PHARMACOGENETICS AND GENOMICS

No effect of *MDR1* C3435T variant on loperamide disposition and central nervous system effects

Background: **The *MDR1* gene encodes the efflux transporter P-glycoprotein, which is highly expressed in the small intestine and in the blood-brain barrier. A major function of P-glycoprotein is to limit the absorption and central nervous system exposure of numerous xenobiotics. A genetic polymorphism in the *MDR1* gene (C3435T) has been associated with changes in the intestinal expression level and function of P-glycoprotein. The aim of this study was to investigate the effect of this polymorphism on disposition and brain entry of the P-glycoprotein substrate loperamide.**

Methods: **Healthy white volunteers were genotyped for the *MDR1* C3435T polymorphism, and a 16-mg oral dose of loperamide was administered to 8 subjects with the 3435TT genotype and 8 subjects with the 3435CC genotype. Plasma levels of loperamide were determined by liquid chromatography–tandem mass spectrometry. Loperamide-induced respiratory depression was detected as the ventilatory response to carbon dioxide and was used as a measure of central nervous system side effects.**

Results: **We found no significant difference in loperamide pharmacokinetics between individuals homozygous for the C and the T alleles in position 3435 of *MDR1*, as follows: peak plasma drug concentration, 3164 ± 1053 pg/mL and 3021 ± 984 pg/mL; area under the concentration-time curve from 0 to 8 hours, 14,414 ± 4,756 pg · h/mL and 14,923 ± 6,466 pg · h/mL; and time to peak plasma drug concentration, 3.9 ± 1.4 hours and 3.9 ± 2.6 hours for the *MDR1* 3435CC and 3435TT genotypes, respectively ($P > .05$, for all parameters). Hypercapnic ventilatory response changed only minimally after ingestion of loperamide (the coefficient of variation during the 0- to 8-hour period was 21% ± 14% for the sample population), and there was no *MDR1* 3435 genotype–related effect on respiratory response. Carriers of the 2 major *MDR1* haplotypes, *MDR1*1* and *MDR1*13*, did not differ in their response to loperamide.**

Conclusion: **There was no association between the *MDR1* C3435T variation and plasma levels or central nervous system effects of the P-glycoprotein substrate loperamide in a white study population. The *MDR1* haplotype structure was quite variable and supports the use of haplotypes instead of single nucleotide polymorphisms in determining clinical consequences of genetic variation. (Clin Pharmacol Ther 2003;74:487-98.)**

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The *MDR1* (*ABCB1*) gene product P-glycoprotein is an integral membrane protein that actively translocates substrates out of the intracellular compartment. The clinical importance of P-glycoprotein was first estab-

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lished in cancer chemotherapy, in which high expression and activity of P-glycoprotein can cause pleiotropic resistance of cancer cells to chemotherapeutic agents.[1,2] It is now recognized that P-glycoprotein is also expressed in tissues with excretory or protective function and is involved in absorption, distribution, and elimination of drug substrates.[3-5] One of the major sites of P-glycoprotein action is the blood-brain barrier. P-glycoprotein is highly expressed in brain capillary endothelial cells and is involved in limiting the access of substrate drugs to the central nervous system (CNS).[6,7] Consequently, P-glycoprotein–deficient *mdr1* knockout mice display higher central sensitivity to systemically given agents, such as digoxin, calcium channel blockers, and human immunodeficiency virus protease inhibitors.[5-8] In addition, there is increasing evidence that P-glycoprotein plays a key role in transporting opioids (ie, β-endorphin, morphine, loperamide, and the model opioid peptide [D-penicillamine] enkephalin [DPDPE]) across the blood-brain barrier.[9-15]

Modification of P-glycoprotein function by comedication with other drugs (ie, quinidine, rifampin [INN, rifampicin]) results in increased or decreased plasma concentrations of P-glycoprotein substrates.[3,4] Inhibition of P-glycoprotein by quinidine was shown to be the underlying mechanism for the potentially life-threatening interaction between digoxin and this antiarrhythmic agent.[4] Recently, inhibition of P-glycoprotein by quinidine in healthy volunteers resulted in respiratory depression under carbon dioxide challenge after administration of the potent μ-opioid receptor agonist loperamide.[16] Under normal conditions, CNS effects of loperamide are absent, because pharmacologically active CNS concentrations are not obtained as a result of limited intestinal absorption and P-glycoprotein function in the blood-brain barrier.

There is increasing evidence that genetic polymorphisms in the *MDR1* gene might affect P-glycoprotein function and expression levels and, therefore, have an impact on absorption and tissue distribution of drug substrates. Decreased P-glycoprotein expression levels were first reported for a synonymous change in the *MDR1* gene (C3435T). Differences in intestinal P-glycoprotein expression levels in healthy white volunteers could be correlated with the presence or absence of the *MDR1* C3435T variant.[17] Specifically, individuals with the 3435TT genotype had significantly lower intestinal P-glycoprotein levels than those with the 3435CC genotype. In accordance with these observations, plasma levels of the P-glycoprotein substrate digoxin were higher in individuals harboring the *MDR1* 3435T allele.[17] Despite extensive characterization of the effect of the *MDR1* C3435T variation on pharmacokinetics and pharmacodynamics, the significance of this variation remains controversial.[17-22]

Compromised P-glycoprotein function in the blood-brain barrier in individuals homozygous for the *MDR1* 3435T allele was recently suggested on the basis of an increased incidence of postural hypotension during nortriptyline treatment.[23] We hypothesize that individuals with the *MDR1* 3435TT genotype will have altered intestinal absorption and CNS penetration of loperamide. This study compared loperamide disposition and CNS effects in individuals with the 3435CC and 3435TT genotypes. Measurement of loperamide-induced respiratory depression was used as an indirect measure of P-glycoprotein function in the blood-brain barrier.[16] Although initial stratification was based on the *MDR1* 3435 genotype, additional analysis allowed for consideration of the association of *MDR1* haplotype with loperamide respiratory depression.

