

# PHARMACOGENETICS AND GENOMICS

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## CYP3A activity in African American and European American men: Population differences and functional effect of the *CYP3A4\*1B* 5'-promoter region polymorphism

**Objective:** Cytochrome P4503A (CYP3A) activity exhibits considerable interindividual variability. Possible differences in CYP3A activity were investigated in European American and African American men with the use of midazolam as an in vivo probe.

**Methods:** Midazolam was simultaneously administered intravenously (1 mg, [<sup>15</sup>N<sub>3</sub>]-labeled) and orally (2 mg, unlabeled in capsule form) to 15 young healthy European American men and a similar group of men of African American descent. Plasma concentration–time curves were measured. The subjects were subsequently genotyped with respect to the *CYP3A4\*B1* polymorphism (A-290G) in the 5'-promoter (nifedipine-specific element) region.

**Results:** The oral bioavailability of midazolam was about equally determined by intestinal and hepatic extraction with CYP3A activity at the former site exhibiting greater variability. Oral bioavailability was related to intestinal metabolism ( $r = 0.98$ ), whereas hepatic CYP3A activity contributed little to the interindividual variability ( $r = 0.03$ ). A lower systemic clearance ( $265 \pm 54$  versus  $310 \pm 56$  mL/min;  $P = .04$ ), but not oral clearance, was observed in African Americans. With one exception, the African Americans possessed a variant *CYP3A4\*1B* allele (4 heterozygotes A/G and 10 homozygote G/G), whereas all of the European Americans were wild-type homozygotes (A/A). Hepatic CYP3A activity and the systemic clearance of midazolam were about 30% lower in G/G homozygotes than in A/A homozygotes ( $252 \pm 53$  versus  $310 \pm 54$  mL/min;  $P = .02$ ), and a gene-dose effect was present ( $P = .01$ ). There was no genotype/phenotype relationship with respect to the oral clearance of midazolam.

**Conclusion:** Comparison of CYP3A activity between populations is complicated by frequency distribution differences in the regulatory *CYP3A4\*1B* polymorphism and lower hepatic CYP3A activity associated with the variant allele. However, this reduction is modest; therefore no major and clinically important difference in CYP3A activity is present between Americans of African or European descent. (Clin Pharmacol Ther 2000;68:82-91.)

Christoph Wandel, MD, John S. Witte, PhD, Jefferey M. Hall, PhD,  
C. Michael Stein, MD, Alastair J. J. Wood, MD, and Grant R. Wilkinson, PhD  
Nashville, Tenn, Cleveland, Ohio, and La Jolla, Calif

From the Departments of Medicine and Pharmacology, Vanderbilt University, Nashville; the Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland; and PPGx Inc, La Jolla.

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Reprint requests: Grant R. Wilkinson, PhD, Department of Pharmacology, Vanderbilt University, Nashville, TN 37232-6600.

E-mail: [grant.wilkinson@mcm.vanderbilt.edu](mailto:grant.wilkinson@mcm.vanderbilt.edu)

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Cytochrome P4503A (CYP3A)<sup>†</sup> is the predominant isoform of the CYP superfamily in both the intestinal epithelium and liver, where it contributes to the often large first-pass metabolism of CYP3A substrates after their oral administration.<sup>1</sup> Considerable interindividual variability is present in the activity of the isoform and, because of its involvement in the metabolism of a large number of drugs,<sup>2</sup> elucidation of the determinants of CYP3A activity is important. Much information has been obtained about factors such as drug interactions that result in enzyme inhibition or induction<sup>3</sup>; however, less insight is available about determinants of the basal activity of the isoform. Given the global development and widespread clinical use of drugs that are CYP3A substrates, population differences in CYP3A-mediated metabolism may have important consequences, and such differences, associated with race, could arise because of different genetic or environmental factors. Studies with the prototypic CYP3A substrate nifedipine suggest that this may indeed be the case; for example, the plasma levels of the drug after oral administration were reported to be twofold to threefold higher in Japanese, Mexican, Southeast Asian, and Nigerian populations compared with white persons residing in various countries.<sup>4-9</sup> However, the mechanisms involved in such differences, and whether they apply to other populations, are not known. In contrast to certain other drug-metabolizing enzymes that exhibit genetic polymorphism (eg, CYP2C19 and CYP2D6), the activity of CYP3A is distributed unimodally and no evidence of a common allelic variant in the coding region of the gene has been described. Accordingly, it has been considered that the large interindividual variability in the activity of CYP3A may reflect differences in transcriptional regulation. Recently, an allelic variant in the 5'-flanking region of CYP3A has been identified (CYP3A4\*1B) that involves an A→G transition at position -290 from the transcriptional initiation site.<sup>10</sup> It has been speculated that this nucleotide substitution may be associated with a reduced level of CYP3A activity<sup>10,11</sup>; however, direct supportive evidence of such lower metabolizing ability in vivo has not been reported.

