Polymorphism in *CYP2C8* is associated with reduced plasma concentrations of repaglinide

Objective: **Our objective was to investigate the effects of genetic polymorphisms of cytochrome P450 (CYP) 2C8 on the pharmacokinetics and pharmacodynamics of the meglitinide analog antidiabetic drug repaglinide.**
Methods: **We genotyped 28 healthy volunteers who had participated in our pharmacokinetic studies on repaglinide for variant alleles of the *CYP2C8* gene. Each subject ingested a 0.25-mg dose of repaglinide, and plasma drug and blood glucose concentrations were monitored for 7 hours after dosing.**
Results: **There were 19 subjects (68%) with the *CYP2C8*1/*1* genotype (wild type), 6 subjects (21%) with the *CYP2C8*1/*3* genotype, and 3 subjects (11%) with the *CYP2C8*1/*4* genotype. In the 3 genotypic groups, the mean ± SD area under the plasma repaglinide concentration–time curve from time 0 to infinity [AUC(0-∞)] was 5.8 ± 2.5 ng · h/mL for *CYP2C8*1/*1*, 3.6 ± 0.9 ng · h/mL for *CYP2C8*1/*3*, and 5.1 ± 3.6 ng · h/mL for *CYP2C8*1/*4*. The mean AUC(0-∞) of repaglinide was 45% ($P < .005$) lower and the peak concentration in plasma was 39% lower ($P < .05$) in subjects with the *CYP2C8*1/*3* genotype compared with those with the *CYP2C8*1/*1* genotype. No statistically significant differences were found in the blood glucose response to repaglinide between the genotypes.**
Conclusions: **Unexpectedly, the *CYP2C8*3* variant allele was associated with reduced plasma concentrations of repaglinide. The effects of *CYP2C8* polymorphisms on the pharmacokinetics of CYP2C8 substrates warrant further study. (Clin Pharmacol Ther 2003;74:380-7.)**

Mikko Niemi, MD, Julian B. Leathart, MPhil, Mikko Neuvonen, MSc, Janne T. Backman, MD, Ann K. Daly, PhD, and Pertti J. Neuvonen, MD *Helsinki, Finland, and Newcastle upon Tyne, United Kingdom*

Repaglinide is a short-acting meglitinide analog antidiabetic drug.[1] It lowers blood glucose concentrations by enhancing glucose-stimulated insulin release from the pancreatic beta cells.[2,3] The oral bioavailability of repaglinide is about 60%, and it is likely that repaglinide undergoes some presystemic metabolism.[4] From the systemic circulation, repaglinide is eliminated completely by biotransformation to inactive metabolites, its major metabolites being a dicarboxylic acid (M2), an aromatic amine (M1), and an acyl glucuronide derivative (M7).[4] The enzymes involved in the metabolism of repaglinide have not been fully characterized, but in vitro studies suggest that cytochrome P450 (CYP) 2C8 and CYP3A4 participate in its oxidative biotransformation.[5,6] The lipid-lowering drug gemfibrozil, which inhibits CYP2C8 both in vitro and in vivo,[7,8] increases the mean area under the plasma concentration–time curve from time 0 to infinity [AUC(0-∞)] of repaglinide about 8-fold.[9] The CYP3A4 inhibitors itraconazole[10,11] and clarithromycin[10,12] raise repaglinide AUC(0-∞) about 1.4-fold.[9,13] Thus it is likely that CYP2C8 plays a major role in the systemic elimination of repaglinide but that CYP3A4 does not.

Genetic polymorphisms of *CYP2C8*, causing reduced activity of the enzyme in vitro, have recently gained increasing interest.[14-17] The *CYP2C8*2* and *CYP2C8*4* variant alleles differ from the wild-type allele (*CYP2C8*1*) by single amino acid substitutions

From the Department of Clinical Pharmacology, University of Helsinki and Helsinki University Central Hospital, Helsinki, and Pharmacogenetics Group, School of Clinical and Laboratory Sciences, University of Newcastle Medical School, Newcastle upon Tyne.
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Reprint requests: Pertti J. Neuvonen, MD, Department of Clinical Pharmacology, Helsinki University Central Hospital, PO Box 340, FIN-00029, Helsinki, Finland.
E-mail: *pertti.neuvonen@hus.fi*