METHODS

Subjects. After approval by the University of California, San Francisco Committee on Human Research (San Francisco, Calif), 54 healthy white subjects aged 26 to 49 years were genotyped for the *MDR1* C3435T variant. The subjects were unrelated residents of California and self-identified their ethnic background. Eight individuals homozygous for the reference allele (3435CC) and eight individuals homozygous for the variant allele (3435TT) were enrolled in the study. Subjects had no history of medical problems, had no cardiovascular or respiratory abnormalities on physical examination, and were not taking regular medication. Cigarette smoking, drug intake, and the consumption of alcohol, coffee, tea, and grapefruit juice were not permitted from 10 PM on the evening before and throughout the remainder of the study. A negative pregnancy test result was required for all female participants. All subjects gave written informed consent.

Study design. After an overnight fast, volunteers ingested 16 mg loperamide hydrochloride (eight 2-mg capsules) with 50 mL of water. Subjects abstained from food for 2 hours after loperamide administration. Thereafter, standardized caffeine-free meals were provided. Venous blood samples (8 mL) were collected before and at 0.5, 1, 1.5, 2, 4, 6, and 8 hours after administration of loperamide. Blood samples were centrifuged, and plasma was stored at −20°C until analysis.

Genotyping. Genomic deoxyribonucleic acid from peripheral lymphocytes was isolated by standard methods with the QIAamp Midi Kit (Qiagen, Valencia,

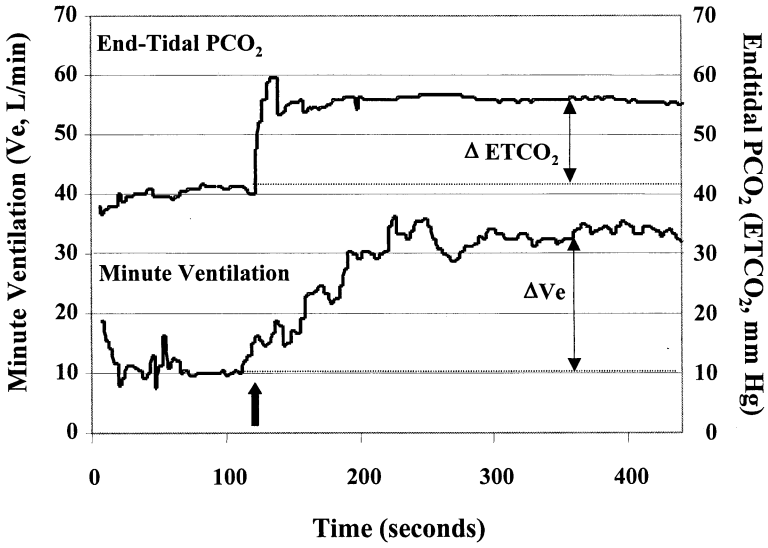

Fig 1. Measurement of hypercapnic ventilatory response (HCVR). HCVR is expressed as the slope of minute ventilation (V_E) and end-tidal partial pressure of carbon dioxide (P_{CO_2})($ETCO_2$) ($\Delta V_E/\Delta P_{CO_2}$). The *thick arrow* indicates the time when carbon dioxide was added to the inhaled gas mixture. The graph shows a representative measurement at a single time point from 1 individual (subject 12 at 6 hours).

Calif). Genotyping was performed by the Program in Human Genetics Core Facility, University of California, San Francisco, by use of polymerase chain reaction amplification and direct sequencing. The primers used have been described online.[24,25] Polymerase chain reaction and sequencing conditions have been described in detail recently.[26]

Measurement of ventilatory response to carbon dioxide. Hypercapnic ventilatory response (HCVR) was determined by a steady-state technique.[27] Subjects wore a nose clip and breathed through a mouthpiece connected by a 3-way valve to a breathing circuit with a fraction of inspired oxygen of at least 50% to avoid peripheral chemoreceptor stimulation. Ventilatory flow was measured with a heated Fleisch No. 3 pneumotachograph (Fleisch, Inc, Lausanne, Switzerland) with a Validyne CD15 demodulator (Validyne Engineering, Northridge, Calif), calibrated with a 3-L syringe. A computer converted flow to minute ventilation, tidal volume, and respiratory rate. A mass spectrometer (Perkin-Elmer 1100 Medical Gas Analyzer; Perkin-Elmer Corp, Norwalk, Conn) continuously measured respiratory gases. Carbon dioxide was added to produce a baseline end-tidal partial pressure of carbon dioxide (P_{CO_2}) 2 to 3 mm Hg above the resting value, and minute ventilation was allowed to stabilize for 5 to 10 minutes. Carbon dioxide was then added to the gas mixture to produce an increase from baseline carbon dioxide of approximately 15 to 20 mm Hg, resulting in end-tidal P_{CO_2} values of 55 to 65 mm Hg. Breathing at constant end-tidal P_{CO_2} continued to a new steady state, for 6 to 7 minutes, until the minute ventilation and tidal volume reached stable levels. End-tidal P_{CO_2}, partial pressure of oxygen, minute ventilation (V_E), tidal volume, respiratory rate, and oxygen saturation (measured by pulse oximetry) were measured and data recorded directly onto a Macintosh Quadra 800 (Apple Computer, Inc, Cupertino, Calif) with the use of LabVIEW 3.1 (National Instruments Corporation, Austin, Tex). HCVR was calculated as the slope ($\Delta V_E/\Delta P_{CO_2}$) (Fig 1) and was measured immediately after each blood collection (before and at 0.5, 1, 2, 4, 6, and 8 hours after loperamide ingestion).