The extensive metabolism of midazolam, mainly by 1'-hydroxylation and, to a lesser extent, 4-hydroxylation, is mediated by CYP3A.<sup>12,13</sup> Because of this, estimation of the clearance of this benzodiazepine has been developed and established as a phenotypic trait measure of CYP3A activity.<sup>14,15</sup> Moreover, by administra-

tion of midazolam both intravenously and orally, it is possible to estimate the separate CYP3A-mediated metabolism in the intestinal epithelium and the liver.<sup>15</sup> Accordingly, studies were undertaken to determine whether differences in CYP3A were present in two American populations—one of African descent and the other of European (white) origin. In addition, post hoc analysis was performed to obtain insight into the possible role of the subsequently discovered CYP3A4\*1B variant in the activity of the isoform and the metabolism of midazolam.

## METHODS

**Clinical protocol.** Subjects were all healthy American men as established by medical history, physical examination, and routine laboratory tests, including those indicative of hepatic and renal function. One group of 15 subjects was by self-identification of African American ancestry through three generations (age range, 26 to 49 years; weight range, 76 to 116 kg), whereas a similarly sized and defined group was of white European descent (age range, 24 to 44 years; weight range, 70 to 100 kg). Subjects were all nonsmokers, and they abstained from medication, including ethanol, for at least 7 days before the study; ingestion of grapefruit juice during the same time period was also not permitted. To reduce other possibly confounding dietary factors, the subjects received standardized daily meals for 7 days immediately before drug administration.

After an overnight fast, each subject simultaneously received an intravenous injection of 1 mg [<sup>15</sup>N<sub>3</sub>]-labeled midazolam over 15 to 30 seconds and 2 mg oral midazolam, formulated with 12% starch 1500 and lactose in a hard capsule. The subjects were maintained in a supine position and continued to fast until the last blood sample had been collected. Blood samples were obtained through an indwelling catheter at 0, 5, 15, and 30 minutes and at 1, 2, 3, 4, 5, 6, and 8 hours after drug administration. Harvested plasma was stored at -20°C until analyzed.

The study was approved by the Vanderbilt University Institutional Review Board. Informed consent was provided by each subject before the study.

**Analysis of midazolam and 1'-hydroxylation in plasma.** Midazolam and its 1'-hydroxy metabolite were determined by use of a method based on validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS-MS). In brief, 1 mL (200 units) β-glucuronidase type H1 from *Helix pomatia* (Sigma Chemical Co, St Louis, Mo) was added to 1 mL plasma, diluted with 0.55 mL water that contained 50 ng diazepam (internal standard), and the mixture was

<sup>†</sup>In adults, CYP3A represents the combined activities of CYP3A4 and CYP3A5, which cannot be separated. However, in the majority of individuals, activity in the intestine and liver predominantly reflects CYP3A4.

incubated at room temperature for 20 to 24 hours. After the addition of 1 mL 2 mol/L bicine buffer saturated with sodium chloride, pH 9.3, the mixture was extracted with 6 mL methyl- *t*-butyl ether that contained 10% ethyl acetate. After centrifugation, the organic phase was evaporated to dryness under nitrogen at 60°C and the residue dissolved in 250  $\mu$ L of an equal mixture of mobile phase A/mobile phase B. After the solution was vortexed briefly (5 seconds) and allowed to stand for 1 minute, it was filtered (2  $\mu$ , Spin-X Costar centrifuge tube filter, Corning Inc, Corning, NY) and a 60- $\mu$ L aliquot was chromatographed with use of an automatic sample injector. Gradient chromatography was performed with a 4.6  $\times$  50 mm Zorbax XDB C<sub>8</sub> column with a flow rate of 0.35 mL/min. The mobile phases consisted of 20 mmol/L ammonium acetate in either water (phase A) or 90% acetonitrile (phase B); chromatography was initiated with an equal mixture of the two phases and then linearly changed over 6 minutes to 10% phase A/90% phase B, after which the gradient was returned to the original 50:50 mixture and equilibrated for 2 minutes before the next injection.

LC-MS-MS analysis was performed with use of a Finnegan 7000 TSQ instrument with an APCI source interfaced with the Waters HPLC system. The APCI settings were as follows: capillary temperature was -200°C, vaporizer temperature was -500°C, and current was -5  $\mu$ A. A collision energy of 27 eV with a collision gas pressure of 2.5 mTorr was used for MS-MS. Selected reaction monitoring and retention characteristics of the various compounds were as follows: [<sup>15</sup>N<sub>0</sub>]-midazolam, 326→291 *m/z* and 4.4 minutes; [<sup>15</sup>N<sub>0</sub>]-1'-hydroxymidazolam, 342→324 *m/z* and 3.2 minutes; diazepam, 285→193 *m/z* and 5.4 minutes; [<sup>15</sup>N<sub>3</sub>]-midazolam, 329→294 *m/z*; and [<sup>15</sup>N<sub>3</sub>]-1'-hydroxymidazolam, 345→327 *m/z* and 3.2 minutes. Interday reproducibility for all compounds was less than 12%.