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(Ile269Phe and Ile264Met, respectively) and the *CYP2C8*3* allele by 2 amino acid substitutions (Arg139Lys and Lys399Arg).[14,17] The *CYP2C8*2* allele has been seen primarily in black subjects, with an estimated allele frequency of about 18% in this population.[14] The *CYP2C8*3* allele is seen primarily in white subjects, with a reported allelic frequency of between 13% and 15% in white subjects and about 2% in black subjects.[14,17] The allelic frequency of the *CYP2C8*4* allele in a white population was 7.5%.[17] The *CYP2C8*2* and *CYP2C8*3* variants have been shown to be defective in the metabolism of the CYP2C8 probe substrate paclitaxel in vitro.[14,15,17] In addition, *CYP2C8*3* has been shown to be defective in the metabolism of arachidonic acid to its biologically active epoxyeicosatrienoic acid metabolites (11,12-EET and 14,15-EET) in vitro.[14] Data regarding the activity of *CYP2C8*4* have been inconclusive.[17] Currently, there appear to be no studies on the possible effects of polymorphisms in *CYP2C8* on the pharmacokinetics of CYP2C8 substrates in vivo.

A very high interindividual variability is evident in the pharmacokinetics of repaglinide. For example, in our recent pharmacokinetic interaction studies, the AUC(0-∞) of repaglinide in the absence of concomitant medication varied nearly 6-fold between individual subjects.[9,13] The cause for this high variability is unknown. We hypothesized that genetic polymorphisms in *CYP2C8* might affect the metabolic clearance of repaglinide and thereby contribute to the high interindividual variability in repaglinide pharmacokinetics. Therefore we have studied whether the *CYP2C8* genotype affects the pharmacokinetics of repaglinide.

METHODS

Subjects. Of the 30 healthy white Finnish volunteers who had participated in our 2 previous pharmacokinetic studies[9,13] and 1 ongoing study with repaglinide, 28 (12 women and 16 men) participated in the analysis of the *CYP2C8* genotype. Their mean age at the beginning of the pharmacokinetic study was 22 ± 2 years (range, 19-27 years), and their mean weight was 69 ± 12 kg (range, 46-97 kg). Four subjects were tobacco smokers. Two female subjects were taking oral contraceptive steroids, but none was taking any other continuous medication. All subjects gave written informed consent.

Study design. The study protocol was approved by the Ethics Committee for Studies in Healthy Subjects of the Helsinki and Uusimaa Hospital District, Finland. The pharmacokinetic and pharmacodynamic variables of repaglinide were recalculated for each subject from control phase data (ie, in the absence of concomitant medication). In all 3 studies, the volunteers ingested a single 0.25-mg dose of repaglinide (one half of a Novonorm 0.5-mg tablet; Novo Nordisk, Bagsvaerd, Denmark) at 9 AM after an overnight fast and received a standardized breakfast 15 minutes after the administration of repaglinide, a standardized snack 1 and 2 hours after repaglinide, and a standardized warm meal after 3 hours, as described previously.[9,13] The study design with respect to ingestion of repaglinide and meals was identical in all studies. Plasma repaglinide concentrations were quantified from samples taken before the administration of repaglinide and at 20, 40, 60, 80, and 100 minutes and 2, 2.5, 3, 4, 5, and 7 hours after administration by liquid chromatography–tandem mass spectrometry, as described previously.[13] The quantification limit was 0.05 ng/mL, and the between-day coefficient of variation was below 13% at relevant drug concentrations. Blood glucose was measured immediately after each blood sampling by the glucose oxidase method with the Precision G Blood Glucose Testing System (Medisense, Bedford, Mass). The between-day coefficient of variation for blood glucose was below 8% at relevant blood glucose concentrations.

Deoxyribonucleic acid preparation. For genotyping, a 10-mL blood sample was drawn from each subject into a tube containing ethylenediaminetetraacetic acid (EDTA). The blood samples were stored at −20°C until deoxyribonucleic acid (DNA) extraction. DNA was extracted from whole-blood samples by the method previously described.[18] In brief, leukocyte nuclei were pelleted and lysed to release DNA, which was deproteinated by sodium perchlorate treatment followed by chloroform extraction. DNA was precipitated by addition of ethanol and spooled and air-dried before dissolving in 10-mmol/L Tris–hydrochloric acid and 1-mmol/L EDTA (pH 7.4) at approximately 0.5 mg/mL.