Measurement of loperamide and its main metabolite in plasma. Loperamide and its main metabolite, monodesmethyl loperamide, were measured by liquid chromatography–tandem mass spectrometry by use of positive electrospray ionization. Loperamide and propranolol (internal standard) were from United States Pharmacopeia (Rockville, Md). All other chemicals were reagent grade and were purchased from Sigma Chemical (St Louis, Mo) or Fisher Scientific (Pittsburgh, Pa). Plasma samples were extracted with methyl *t*-butyl ether before separation by liquid chromatogra-

phy–tandem mass spectrometry. Human plasma samples (0.5 mL) were analyzed for loperamide in a PE Sciex-API III system (Perkin-Elmer Corp) equipped with a Hypersil silica column (4.6 × 50 mm, 3-μm particle size; Thermo Hypersil-Keystone, Bellefonte, Pa). A mobile phase of acetonitrile/water/trifluoroacetic acid (90:10:0.06 [vol/vol]) was used at a flow rate of 1.2 mL/min. Retention times were 1.20 minutes for loperamide and 1.33 minutes for propranolol. Mass spectrometric detection was performed with sample inlet by heated nebulizer, positive ionization by atmospheric pressure chemical ionization, and mass scanning by multiple reaction monitoring analysis, in which parent-to-daughter ions were as follows: loperamide, 477.2 to 266.1 mass-to-charge ratio (*m/z*), and propranolol, 260 to 116 *m/z*. The linear standard curve range is 20 to 4000 pg/mL. Coefficients of variation for the lower limit of quantitation and interday and intraday precision and accuracy were within 10%. Stability studies demonstrated stability for freeze/thaw, bench top, and processed sample (up to 6 days). Sample reinjection variation and accuracy remained within acceptable limits. Matrix studies also showed no interference by this method.

Data analysis. The area under the concentration-time curve (AUC) was measured for 8 hours after administration of loperamide. This observation period was chosen on the basis of a previous report of maximum respiratory effect after 1.5 to 2 hours and return to baseline respiratory function after 6 hours.[16] Plasma concentrations of loperamide hydrochloride and monodesmethyl loperamide were plotted semilogarithmically against time, and the AUC was calculated by the trapezoidal rule for the periods from 0 to 2 hours [AUC(0-2)], 0 to 4 hours [AUC(0-4)], and 0 to 8 hours [AUC(0-8)]. Statistical comparison for pharmacokinetic parameters (peak plasma drug concentration [C_{max}], time to C_{max} [t_{max}], and AUC) between the *MDR1* 3435CC and 3435TT groups and the 2 major haplotype groups was performed by Mann-Whitney *U* test. Differences in HCVR from baseline over time and between the genotype groups were analyzed by repeated-measures ANOVA. Analysis was done with StatView 5 Software (SAS Institute Inc, Cary, NC). $P < .05$ was considered statistically significant.

Power calculation. The aim of this study was to compare loperamide-induced respiratory depression for volunteers homozygous for the CC and TT alleles at position 3435 of the *MDR1* gene. In a previous study on the effect of quinidine coadministration on loperamide-induced respiratory depression, the mean value of the baseline carbon dioxide response slope in the presence of loperamide was 108% ± 17% and quinidine coadministration reduced it by 27%.[16] On the basis of the variance estimate from the previous loperamide study and a significance level of .05, a study population of 8 volunteers for each allele group was chosen to allow detection of intragroup differences in respiratory depression of 27% to 35% with a power of at least 90%. The *MDR1* 3435TT genotype was previously associated with a 1.3-fold higher C_{max} relative to the 3435CC genotype, a difference that was even more pronounced (2-fold) after rifampin induction.[17] If we assume a similar increase in loperamide AUC(0-8) in the *MDR1* 3435TT group and loperamide AUC values similar to those reported from a previous 16-mg oral dose study,[16] a study population of 8 volunteers for each allele group yields a power of 76% (1.3-fold difference) to 100% (2-fold difference) for a 2-sided test and an α of .05. Power calculations were done with SamplePower 2.0 from SPSS Inc (Chicago, Ill).

RESULTS

MDR1 C3435T variation and loperamide disposition. Mean plasma concentration–time curves of loperamide and monodesmethyl loperamide were similar for the *MDR1* 3435CC and 3435TT groups (Figs 2 and 3). There were no significant differences in loperamide C_{max} ($P = .92$), t_{max} ($P = .67$), and AUC(0-8) ($P = .83$) between the 2 groups (Table I). The AUC(0-2) and AUC(0-4) values were analyzed to allow detection of *MDR1* genotypic effects on early plasma concentrations, which more accurately reflect the initial phase of intestinal absorption. However, no intragroup differences could be detected for AUC(0-2) and AUC(0-4). Estimation of a terminal half-life was not possible during this 8-hour period.