**Pharmacokinetic analysis.** Systemic clearance (CL<sub>S</sub>), oral clearance (CL<sub>S</sub>/F), steady-state volume of distribution (V<sub>ss</sub>), and oral bioavailability (F<sub>obs</sub>) of midazolam were determined by standard statistical moment analysis, with the terminal elimination rate constant (*k*) being estimated by log-linear regression of the last 4 to 6 data points. The midazolam elimination half-life (*t*<sub>1/2</sub>) was obtained from the relationship: *t*<sub>1/2</sub> = 0.693/*k*. The total area under the plasma level-time curve (AUC) for 1'-hydroxymidazolam was also determined. Estimates of CYP3A-mediated extraction ratios in the liver (E<sub>H</sub>) and intestinal tract (E<sub>GI</sub>) were obtained from equations 1 and 2, respectively, as described previously<sup>15</sup>:

$$E_H = CL_S / Q_{H,plasma} \quad (1)$$

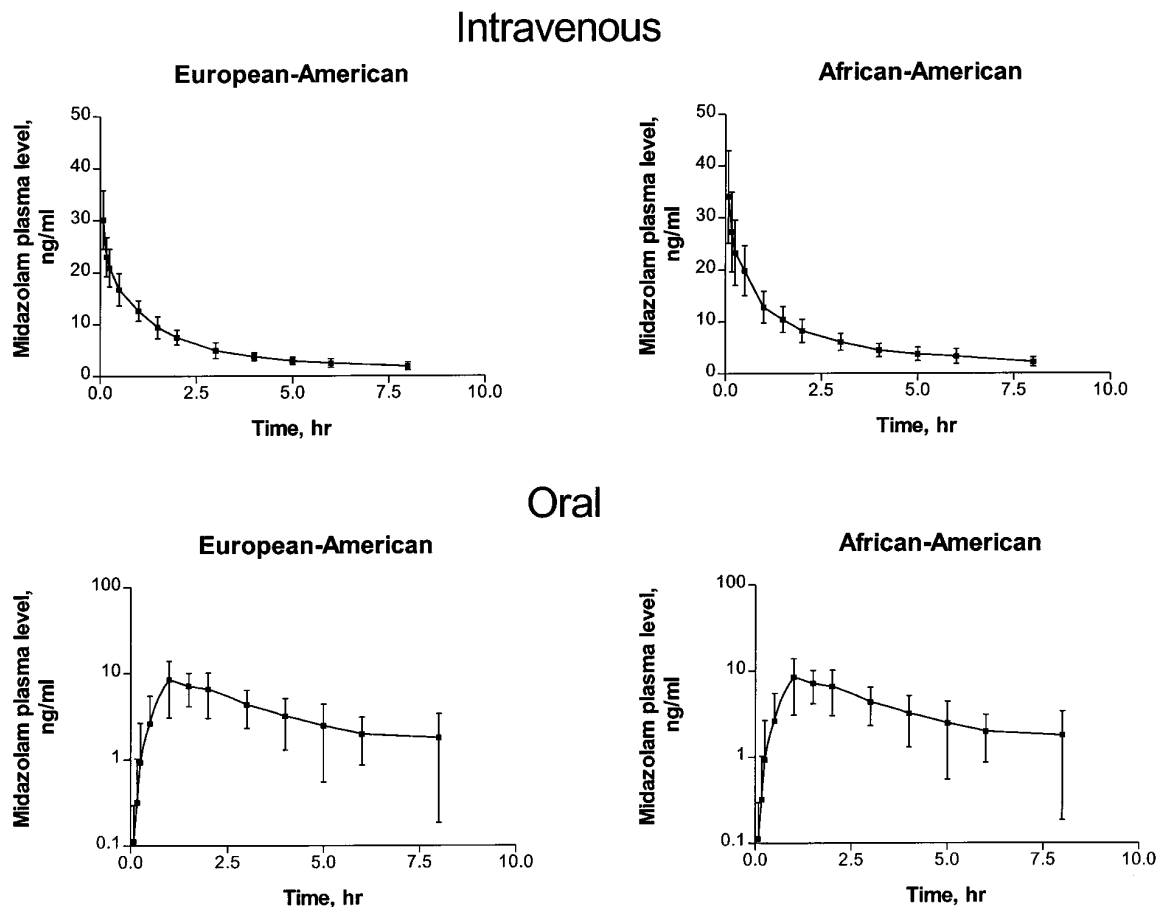
$$E_{GI} = (1 - F_{obs}) / (1 - E_H) \quad (2)$$

in which liver plasma flow (Q<sub>H,plasma</sub>) was equal to (1 - hematocrit) · Q<sub>H,blood</sub>, with the hematocrit value assumed to equal 0.48, and liver blood flow (Q<sub>H,blood</sub>) was equal to 21.6 body weight (in milliliters per minute per kilogram).<sup>15</sup>