CYP2C8 genotyping. All polymerase chain reactions (PCRs) were performed by a standard method. Approximately 0.2 to 0.5 μg of DNA was amplified in 1× buffer (10-mmol/L Tris–hydrochloric acid [pH 9.0], 1.5-mmol/L magnesium chloride, 50-mmol/L potassium chloride, and 0.1% [vol/vol] Triton X-100), containing 100-μmol/L deoxyribonucleoside triphosphates (supplied as Li salt, 100 mmol/L; Promega, Madison, Wis), 0.025 U/μL Bio*Taq* polymerase (Bioline, London, United Kingdom), and 0.25 μmol of forward and reverse primer per liter. Reactions were carried out in a final volume of 25 or 50 μL. All PCR reactions were performed with 1-minute denaturation at 94°C, followed by annealing for 1 minute at the temperature specific to the primers used and 1 minute of extension at 72°C. Controls of known genotype that

had been verified by DNA sequencing (homozygous wild type, heterozygous, and for *CYP2C8*3* only, homozygous mutant) were included in all assay sets together with a blank tube containing the PCR mixture but no DNA. Only assay sets giving the correct genotype assignments for the controls and no band in the blank tube were accepted.

Two separate assays to detect the 2 linked polymorphisms (C792G and A1196G) associated with the *CYP2C8*3* allele were performed.

For the C792G polymorphism (Arg139Lys), forward and reverse primers specific for CYP2C8 and spanning the intron and exon boundaries of exon 3 (N3.1 TTTTTATTAGGAATCATTTC, N3.2 AGTCACCCACCCTTGGTTTT) were used to amplify a 170–base pair (bp) fragment by PCR at an annealing temperature of 48°C. The product was incubated with *Bse*RI (New England Biolabs, Beverley, Mass) at 37°C for 16 hours, which digested the wild-type product into 3 fragments of 110 bp, 40 bp, and 20 bp and the variant product into fragments of 150 bp and 20 bp. Digestion fragments were resolved by electrophoresis on a 10% polyacrylamide gel with the use of TBE (0.09-mol/L Tris-borate and 2-mmol/L EDTA [pH 8.0]) buffer.

The A1196G polymorphism (Lys399Arg) was detected by minor modification of the single-strand conformational polymorphism–based method described previously[17] with the use of the forward and reverse primers ACTACTTCTCCTCACTTCTG and TGCCATGTAAATTCCAACTA, respectively, and an annealing temperature of 57°C. Single-strand conformational polymorphism analysis was performed on 1× Mutation Detection Enhancement (MDE; BioWhittaker Molecular Applications, Rockland, Me) in TME buffer (0.3-mol/L Tris, 0.35-mol/L 2-[*N*-morpholino]ethanesulfonic acid [MES], and 10-mmol/L EDTA [pH 6.8]) containing 2.5-mol/L urea as previously described. Eight microliters of PCR product was denatured at 95°C for 3 minutes in 25 μL of denaturing loading buffer (95% [vol/vol] formamide, 10-mmol/L sodium hydroxide, and 0.05% xylene cyanol). Electrophoresis was performed at a constant 225 V for 28 hours at 4°C. After electrophoresis, DNA was visualized by staining with SYBR Gold (Molecular Probes, Eugene, Ore) and photographed with a Fluor-S multiImager (Biorad, Hercules, Calif). Genotypes were read by reference to the band patterns seen for control homozygous wild-type, heterozygous, and homozygous mutant samples, which were included on each gel.

Genotyping for the *CYP2C8*4* allele was performed by PCR followed by digestion with *Taq*I exactly as described previously.[17]

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 area under the plasma concentration–time curve from time 0 to 7 hours [AUC(0-7)] and from 0 hours 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 following equation: $t_{1/2} = \ln 2/k_e$. The AUC was calculated by the linear trapezoidal rule for the rising phase of the plasma concentration–time curve and by the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by division of the last measured concentration by k_e.

Pharmacodynamics. The blood glucose response was characterized by the mean change in blood glucose concentration calculated by dividing the net AUC of blood glucose by the corresponding time interval. In addition, the maximum increase and maximum decrease from the baseline blood glucose concentration were calculated.