MDR1 C3435T variation and respiratory depression. Baseline HCVR varied almost 4-fold across the study population (Fig 4), and there was only minimal change in HCVR after administration of loperamide. There was no significant difference in respiratory response between the *MDR1* 3435CC and 3435TT genotype groups (repeated-measures ANOVA, $P = .42$) (Table II). The coefficient of variation in HCVR over the 8-hour period in a given individual ranged from 11% to 25%, with the exception of subjects 4 and 15, who showed a significant decrease in respiratory response relative to baseline. HCVR decreased by a maximum of 76% and 93% in subjects 4 and 15, respectively. In both subjects HCVR was significantly decreased within 30 minutes of loperamide administration and remained depressed for the entire 8-hour study

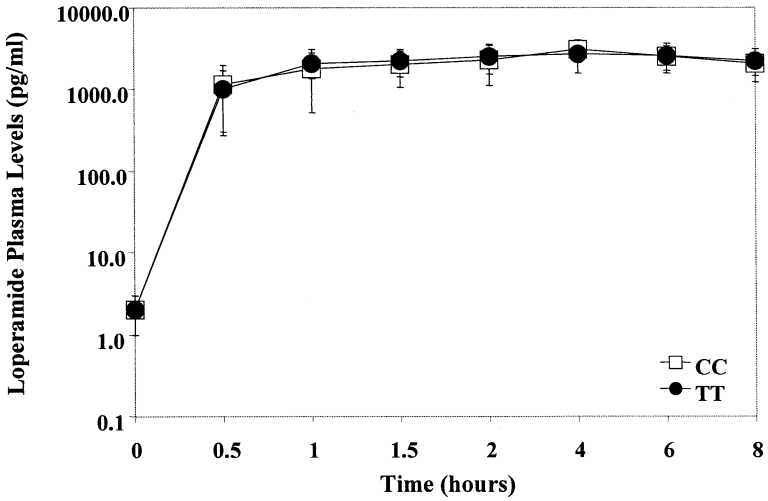

Fig 2. Plasma concentration–time profiles for loperamide in individuals with *MDR1* 3435CC and 3435TT genotypes. Loperamide plasma levels were measured for 8 hours after a 16-mg oral dose of loperamide to healthy white subjects. Data are presented as mean ± SD for 8 subjects per group. There were no significant differences in loperamide plasma levels between the *MDR1* 3435 genotype groups.

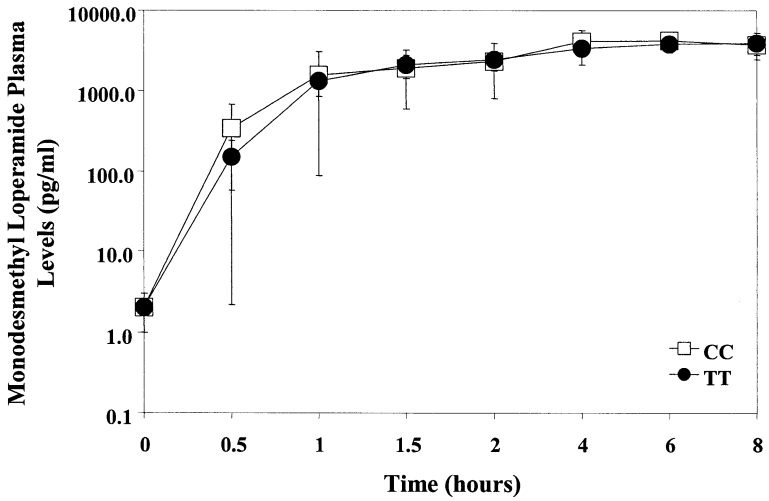

Fig 3. Plasma concentration–time profiles for monodesmethyl loperamide in individuals with *MDR1* 3435CC and 3435TT genotypes. Monodesmethyl loperamide plasma levels were measured for 8 hours after a 16-mg oral dose of loperamide to healthy white subjects. Data are presented as mean ± SD for 8 subjects per group. There were no significant differences in monodesmethyl loperamide plasma levels between the *MDR1* 3435 genotype groups.

period. Plasma loperamide levels in these 2 individuals were in the range observed in the other subjects.

***MDR1* haplotypes and loperamide disposition and respiratory depression.** Subjects were genotyped for 25 additional *MDR1* variants previously described for the white population, and haplotypes were assigned where possible according to our earlier analysis.[26] *MDR1* haplotypes for our study population are shown in Fig 5. In addition to the C3435T polymorphism, 10 variant sites were found in our sample. These additional

Table I. Loperamide pharmacokinetic parameters

	*MDR1 haplotype**	C_{max} *(pg/mL)*	t_{max} *(h)*	*AUC(0-2) (pg · h/mL)*	*AUC(0-4) (pg · h/mL)*	*AUC(0-8) (pg · h/mL)*
CC genotype group						
1	**1/*24*	2310	4.0	1570	5,180	12,680
2	Unassigned	2530	6.0	3072	7,682	12,612
3	**25/*26*	3630	4.0	1995	7,525	14,015
4	**25/*26*	2270	4.0	3085	7,375	11,465
5	**1/*3*	3740	4.0	2314	8,064	14,464
6	**1/*1*	4790	1.0	6888	15,828	23,738
7	**1/*1*	4160	4.0	3413	10,653	18,323
8	**1/*32*	1880	4.0	1960	4,850	8,010
Mean		3164	3.9	3037	8,395	14,414
SD		1053	1.4	1686	3,498	4,756
TT genotype group						
9	**14/*14*	3690	8.0	1484	8,404	23,264
10	**13/*13*	2690	1.0	3122	7,122	10,712
11	**13/*13B*	2920	1.5	3488	8,088	12,648
12	**13/*13*	3630	6.0	4375	10,195	16,865
13	Unassigned	2150	1.0	2922	6,632	10,132
14	**13/*14*	3980	4.0	1598	15,278	22,948
15	Unassigned	3920	4.0	3660	11,020	17,900
16	**13/*13*	1190	6.0	1064	2,751	4,915
Mean		3021	3.9	2714	8,686	14,923
SD		984	2.6	1192	3,655	6,466
Difference		$P = .92$	$P = .67$	$P = .92$	$P = .99$	$P = .83$