**Genotyping for the CYP3A4\*1B polymorphism.**

The genotype of each subject with respect to the A/G genetic polymorphism at position -290 from the start codon in a sequence motif of the 5'-flanking region of the CYP3A4 gene, termed the *nifedipine-specific element*, was determined by a recently described method.<sup>16</sup> In brief, this involved the isolation of genomic DNA obtained from a blood sample of each subject, followed by genotyping with use of the Perkin-Elmer TaqMan method. DNA was amplified in a reaction containing 900 nmol/L forward unlabeled primer (5'-TGGCTTGGTGGGATGAATTTCAAG-3'), 900 nmol/L reverse unlabeled primer (5'-TTACTGGGGA-GTCCAAGGGTTCTG-3'), 200 nmol/L 6-carboxyfluorescein-labeled probe (5'-TTAAATCGCCTCTCTCTTGGCCTTGTCTCTAT-3'), 200 nmol/L tetrachloro-6-carboxyfluorescein-labeled probe (5'-AATCGCCTCTCTCTCTGCTTGTCTCTAT-3'), and 1X Perkin-Elmer TaqMan reagent mixture 43C4447. The polymerase chain reaction reaction mixture was pre-incubated at 50°C for 2 minutes and then 95°C for 10 minutes. Two-step thermocycling was performed for 45 cycles; each cycle consisted of denaturing at 95°C for 30 seconds and annealing at 64°C for 30 seconds. After completion of thermocycling, the fluorescence was read on an ABI 7700 Sequence Detector with use of allelic discrimination software. 6-Carboxyfluorescein/tetrachloro-6-carboxyfluorescein ratios for each DNA sample, normalized against the 6-carboxytetramethyl rhodamine signal, indicated the CYP3A4\*1B genotype of each patient and was further confirmed by comparison with the signals from known control DNA.

**Statistical analysis.** Differences between the two populations were analyzed nonparametrically by the Wilcoxon two-sample test, and *P* < .05 was interpreted as being statistically significant. The same analysis was also used to evaluate possible differences between the pharmacokinetic estimates on the basis of CYP3A\*1B genotype. In addition, linear regression analysis was used to investigate any gene-dose effect. Specifically, the homozygous wild-type genotype (A/A) was coded as 0, the heterozygotes genotype (A/G) was coded as 1, and the homozygous variant (G/G) was coded as 2, that is, a log-additive model. The resulting regression coefficient was then tested for trend. All statistical



**Fig 1.** Mean  $\pm$  SD plasma concentration–time curves of midazolam after intravenous (**upper panels**) and oral (**lower panels**) in European American (**left panels**) and African American (**right panels**) men.

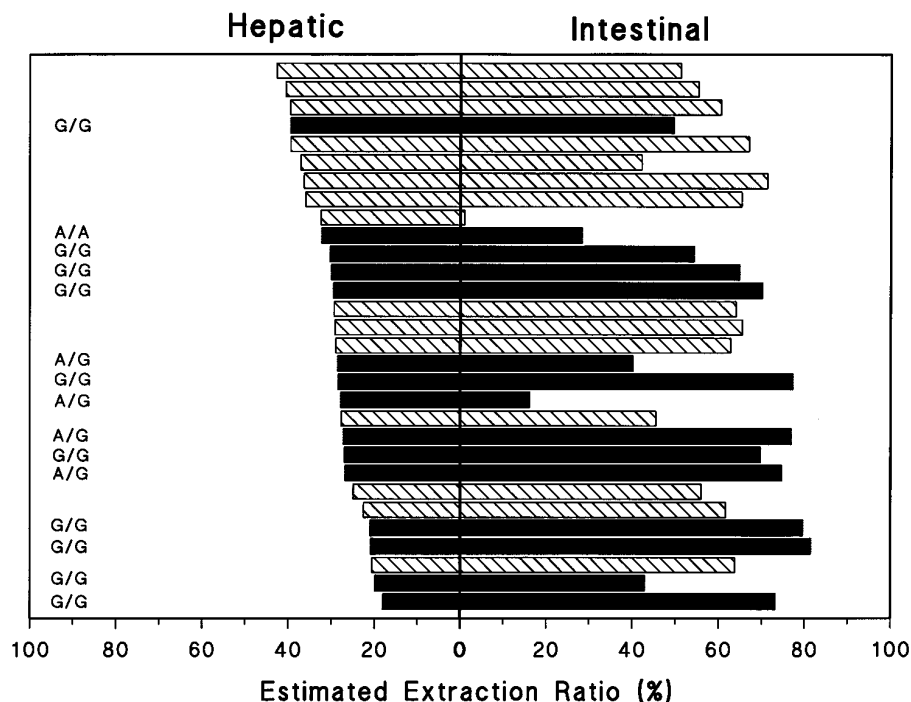
**Table I.** Mean  $\pm$  SD pharmacokinetic parameters of midazolam in American men of African and European descent