Statistical analysis. Results are expressed as mean ± SD in the text and tables and, for clarity, as mean ± SEM in the figures. The 95% confidence interval (CI) was calculated for the mean values of all pharmacokinetic variables, except for t_{max}. Statistical comparisons between the wild-type and mutant genotypes were made with the 2-sample *t* test (after log transformation of C_{max} and AUC values) or, in the case of t_{max}, with the Mann-Whitney *U* test, with Bonferroni correction. Differences were considered statistically significant at $P < .05$. On the basis of intersubject variability of 27% for the log-transformed AUC(0-∞) of repaglinide, it was calculated that a minimum of 6 subjects would be required for a mutant genotype group and 16 for the wild-type genotype group to detect a potentially clinically significant difference of 30% in the AUC(0-∞) of repaglinide with a statistical power of 80%.

RESULTS

Genotypes found. Of the 28 subjects, 19 (68%) had the *CYP2C8*1/*1* genotype (wild type), 6 (21%) had the *CYP2C8*1/*3* genotype, and 3 (11%) had the *CYP2C8*1/*4* genotype. None was homozygous for a mutant allele. The observed frequencies were in the Hardy-Weinberg equilibrium.

Effect of CYP2C8 genotype on pharmacokinetics of repaglinide. The *CYP2C8* genotype significantly affected the pharmacokinetics of repaglinide (Table I, Fig 1). The mean AUC(0-∞) of repaglinide was 45% lower ($P < .005$) and the C_{max} was 39% lower ($P < .05$) in

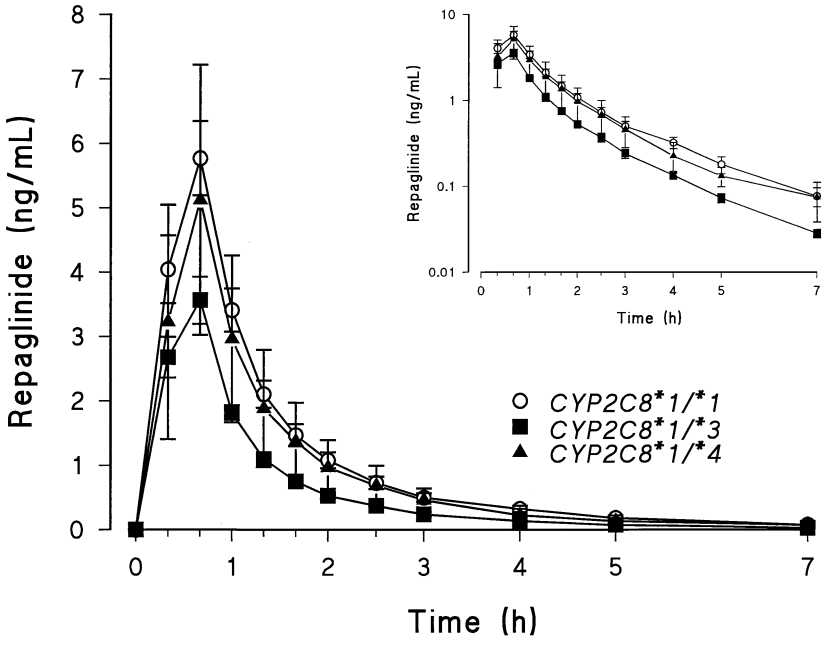

Fig 1. Mean ± SEM plasma concentrations of repaglinide in 28 healthy volunteers with different *CYP2C8* genotypes after single oral dose of 0.25 mg repaglinide. *Inset* depicts the same data on a semilogarithmic scale. *Circles*, Subjects with *CYP2C8*1/*1* genotype; *squares*, subjects with *CYP2C8*1/*3* genotype; *triangles*, subjects with *CYP2C8*1/*4* genotype.