Data are shown for individuals grouped according to *MDR1* 3435 genotype. There were no significant differences in any parameter between the *MDR1* 3435CC and 3435TT groups. C_{max}, Peak plasma drug concentration; t_{max}, time to peak plasma drug concentration; AUC(0-2), area under concentration-time curve from 0 to 2 hours; AUC(0-4), area under concentration-time curve from 0 to 4 hours; AUC(0-8), area under concentration-time curve from 0 to 8 hours.

*Where possible, haplotypes were assigned according to recent nomenclature.[26]

variants included 2 in the noncoding exonic region (T-129C and G-1A), 5 in the intronic region (introns 4, 9, 13, 14, and 20), and 3 in the coding exonic region (A61G, C1236T, and G2677T/A). In the *MDR1* 3435CC group, only 2 individuals were homozygous and 3 individuals heterozygous for the reference haplotype *MDR1*1*. Two individuals in the *MDR1* 3435CC group carried *MDR1*25*/*MDR1*26* haplotypes. *MDR1*25* contains the G(+24)A variation in intron 20, and *MDR1*26* contains a second variant at (−25) in intron 4. One individual had a haplotype that encoded the G2677A variation, which results in an Ala893Thr change in P-glycoprotein (*MDR1*24*). Haplotypes could not be called for subject 2, and 1 chromosome of subject 8 was assigned a novel haplotype not described in our previous study (*MDR1*32*).[26] In the *MDR1* 3435TT group 3 individuals were homozygous and 2 individuals heterozygous for the *MDR1*13* haplotype, which has 3 exonic and 3 intronic changes relative to the reference haplotype. The *MDR1*13* haplotype encodes for Ser893 P-glycoprotein. One individual was homozygous for *MDR1*14*, which contains the G2677T variation, leading to the Ser893 coding change plus an additional A61G variant resulting in an Asn21Asp change in P-glycoprotein. Haplotype assignments could not be made for 2 subjects in the *MDR1* 3435TT group. Comparison of carriers of *MDR1*1* and *MDR1*13* revealed no significant differences in plasma loperamide pharmacokinetics or the respiratory response to loperamide. Subject 4, who had a large decrease in respiratory response, had the same haplotype as subject 3, who had no significant respiratory response to loperamide. Subject 15, the second individual with a dramatic respiratory response to loperamide, had a unique haplotype that could not be deduced from genotyping data alone.

DISCUSSION

This study compared disposition and brain entry of the P-glycoprotein substrate loperamide in white volunteers who were homozygous for the reference or the variant allele at nucleotide 3435 in the coding region of the *MDR1* gene. This genetic polymorphism has recently been described as a determinant of intestinal P-glycoprotein expression levels and of intestinal ab-

