = 4)	1.1 ± 0.8	1.5 ± 1.1	−0.6 ± 1.1
P value, versus TT genotype	.6913	.9032	.5521
ANOVA *P* value	.6256	.8337	.4799
*CYP2C8*1/*1* (n = 41)	1.5 ± 0.8	1.3 ± 0.6	−0.3 ± 0.6
*CYP2C8*1/*3* (n = 10)	1.5 ± 0.8	1.3 ± 0.6	−0.2 ± 0.4
P value	1.000	.9969	.8359
*CYP2C8*1/*4* (n = 5)	1.5 ± 1.0	1.1 ± 0.5	−0.1 ± 0.4
P value, versus **1/*1* genotype	.9945	.7437	.6313
ANOVA *P* value	.9949	.7513	.5979

Data are given as mean ± SD.

what higher than that observed in a large white population (50%) ($P < .0001$ and $P = .0168$, Fisher exact test).[38] Interethnic and geographic differences in *ABCB1* SNP frequencies are well-known phenomena.[38,39] The frequencies of the *CYP2C8*3*, *CYP2C8*4*, and *CYP3A5*3* alleles were similar to those reported previously in white populations.[20,34,36,37]

Because subjects with the *SLCO1B1* 521CC genotype showed markedly increased plasma concentrations of repaglinide, it is possible that a lower repaglinide dose (50% to 75% lower than in subjects with the 521TT genotype) would suffice in patients with this genotype. It is unclear why only the −11187G>A SNP was significantly associated with the blood glucose response to repaglinide, but the partial linkage between this SNP and the 521T>C SNP[26] may be involved. The *SLCO1B1* 521T>C or −11187G>A SNP may be associated with an increased risk of hypoglycemia, especially at the beginning of repaglinide treatment.

In conclusion, genetic polymorphism in *SLCO1B1*, which encodes for the hepatic uptake transporter OATP1B1, is a major determinant of interindividual variability in the plasma concentrations of repaglinide. The effect of *SLCO1B1* polymorphism on the pharmacokinetics of repaglinide may be clinically important.

The authors have identified no conflicts of interest in relation to this manuscript.

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