Table I. Pharmacokinetic variables of 0.25 mg repaglinide in healthy volunteers with *CYP2C8*1/*1*, *CYP2C8*1/*3*, and *CYP2C8*1/*4* genotypes

	*CYP2C8*1/*1 (n = 19)*			*CYP2C8*1/3 (n = 6)*			*CYP2C8*1/*4 (n = 3)*		
Variable	*Mean ± SD*	*95% CI*	*Range*	*Mean ± SD*	*95% CI*	*Range*	*Mean ± SD*	*95% CI*	*Range*
C_{max} (ng/mL)	5.8 ± 2.4	4.4-6.6	2.3-11.7	3.6 ± 0.9*	2.6-4.5	2.5-4.6	5.1 ± 3.6	0.9-22.2	2.9-9.3
t_{max} (min)	40	—	20-60	40	—	40-40	40	—	40-40
$t_{1/2}$ (h)	1.3 ± 0.5	1.0-1.5	0.6-2.3	1.2 ± 0.4	0.8-1.7	0.9-2.1	1.3 ± 0.1	1.1-1.5	1.2-1.4
AUC(0-12) (ng · h/mL)	7.3 ± 3.4	5.4-8.3	3.1-17.0	4.1 ± 0.7†	3.3-4.8	3.1-4.8	6.3 ± 4.9	0.9-31.4	3.2-12.0
AUC(0-∞) (ng · h/mL)	7.5 ± 3.7	5.5-8.5	3.1-18.2	4.1 ± 0.7†	3.4-4.9	3.1-4.9	6.5 ± 5.1	0.9-32.4	3.2-12.3

CI, Confidence interval; C_{max}, peak concentration in plasma; t_{max}, time to peak concentration in plasma; $t_{1/2}$, elimination half-life; AUC(0-12), area under plasma concentration–time curve from 0 to 12 hours; AUC(0-∞), area under plasma concentration–time curve from time 0 to infinity.
*$P < .05$, versus *CYP2C8*1/*1*.
†$P < .005$, versus *CYP2C8*1/*1*.

subjects with the *CYP2C8*1/*3* genotype compared with those with the *CYP2C8*1/*1* genotype. Because the number of subjects in the *CYP2C8*1/*4* genotype group did not reach the number (n = 6) required to show a potentially clinically significant effect on the AUC(0-∞) of repaglinide, the data regarding the effects of the *CYP2C8*4* allele on the pharmacokinetics of repaglinide remain inconclusive. There was marked interindividual variation in the AUC(0-∞) of repaglinide among subjects with the *CYP2C8*1/*1* and *CYP2C8*1/*4* genotypes but only modest variation among subjects with the *CYP2C8*1/*3* genotype (Fig 2).

Effect of CYP2C8 genotype on pharmacodynamics of repaglinide. No significant differences were seen in the blood glucose response to repaglinide between the genotypes (Table II, Fig 3).

DISCUSSION

To our knowledge, this is the first demonstration of an association between genetic polymorphism in *CYP2C8* and altered pharmacokinetics of a CYP2C8 substrate. Repaglinide plasma concentrations were lower in subjects carrying the *CYP2C8*3* allele than in those homozygous for the wild-type (*CYP2C8*1*) al-

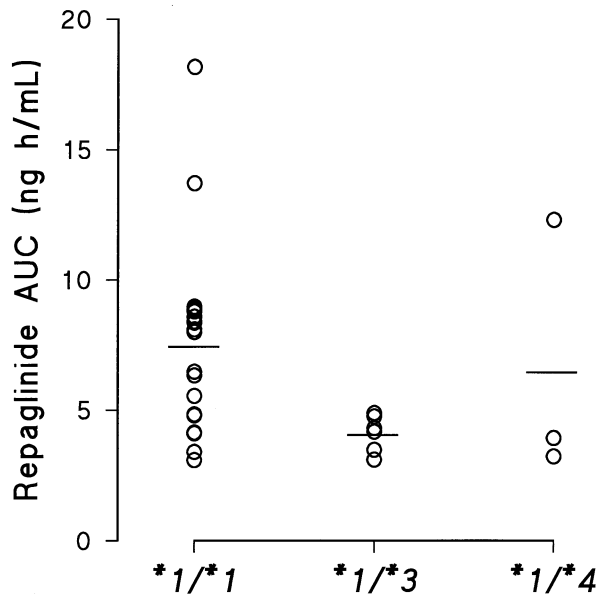

Fig 2. Individual area under plasma repaglinide concentration–time curve (AUC) from time 0 to infinity values of repaglinide in subjects with different *CYP2C8* genotypes after single oral dose of 0.25 mg repaglinide. *Horizontal rules* show mean values for each genotype group.