	European Americans (n = 15)	African Americans (n = 15)
Age (y)	29.8 $\pm$ 10.02	33.6 $\pm$ 11.0
Weight (kg)	86.0 $\pm$ 9.0	87.7 $\pm$ 11.9
V <sub>SS</sub> (L)	61.1 $\pm$ 11.2	58.6 $\pm$ 35.8
V <sub>SS</sub> (L · kg <sup>-1</sup> )	0.71 $\pm$ 0.12	0.65 $\pm$ 0.12
CL <sub>S</sub> (mL · min <sup>-1</sup> )	310 $\pm$ 56	265 $\pm$ 54*
CL <sub>S</sub> (mL · min <sup>-1</sup> · kg <sup>-1</sup> )	3.65 $\pm$ 0.78	3.05 $\pm$ 0.61†
E <sub>H</sub> (%)	32 $\pm$ 7	27 $\pm$ 5†
Intravenous t <sub>1/2</sub> (h)	2.9 $\pm$ 0.8	3.2 $\pm$ 1.2
CL <sub>S</sub> /F (mL · min <sup>-1</sup> )	1143 $\pm$ 413	1079 $\pm$ 391
CL <sub>S</sub> /F (mL · min <sup>-1</sup> · kg <sup>-1</sup> )	13.6 $\pm$ 5.4	12.5 $\pm$ 4.7
F <sub>obs</sub> (% dose)	30 $\pm$ 12	28 $\pm$ 14
E <sub>GI</sub> (%)	56 $\pm$ 17	60 $\pm$ 20
AUC of intravenous 1'-hydroxymidazolam (ng · mL <sup>-1</sup> · h)	36.1 $\pm$ 7.7	37.8 $\pm$ 9.0
AUC of oral 1'-hydroxymidazolam (ng · mL <sup>-1</sup> · h)	53.9 $\pm$ 15.2	55.0 $\pm$ 16.2

V<sub>SS</sub>, Steady-state volume of distribution; CL<sub>S</sub>, systemic clearance; E<sub>H</sub>, estimate of CYP3A-mediated extraction ratio in the liver; t<sub>1/2</sub>, elimination half-life; CL<sub>S</sub>/F, oral clearance; F<sub>obs</sub>, oral bioavailability; E<sub>GI</sub>, estimate of CYP3A-mediated extraction ratio in the intestinal tract; AUC, total area under the plasma level–time curve.

\*P = .04 by Wilcoxon two-sample test obtained from *t* test of means.

†P = .03 by Wilcoxon two-sample test obtained from *t* test of means.



**Fig 2.** Estimated intestinal ( $E_{GI}$ ) and hepatic ( $E_H$ ) extraction ratios of midazolam in European American men (hatched bars) and African American men (solid bars). The individual *CYP3A4\*1B* genotypes of the African Americans are indicated on the left-hand side; (A/A, homozygous wild-type; A/G, heterozygotes; G/G, homozygous mutant). All of the European Americans were wild-type homozygotes.

analyses were performed with the SAS program (SAS Institute, Cary, NC).

## RESULTS

Plasma concentrations of midazolam declined rapidly after intravenous administration and in a mono-exponential fashion at times beyond 15 minutes after injection. Modest interindividual variability was present (Fig 1), but there did not appear to be any obvious difference between the plasma level–time profiles in African Americans and European Americans. However, comparison of the pharmacokinetic parameters in the two groups indicated differences (Table I), in particular the mean systemic clearance in African Americans was about 15% lower ( $P = .03$  to  $P = .04$ ) than that in European Americans (Table I).

Interindividual variability was greater after oral drug administration (Fig 1). In general, the absorption of midazolam was relatively rapid with the peak plasma level being attained within 1 to 1½ hours, although slower absorption was observed in a few individuals in both study groups. No statistically significant differ-

ences were present in the mean pharmacokinetic parameters in the two populations after oral administration (Table I). A similar situation was present with respect to the area under the curve for 1'-hydroxymidazolam, regardless of the route of administration (Table I).

Further pharmacokinetic analysis on the basis of equations 1 and 2 indicated considerable interindividual variability in the estimated  $E_H$  value and, to a greater degree, in  $E_{GI}$ . For example, a greater than threefold range of estimated  $E_H$  values was present, whereas estimated  $E_{GI}$  values ranged from less than 5% to 80% (Fig 2). Not unexpectedly, given the lower systemic clearance value in African Americans compared with European Americans,  $E_H$  was smaller ( $P < .03$ ) in the former group (Table I); however, there was considerable overlap between the two groups (Fig 2). Overlap was also present with respect to  $E_{GI}$ , but no population difference was present in this parameter (Table I). The overall mean  $E_{GI}$  value for the combined groups ( $58\% \pm 18\%$ ) was about twofold greater than the  $E_H$  estimate ( $30\% \pm 7\%$ ). Because  $F_{obs} = (1 - E_{GI})(1 - E_H)$ , it was possible to assess the relative importance of CYP3A-mediated

**Table II.** Relation between *CYP3A4\*1B* (A-290G) genotype and the pharmacokinetic parameters (mean  $\pm$  SD) of midazolam

Parameters	Genotype			P Values <sup>†</sup>	P Values <sup>‡</sup>
	A/A (n = 16)	A/G (n = 4)	G/G (n = 10)		
Systemic clearance					
CL <sub>S</sub> (mL · min <sup>-1</sup> )	310 $\pm$ 54	288 $\pm$ 60	252 $\pm$ 53	.02	.01
CL <sub>S</sub> (mL · min <sup>-1</sup> · kg <sup>-1</sup> )	3.6 $\pm$ 0.75	3.1 $\pm$ 0.84	3.0 $\pm$ 0.73	.05	.02
Oral clearance					
CL <sub>S</sub> /F <sub>obs</sub> (mL · min <sup>-1</sup> )	1111 $\pm$ 420	1084 $\pm$ 576	122 $\pm$ 321	.85	.96
CL <sub>S</sub> /F <sub>obs</sub> (mL · min <sup>-1</sup> · kg <sup>-1</sup> )	13 $\pm$ 5.4	12 $\pm$ 6.4	13 $\pm$ 4.1	.77	.99

<sup>†</sup>From Wilcoxon two-sample test comparing G/G versus A/A.