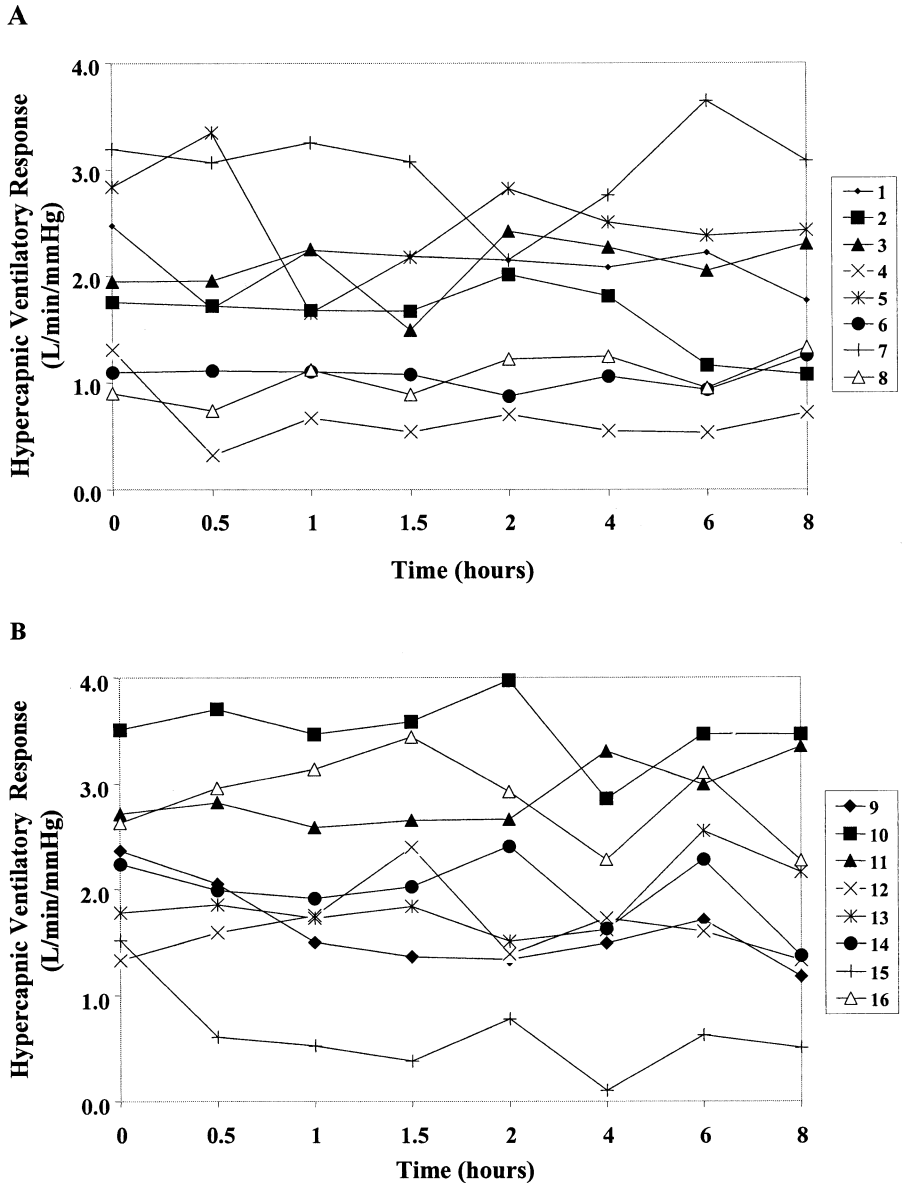

Fig 4. Repeated-measures plot of HCVR. HCVR was measured before and at the indicated times after loperamide administration to 16 subjects. Each *line* represents a single individual in the *MDR1* 3435CC (**A**) or 3435TT (**B**) genotype group. In 2 individuals (subjects 4 and 15) a notable decrease in respiratory response was shown compared with the response in the remaining subjects.

sorption and plasma levels of different P-glycoprotein substrates.[17-20,28,29] Furthermore, P-glycoprotein expression in blood leukocytes was shown to be influenced by the *MDR1* 3435 genotype.[22] The possible impact of genetic variation in *MDR1* on P-glycoprotein function in the blood-brain barrier has not been directly studied. In this study HCVR to loperamide was used as a marker for P-glycoprotein function in the blood-brain barrier, on the basis of a recent study that demonstrated loperamide-induced respiratory depression after coadministration of the P-glycoprotein inhibitor quinidine.[16] On the basis of earlier work showing *MDR1* 3435 genotype–related changes in the intestinal absorption of P-glycoprotein substrates, we hypothesized that loperamide plasma and CNS levels would be influenced by this common genetic polymorphism.

Table II. Loperamide pharmacodynamic measurements

	MDR1 haplotype*	*Hypercapnic respiratory response (% of baseline)*							
		0 h	*0.5 h*	*1 h*	*1.5 h*	*2 h*	*4 h*	*6 h*	*8 h*
CC genotype group									
1	**1/*24*	1.00	0.68	0.91	0.89	0.87	0.84	0.90	0.72
2	Unassigned	1.00	0.98	0.96	0.95	1.15	1.04	0.66	0.62
3	**25/*26*	1.00	1.00	1.16	0.77	1.24	1.17	1.05	1.18
4	**25/*26*	1.00	0.24	0.51	0.41	0.54	0.42	0.41	0.55
5	**1/*3*	1.00	1.18	0.58	0.77	0.99	0.88	0.84	0.86
6	**1/*1*	1.00	1.01	1.01	0.98	0.80	0.96	0.86	1.14
7	**1/*1*	1.00	0.96	1.02	0.96	0.67	0.86	1.14	0.97
8	**1/*32*	1.00	0.82	1.25	1.00	1.36	1.39	1.06	1.49
Mean			0.86	0.92	0.84	0.95	0.95	0.86	0.94
SD			0.29	0.26	0.20	0.29	0.28	0.24	0.32
TT genotype group									
9	**14/*14*	1.00	0.87	0.63	0.58	0.57	0.63	0.72	0.50
10	**13/*13*	1.00	1.05	0.99	1.02	1.13	0.81	0.99	0.99
11	**13/*13B*	1.00	1.04	0.95	0.98	0.98	1.22	1.10	1.23
12	**13/*13*	1.00	1.20	1.32	1.80	1.04	1.30	1.20	1.00
13	Unassigned	1.00	1.04	0.97	1.03	0.85	0.91	1.43	1.21
14	**13/*14*	1.00	0.89	0.85	0.91	1.08	0.72	1.02	0.61
15	Unassigned	1.00	0.40	0.35	0.25	0.52	0.07	0.42	0.34
16	**13/*13*	1.00	1.13	1.20	1.31	1.11	0.87	1.18	0.87
Mean			0.95	0.91	0.98	0.91	0.82	1.01	0.84
SD			0.25	0.31	0.46	0.24	0.38	0.31	0.33

Data are shown for individuals grouped according to *MDR1* 3435 genotype. Respiratory response at each time point is expressed relative to the baseline measurement in a given individual. There was no significant difference in respiratory response between the *MDR1* 3435CC and 3435TT groups as measured by repeated-measures ANOVA ($P = .42$).