lele. The mean AUC(0-∞) of repaglinide was about 45% lower in subjects with the *CYP2C8*1/*3* genotype than in those with the *CYP2C8*1/*1* genotype. The possible effect of the *CYP2C8*1/*4* genotype on the pharmacokinetics of repaglinide could not be detected because of the small number of subjects with this genotype. Marked variation was evident in the plasma concentrations of repaglinide among subjects with the wild-type and *CYP2C8*1/*4* genotypes, but there was only modest variation among subjects with the *CYP2C8*1/*3* genotype. No statistically significant differences were seen in the blood glucose response to repaglinide followed by meals between subjects with different *CYP2C8* genotypes. This is probably partly the result of the small number of subjects included, as well as the use of a subtherapeutic dose of repaglinide, which was chosen for safety reasons to prevent severe hypoglycemia.

The finding that the plasma concentrations of repaglinide were reduced in subjects with the *CYP2C8*1/*3* genotype was unexpected in view of the previous in vitro findings suggesting a reduced metabolic capacity for this genotype. In one in vitro study using recombinant enzymes, the activity of *CYP2C8*3* for the metabolism of paclitaxel was decreased by 85% and that for arachidonic acid was decreased by 60% to 70% compared with the activity of the wild-type enzyme (*CYP2C8*1*).[14] However, in another study the maximum rate of metabolism of *CYP2C8*3* for paclitaxel 6α-hydroxylation was only 34% lower than that of *CYP2C8*1*, and the Michaelis-Menten constant values were similar.[15] Furthermore, in human liver microsomes, heterozygosity for the *CYP2C8*3* allele was associated with a relatively small reduction (about 40%) in the metabolism of paclitaxel.[17]

A number of explanations for these findings can be suggested. First, it is possible that the activity of the *CYP2C8*3* variant is increased toward repaglinide, although its activity is reduced toward some other CYP2C8 substrates. In line with such a possibility, the effects of the *CYP2C8*3* mutant allele seem to be substrate-specific, because its ability to catalyze the CYP2C8-mediated *N*-deethylation of amiodarone was not reduced in a recent study with HepG2 cells transiently expressing *CYP2C8*1* and *CYP2C8*3* alleles.[16] Although amino acid substitutions resulting in substrate-specific differences as substantial as this appear not to have been reported previously for CYPs, there is a well-established polymorphism in the esterase paraoxonase 1 (PON1) that increases catalytic activity with some substrates and decreases it toward others.[19]

Second, it is possible that the presence of the *CYP2C8*3* allele results in a compensatory upregulation of the expression of some CYP enzymes in humans. Because repaglinide is a substrate of both CYP2C8 and CYP3A4,[5,6] increased expression of either CYP2C8 or CYP3A4, or both, in subjects with the *CYP2C8*1/*3* genotype would explain the apparently increased clearance of repaglinide in these subjects.

Third, it is possible that the *CYP2C8*3* allele is linked to an as yet unknown polymorphism that causes the reduced plasma concentrations of repaglinide. In this respect, it is interesting that the *CYP2C8*3* allele was recently found to be linked to the *CYP2C9*2* allele in a large white population.[20] However, this linkage cannot explain the current findings because repaglinide is not metabolized by CYP2C9[5,6] and because the *CYP2C9*2* allele is associated with reduced enzymatic activity both in vitro and in vivo.[21] Furthermore, linkage with a polymorphism in the *CYP3A4* gene cannot explain the observation because *CYP2C8* and *CYP3A4* are located in different chromosomes.[22,23]

Fourth, because *CYP2C8*3* is defective in the production of the 11,12-EET and 14,15-EET metabolites of arachidonic acid,[14] which are important for the regulation of various physiologic functions, such as vascular smooth muscle tone[24] and the contractility of