<sup>‡</sup>From test of trend across genotypes, assuming a log-additive model.

metabolism at the two anatomical sites to the overall oral bioavailability of midazolam. No relationship was observed between F<sub>obs</sub> and hepatic bioavailability (1 - E<sub>H</sub>), whereas a highly statistically significant ( $P < .0001$ ) linear relationship (Fig 3) was present between F<sub>obs</sub> and intestinal bioavailability (1 - E<sub>GI</sub>).

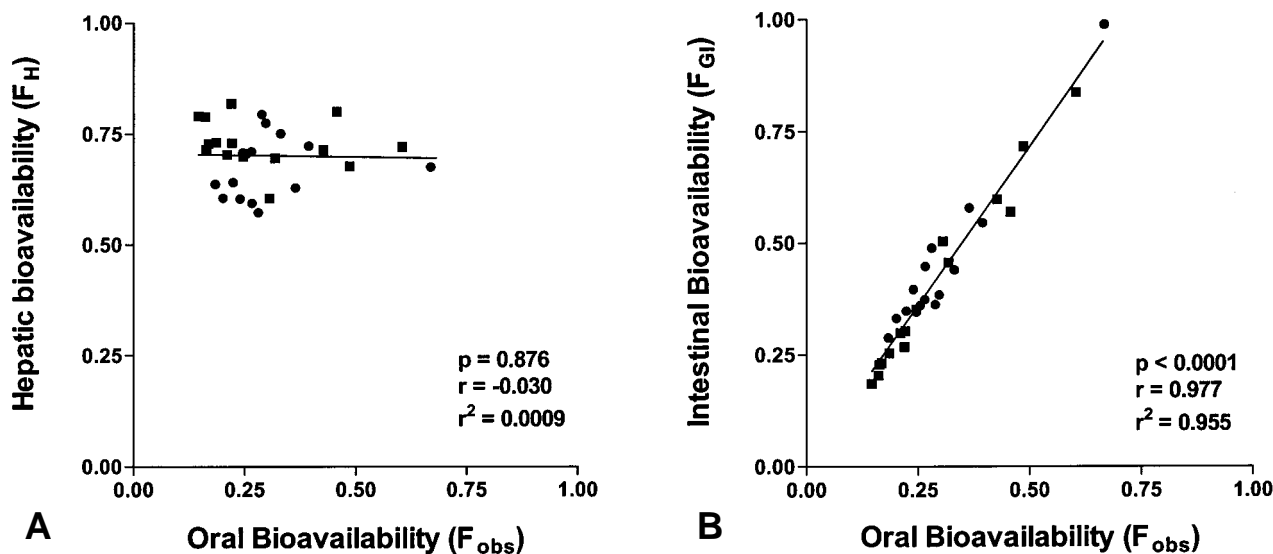
All of the European Americans and one African American were homozygous for the wild-type genotype (A/A), four African Americans were heterozygous (A/G), and the remaining 10 African American subjects were homozygous mutants (G/G). The systemic clearance of midazolam was about 30% lower ( $P = .05$ ) in the homozygous mutant group compared with individuals who were homozygous for the wild-type allele (Table II); moreover, a gene-dose effect was apparent ( $P = .02$  for trend). By contrast, no such genotype-phenotype relationship was apparent with respect to the oral clearance of midazolam.

## DISCUSSION

Midazolam has been increasingly used as an in vivo probe of CYP3A activity<sup>3,14,15</sup> because its metabolism is essentially complete and is totally mediated by CYP3A<sup>12,13</sup>; furthermore, the drug may be safely given by both the intravenous and oral routes.<sup>17,18</sup> In addition, midazolam is not a substrate for P-glycoprotein,<sup>19</sup> and therefore its disposition and elimination are not confounded by this factor. It also appears that the systemic clearance of midazolam after intravenous administration predominantly reflects hepatic CYP3A activity.<sup>14,15</sup> By contrast, after oral administration, absorption per se is complete, with bioavailability being determined by both hepatic extraction and extensive intestinal metabolism associated with CYP3A located in the enterocyte.<sup>15</sup> Recently, an approach has been developed to obtain separate estimates of these two independent processes.<sup>15</sup> The disposition characteris-

tics of midazolam and its 1'-hydroxy metabolite observed in the present study are, in general, similar to those reported previously in healthy white subjects of European ancestry.<sup>15,17,18</sup> However, oral administration of the drug formulated as a hard capsule, not unexpectedly, reduced the rate of absorption compared with administration as a solution. On the other hand, the extent of bioavailability (F<sub>obs</sub>) was not different from that observed in other studies.<sup>15</sup>