*Where possible, haplotypes were assigned according to recent nomenclature.[26]

Variability in ventilatory drive is well known, and our population of subjects is reasonably typical.[30] Baseline HCVR varied across the study population but did not differ between groups. No statistically significant ventilatory depression occurred as a result of loperamide in either group, although loperamide did appear to affect 2 subjects (subjects 4 and 15). Variability of ventilatory drive during the study period could obscure a significant effect of loperamide; however, a repeated-measures design is extremely robust. Baseline differences in responsiveness are not important, and the ventilatory depression of most drugs is easily determined when the drug has a consistent effect. Examination of Fig 4 shows that most subjects had consistent ventilatory drive throughout the multiple measurements, without evidence of ventilatory depression, making it unlikely that larger numbers of subjects would show a significant effect. The finding of 2 subjects with apparent ventilatory depression from loperamide suggests that there is a possibility that CNS penetration occurred but that it did not follow the pattern of known polymorphism. This raises the question of whether other *MDR1* genotypes or haplotypes are more important determinants of loperamide CNS levels. Alternatively, additional transporters may play a significant role in the blood-brain barrier penetration of loperamide.

Compromised P-glycoprotein function on the blood-brain barrier in individuals homozygous for the *MDR1* 3435T allele was recently suggested on the basis of an increased incidence of postural hypotension during nortriptyline treatment.[23] In another study the *MDR1* 3435TT genotype was overrepresented in patients with early- and late-onset Parkinson's disease, supporting a role for P-glycoprotein expression and function in modulating interindividual susceptibility for neurologic disorders.[31] Furthermore, increased tacrolimus neurotoxicity in liver transplant patients could be correlated with a nonsynonymous *MDR1* polymorphism (G2677T leading to an Ala893Ser change in P-glycoprotein),[32] which is highly linked to the synonymous variant in position 3435.[26] Surprisingly, despite this significant linkage disequilibrium, *MDR1* G2677T is a positive predictor for the development of tacrolimus neurotoxicity whereas *MDR1* C3435T is a negative predictor of this phenomenon.

Available data suggest that differences in total P-glycoprotein expression levels in the small intestine

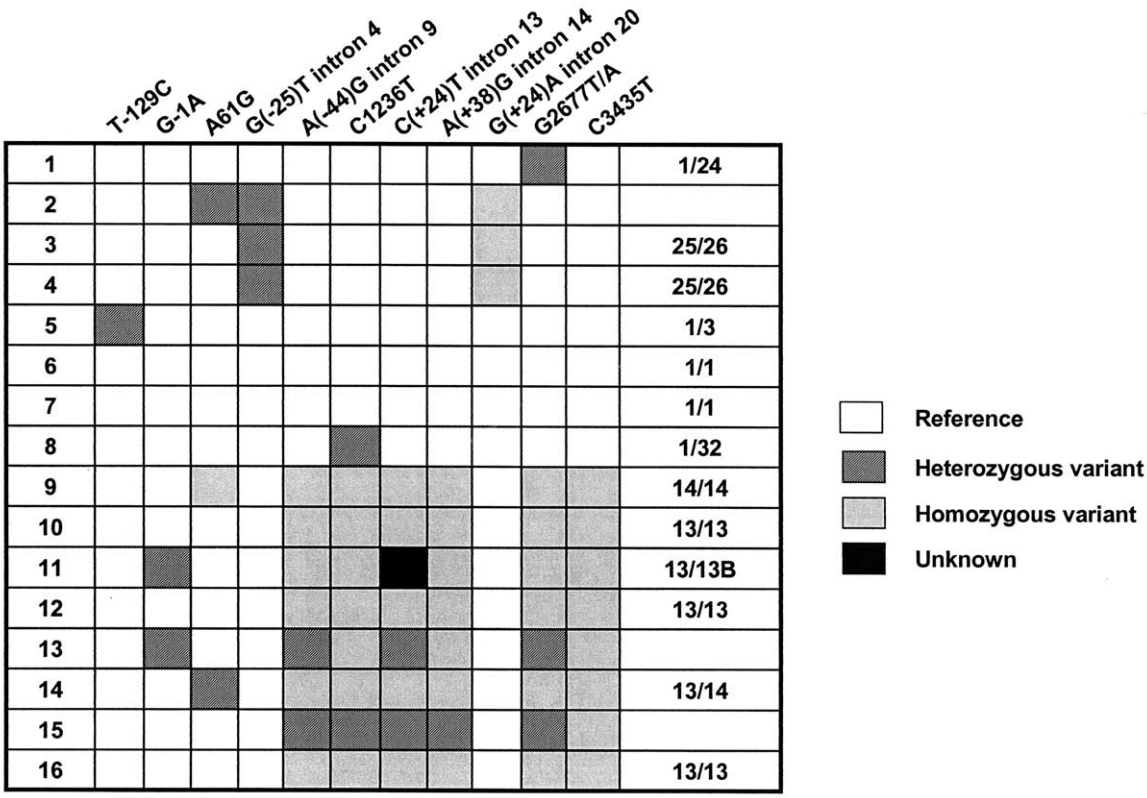

Fig 5. *MDR1* haplotype structure for study participants. *MDR1* genotypes were determined for 26 variants previously described in a large white population. The genotypes at each of 10 variant sites detected in the current study population are shown. Haplotype assignments correspond to the nomenclature recently described.[26] In the one case of missing genotype data, haplotypes were tentatively assigned and a variant genotype in intron 13 was assumed. Haplotypes *MDR1*32* and *MDR1*13B* are newly defined in this study. Subject 1 carries the G2677A variant, whereas all other variant genotypes are G2677T.

between homozygous carriers of the C and the T allele are in the range of 40%.[17] If we assume the C3435T-related differences in P-glycoprotein expression in the blood-brain barrier to be in the same range, functional P-glycoprotein levels in the blood-brain barrier might still be high enough to effectively protect the CNS against toxic loperamide levels. In contrast, chemical inhibition of P-glycoprotein by quinidine (median inhibitory concentration values, 2-3 μmol/L[33]) is likely to result in an almost complete inhibition of P-glycoprotein in the blood-brain barrier and to be more potent than the effect attributed to the genetic polymorphism in *MDR1*.[16] After a single oral dose of 600 mg, plasma levels of quinidine are in the range of 3 mg/L (9 μmol/L),[16] which exceeds the median inhibitory concentration value.