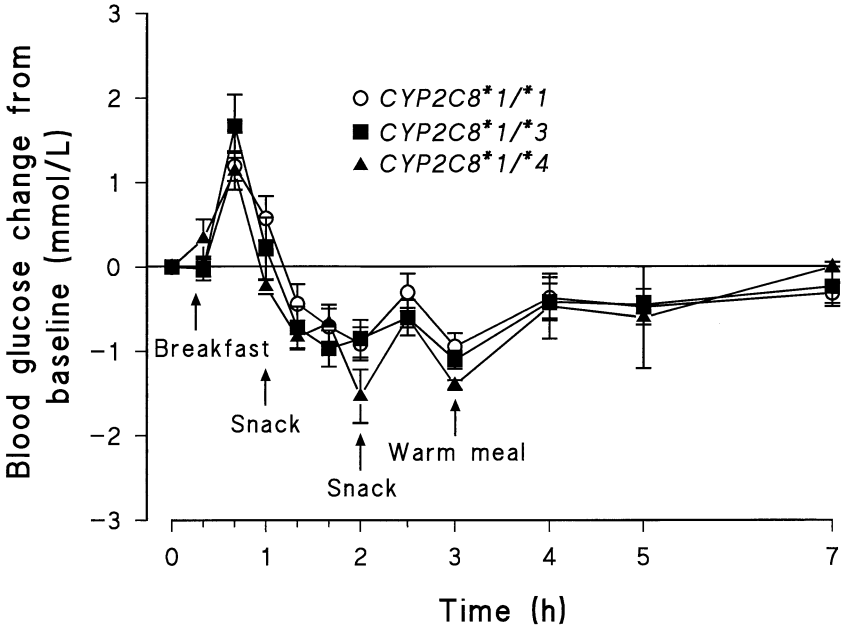

Fig 3. Mean ± SEM change in blood glucose concentrations in 28 healthy volunteers with different *CYP2C8* genotypes after single oral dose of 0.25 mg repaglinide. *Circles*, Subjects with *CYP2C8*1/*1* genotype; *squares*, subjects with *CYP2C8*1/*3* genotype; *triangles*, subjects with *CYP2C8*1/*4* genotype.

Table II. Blood glucose response to 0.25 mg repaglinide followed by meals in healthy volunteers with *CYP2C8*1/*1*, *CYP2C8*1/*3*, and *CYP2C8*1/*4* genotypes

Blood glucose variable	*CYP2C8*1/*1 (n = 19)*	*CYP2C8*1/*3 (n = 6)*	*CYP2C8*1/*4 (n = 3)*
Mean change, 0-3 h (mmol/L)	–0.2 ± 0.7	–0.3 ± 0.3	–0.5 ± 0.0
Mean change, 0-7 h (mmol/L)	–0.3 ± 0.6	–0.4 ± 0.2	–0.5 ± 0.3
Maximum increase (mmol/L)	1.4 ± 0.8	1.7 ± 0.9	1.1 ± 0.4
Maximum decrease (mmol/L)	1.3 ± 0.6	1.3 ± 0.2	1.7 ± 0.3

Data are presented as mean ± SD.

cardiac muscle,[25] it is possible that the *CYP2C8*3* allele alters some physiologic function so that the clearance of repaglinide is increased.

In any case, it is evident that further in vitro and in vivo studies are needed to uncover the mechanistic link between the *CYP2C8*3* allele and reduced plasma concentrations of repaglinide. In addition, further studies are needed to evaluate whether *CYP2C8* polymorphisms alter the plasma concentrations of other CYP2C8 substrates. CYP2C8 is involved in the metabolism of several drugs including amiodarone,[26] amodiaquine,[27] carbamazepine,[28] cerivastatin,[7,29] paclitaxel,[30] pioglitazone,[31] rosiglitazone,[32] verapamil,[33] and zopiclone.[34]

In the clinical setting the potential problems associated with high interpatient variability in the exposure to repaglinide are overcome by a dosing schedule in which repaglinide treatment is begun with a low dose in all patients and increased later according to the pharmacodynamic response. Genotyping of patients with respect to their *CYP2C8* genotype might allow detection of some patients who ultimately require a higher repaglinide dose. However, it should be noted that in our study the *CYP2C8* genotype explained only a part of the high interindividual variability in the exposure to repaglinide, because the variation was still high in subjects with the homozygous wild-type genotype. Therefore further studies are needed to determine other

possible sources of variability in the plasma concentrations of repaglinide.

In conclusion, the *CYP2C8*1/*3* genotype was associated with reduced plasma concentrations of the CYP2C8 substrate repaglinide. Genotyping of patients with respect to the *CYP2C8*3* allele may allow detection of patients who require a higher dosage of repaglinide. Further studies on the effects of the *CYP2C8* genotype on the pharmacokinetics of other CYP2C8 substrates and on the effects of the variant *CYP2C8* alleles on the metabolism of repaglinide in vitro are warranted.

The authors have identified no conflicts of interest.

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