Estimated values of E<sub>GI</sub> and E<sub>H</sub> were also similar to those reported previously, both with respect to their values and interindividual variability.<sup>15</sup> Thus, presystemic metabolism by intestinal CYP3A contributed to the low oral bioavailability of midazolam to an equal or greater extent than hepatic metabolism; interindividual variability in intestinal CYP3A activity was greater than that in the liver, and in one individual CYP3A-mediated metabolism by the intestinal epithelium was essentially absent; and CYP3A activity at the two anatomical sites did not appear to be coordinately regulated. Of particular interest was the observed relationship between the sevenfold interindividual variability in the overall oral bioavailability (F<sub>obs</sub>) of midazolam and the individual bioavailabilities of the intestine (F<sub>GI</sub>) and liver (F<sub>H</sub>) resulting from CYP3A-mediated metabolism. Interestingly, no relationship was observable between oral bioavailability and the extent of first-pass hepatic metabolism. By contrast, an extremely strong linear relationship ( $r^2 = 0.977$ ) was present between overall oral bioavailability and intestinal bioavailability. A similar finding has been observed recently in studies of the interaction of clarithromycin with midazolam.<sup>20</sup> These data indicate that the oral bioavailability of midazolam is almost entirely determined by CYP3A activity in the intestinal epithelium. This presumably occurs because the bioavailability of this organ is equal to or less than that of the liver and because its



**Fig 3.** Relationships between the overall oral bioavailability of midazolam ( $F_{obs}$ ) and hepatic bioavailability of midazolam ( $F_H = 1 - E_H$ ; **A**) and the estimated intestinal bioavailability of midazolam ( $F_{GI} = 1 - E_{GI}$ ; **B**) in European American men (*circles*) and African American men (*squares*).

variability is greater. Whether this same situation applies to other drugs that are CYP3A substrates is not currently known and presumably would depend, in a drug-specific fashion, on the absolute and relative extraction ratios and bioavailabilities of the intestinal epithelium and liver, as well as their variability. The importance of P-glycoprotein as an efflux transporter in the enterocyte would also likely be a factor. This consideration does not apply to midazolam because it is not transported by P-glycoprotein<sup>19</sup> but, for substrates that are both CYP3A and P-glycoprotein substrates, the relationship between oral bioavailability and intestinal bioavailability would presumably be confounded by the transport process.

The issue of whether CYP3A activity differs between populations of different racial backgrounds is important because of the key role of this isoform in the metabolism of a large number of widely used drugs.<sup>2</sup> The most direct evidence in support of this possibility has been obtained with nifedipine, whose extensive and primary metabolism is mediated by CYP3A. For example, after an oral dose, plasma AUC values have been reported to be substantially and significantly higher in Japanese, Mexicans, Southeast Asians from the Indian subcontinent, and Nigerians compared with white populations.<sup>4-9</sup> In the case of Mexicans and Southeast Asians, it has been suggested that the interracial differences are a result of a lower systemic clearance of nifedipine in these populations that reduces first-pass

metabolism and enhances oral bioavailability.<sup>8,21</sup> On the other hand, trough plasma levels of nifedipine after long-term administration of a controlled-release formulation were similar in white British patients compared with those with West Indian ancestry.<sup>22</sup>

Neither midazolam nor nifedipine is a substrate of P-glycoprotein<sup>19</sup>; therefore their clearances presumably reflect only CYP3A-mediated metabolism. Accordingly, the absence of any significant differences in the oral disposition of midazolam between African American and European American populations compared with the findings of twofold higher nifedipine levels after oral drug administration in a group of Nigerians relative to white British patients<sup>9</sup> is somewhat unexpected. One explanation is the possibility that racial differences exist between the two African-derived populations that affect drug metabolism; for example, a substantial admixture of white genes into the African gene pool in African Americans.<sup>23</sup> Other possible factors include diet and additional environmental differences. An alternative possibility is that different CYP3A substrates interact in different ways with the active site of the enzyme and that such microsubstrate specificity differs among various populations. The difficulty in obtaining quantitative correlations between the clearances of various CYP3A substrates is consistent with such a possibility, although it is now clear that the *in vivo* probes used in such studies were not ideal.<sup>24,25</sup> However, the recent *in vitro* findings of two different

CYP3A substrate subgroups with respect to catalytic activity and its modulation, using a recombinant CYP3A4 expression system,<sup>26</sup> provides some support for this possibility. Clearly, further studies in different populations using a number of well-characterized CYP3A *in vivo* probes is warranted.

Interestingly, the oral bioavailabilities of cyclosporine (INN, ciclosporin) and tacrolimus have been reported to be lower in African American transplant recipients compared with other transplant patients.<sup>27,28</sup> Both of these drugs are extensively metabolized by CYP3A and are also excellent substrates for P-glycoprotein.<sup>29</sup> Because the mean activity of CYP3A in and of itself appears to be either not significantly different between African American and European American populations (as measured by the metabolism of midazolam), or even impaired if the nifedipine data in Nigerians can be extrapolated to African Americans, the observations with these orally administered immunosuppressant agents raise the possibility that population differences may exist in the functional level of P-glycoprotein activity in the intestinal epithelium. For drugs whose overall oral bioavailability is determined by this transporter rather than first-pass metabolism, population differences in oral bioavailability may therefore be much different than if intestinal or hepatic CYP3A activities alone are the critical factors.