There is an indication that, in addition to P-glycoprotein, members of the organic anion transporting polypeptide (OATP) gene family are involved in the transport of opioid peptides across the blood-brain barrier. The δ-receptor agonist [D-penicillamine(2,5)] enkephalin (DPDPE) and deltorphin II were identified as substrates of human OATP-A, which is expressed in the blood-brain barrier.[16] Overlapping substrate specificity between OATPs and P-glycoprotein has been described for many drugs such as digoxin, fexofenadine, and the opioid DPDPE.[34,35] Although OATP-dependent intestinal and CNS transport of loperamide has not yet been established, involvement of one or several uptake transporters would provide an explanation for the lack of association between the *MDR1* CC and TT genotype and CNS effects in our study.

Concordance was observed between the effect of the *MDR1* 3435 genotype on loperamide disposition and CNS effects. We could not observe differences in loperamide intestinal absorption or disposition between the *MDR1* genotype groups. Altered loperamide absorption in the *MDR1* 3435TT genotype group would have been expected on the basis of the finding of impaired intestinal absorption of digoxin in individuals with this genotype.[17,29] However, other reports suggest that digoxin absorption is enhanced or unaffected by the *MDR1* 3435 genotype.[19,36-38] Similar confusion about the effect of the *MDR1* 3435 genotype on intestinal absorption also exists for fexofenadine and tacrolimus.[18,20,39,40] The lack of an effect of the *MDR1* 3435 genotype on loperamide absorption is similar to results reported for cyclosporine (INN, ciclosporin).[41,42] Differences in substrate affinity to P-glycoprotein with regard to the extent of biotransformation or the existence of alternative transport pathways might influence whether a clinical phenotype is observed in individuals homozygous for the *MDR1* 3435 variant. Unlike digoxin, loperamide undergoes biotransformation via oxidative metabolism, and there are data indicating that cytochrome P450 (CYP) 3A4 is involved in loperamide metabolism.[43] The CYP3A family displays large interindividual variability in expression and function,[44] which might contribute to variability in loperamide disposition and, therefore, mitigate a contribution of genetically determined differences in transporter function. Furthermore, as mentioned earlier, it is conceivable that, in addition to P-glycoprotein, other transporters, such as members of the OATP or multidrug resistance–associated protein (MRP) family, might be involved in intestinal loperamide transport. Overlapping substrate specificity between P-glycoprotein and OATPs or MRPs has been described for many drugs and might contribute to loperamide disposition.[45,46]

Of importance, previous studies have focused largely on the *MDR1* C3435T variation, but this synonymous variation alone might not define a clinical phenotype. A recent haplotype analysis of *MDR1* revealed that the genetic polymorphism in position 3435 is in tight linkage disequilibrium with other intronic and coding region polymorphisms in the *MDR1* gene.[26] The most common haplotypes are *MDR1*1* and *MDR1*13*, which differ at 3 coding and 3 intronic positions. Importantly, the *MDR1*13* haplotype encodes the Ala893Ser change in P-glycoprotein. Although our primary goal was to investigate the impact of the *MDR1* C3435T variation on loperamide respiratory depression, we performed a post hoc analysis considering *MDR1* haplotypes. There was no difference in the plasma pharmacokinetics or respiratory response in individuals who carried the *MDR1*1* and *MDR1*13* haplotypes. However, the current study illustrates the diversity of *MDR1* haplotypes found in a sample selected for a common *MDR1* polymorphism. In the *MDR1* 3435CC group, 2 individuals had haplotypes encoding P-glycoprotein variants (Ala893Thr and Asn21Asp), and all subjects in the *MDR1* 3435TT group were at least heterozygous for a haplotype encoding for the P-glycoprotein Ala893Ser variant. The functional significance of these *MDR1* variants remains undefined, but the possibility exists that at least several of these variants might influence P-glycoprotein expression or function or both. In an earlier study, heterozygous carriers of the *MDR1* −129C allele showed significantly decreased placental P-glycoprotein expression.[21] One of the subjects with an exaggerated respiratory response to loperamide (subject 15) was heterozygous for the 1236 and 2677 variants and at least 2 of the intronic variants found in the *MDR1*13* haplotype yet was homozygous for the 3435 polymorphism. Previous analysis revealed a tight linkage disequilibrium between the 6 variants in *MDR1*13*, and the allele combinations found in subject 15 were not observed in our earlier study.[26]

In summary, these findings provide no evidence that the *MDR1* C3435T polymorphism is a significant determinant of the disposition and brain entry of the μ-receptor agonist loperamide in white subjects. However, the importance of P-glycoprotein in maintaining an intact blood-brain barrier warrants further studies to clarify the impact of *MDR1* haplotypes on P-glycoprotein function in the blood-brain barrier. The diversity of haplotypes found in our small study population illustrates the importance of analyzing *MDR1* haplotypes instead of single polymorphisms to characterize the effect of *MDR1* variation on P-glycoprotein function.

The data have been submitted to http://www.pharmgkb.org and http://www.pharmacogenetics.ucsf.edu.

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