In contrast to other CYP isoforms (eg, CYP2C19 and CYP2D6), the activity of CYP3A, although highly variable, appears to be unimodally distributed. No evidence of any common functionally important allelic variability in the *CYP3A4* or *CYP3A5* coding regions has been found, suggesting that the large interindividual variability in activity may be the result of transcriptional regulation.<sup>2</sup> In support of this notion, a variant in the so-called nifedipine-specific element located in the 5'-promoter region (-287 to 296 base pairs from the start codon) of *CYP3A4* (*CYP3A4\*1B*) has recently been described, and its possible role in prostate cancer<sup>10,11</sup> and the development of cancer treatment-related leukemias<sup>30</sup> has been discussed. The frequency of this A-290G transition polymorphism varies considerably between different racial populations; for example, the mutant allele frequency is present in only about 4% to 9% in European Americans but it is the predominant (55%) allele in African Americans.<sup>11,16,31</sup> Genotyping of the present study populations confirm this frequency difference in that all of the European American subjects had the homozygous A/A genotype, whereas the majority of African Americans were G/G homozygotes, with a small number of heterozygotes (A/G) and one A/A individual.

It has been speculated that the *CYP3A\*1B* allele is associated with reduced activity, presumably because

of an altered level of expression.<sup>10,11</sup> However, measurement of CYP3A4 activity based on microsomal nifedipine oxidation by 15 human liver samples (10 A/A, four A/G, and one G/G) provided no evidence of a relationship between genotype and phenotype,<sup>32</sup> although it was recognized that the sample size was small and interindividual variability was quite high. A similar conclusion was also reached on the basis of the results of gel mobility shift experiments with use of human liver nuclear extracts and oligonucleotides that corresponded to the wild-type and variant forms of the nifedipine-specific element region of CYP3A4.<sup>33</sup> By contrast, the present findings show a functional consequence of the A-290G promoter region mutation; namely, that the *CYP3A4\*1B* allele is associated with a reduced CYP3A4 catalytic *in vivo*. However, the genotype-phenotype relationship is only present with respect to CYP3A4 localized in the liver whose catalytic activity determines the systemic clearance of midazolam. No such association was observed with respect to the oral clearance of midazolam, suggesting that this particular genetic determinant of CYP3A4 expression is overridden in the developing enterocyte by other local factors, perhaps dietary in nature. In addition, the functional effect of the mutation is not particularly large—a mean 30% difference between the homozygous wild-type and mutant genotypes. This probably accounts for the difficulty in observing the effect of the mutation in studies with human liver microsomes when interindividual variability in wild-type homozygote samples was large.<sup>31</sup>

A previous investigation of the *in vivo* functional consequences of the *CYP3A4\*1B* allele did not find any genotype-phenotype differences.<sup>16</sup> In that study, the erythromycin breath test was used as the *in vivo* probe for hepatic CYP3A4 activity. However, as noted in recent reports,<sup>24,34</sup> this test is likely to be confounded by the fact that erythromycin is a substrate for hepatic P-glycoprotein and for CYP3A4 and therefore reflects factors other than CYP3A4-mediated metabolism. Moreover, it is possible that the erythromycin breath test is not as sensitive as midazolam to modulation of CYP3A4 activity. On the other hand, given the present finding that the activity of intestinal CYP3A4 is not affected by the *CYP3A4\*1B* allele, it is not unexpected that the oral clearance of nifedipine which, like midazolam, is not transported by P-glycoprotein but is a specific CYP3A4 substrate,<sup>19</sup> was found to be similar in individuals with different *CYP3A4\*1B* genotypes.<sup>16</sup>

Despite the fact that a clear genotype-phenotype relationship was observed for hepatic CYP3A4 activity, it should be noted that this was a post hoc analysis and, as

a result, the subpopulations were not balanced. Another possible confounding effect is that the two homozygous groups essentially represented different racial populations. Significantly, based on the *CYP3A4\*1B* polymorphism, there was considerable overlap and variability in hepatic CYP3A4 activity between the various genotypes. This observation and the low frequency of the A-290G mutation in European Americans, in whom large interindividual variability was found, indicate that additional (genetic) determinants other than *CYP3A4\*1B* are involved in the regulation of the basal activity of the isoform.

In summary, this study confirms the interindividual variability in CYP3A activity after both oral and intravenous administration. It also highlights the important and determining role that CYP3A-mediated metabolism has in the oral first-pass effect of midazolam. In addition, the study results show a modest population difference in CYP3A activity between African Americans and European Americans that is associated with different frequencies of the *CYP3A4\*1B* allele in the two groups. However, this is limited to hepatic CYP3A, and the intergenotypic difference is again modest and of little clinical importance. In contrast, no significant population differences were noted in the disposition of midazolam after oral administration, suggesting that similar oral dosages of midazolam and possibly other CYP3A substrates do not need to be adjusted on the basis of racial ancestry or *CYP3A\*1B* genotype. Finally, the findings of this study indicate that an important criterion for future studies of population differences should take into account differences in the frequency of allelic variants that affect the activity level of CYP3A rather than racial background alone.

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