CYP3A5 genotype predicts renal CYP3A activity and blood pressure in healthy adults

Raymond C. Givens, Yvonne S. Lin, Amy L. S. Dowling, Kenneth E. Thummel, Jatinder K. Lamba, Erin G. Schuetz, Paul W. Stewart, and Paul B. Watkins. CYP3A5 genotype predicts renal CYP3A activity and blood pressure in healthy adults. J Appl Physiol 95: 1297–1300, 2003. First published May 16, 2003; 10.1152/japplphysiol.00322.2003.—A single-nucleotide polymorphism (A6986G) in the cytochrome P-450 3A5 (CYP3A5) gene distinguishes an expressor (*1) and a reduced-expressor (*3) allele and largely predicts CYP3A5 content in liver and intestine. CYP3A5 is the prevailing CYP3A isofrom in kidney. We report that, among renal microsomes from 21 organ donors, those from *1/*3 individuals had at least eightfold higher mean kidney microsomal CYP3A5 content and 18-fold higher mean CYP3A catalytic activity than did those from *3/*3 individuals (P = 0.0001 and P = 0.0137, respectively). We also report significant associations between the A6986G polymorphism and systolic blood pressure (P = 0.0007), mean arterial pressure (P = 0.0075), and creatinine clearance (P = 0.0035) among 25 healthy African-American adults. These associations remained significant when sex, age, and body mass index were taken into account. The mean systolic blood pressure of homozygous CYP3A5 expressors (*1/*1) exceeded that of homozygous nonexpressors (*3/*3) by 19.3 mmHg. We speculate that a high CYP3A5 expressor allele frequency among African-Americans may contribute to a high prevalence of sodium-sensitive hypertension in this population. CYP3A5 activity similarly predicts renal CYP3A5 expression. We type similarly predicts renal CYP3A5 expression. We also speculate that a high CYP3A5 expressor allele frequency among African-Americans may contribute to a high prevalence of sodium-sensitive hypertension in this population.

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METHODS

We assayed CYP3A catalytic activity and CYP3A5 immunoactivity in renal tissue samples from 21 organ donors: 17 were Caucasian, 1 was Hispanic, and 3 were of unknown ethnicity. All methods were described previously (10). CYP3A5 genotypes were determined by direct genomic DNA sequencing. Microsomes were isolated by differential centrifugation. CYP3A5 content in 50 μg H9262/H9262 g of microsomes was measured by Western blot with a CYP3A5-specific antibody (BD Gentest, Woburn, MA); undetectable bands were assigned a value of 0.0001. Methods were described previously (10). CYP3A5 genotypes were determined by direct genomic DNA sequencing. Microsomes were isolated by differential centrifugation. CYP3A5 content in 50 μg H9262/H9262 g of microsomes was measured by Western blot with a CYP3A5-specific antibody (BD Gentest, Woburn, MA); undetectable bands were assigned a value of 0.0001.

Table 1. Blood pressure and creatinine clearance by CYP3A5 genotype group

<table>
<thead>
<tr>
<th></th>
<th>*1/*1</th>
<th>*1/*3</th>
<th>*3/*3</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>24.5±10.7</td>
<td>25.0±8.0</td>
<td>25.0±8.5</td>
<td>0.9933</td>
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<tr>
<td>BMI, kg/m²</td>
<td>30.3±8.1</td>
<td>33.9±4.6</td>
<td>26.2±2.8</td>
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<tr>
<td>SBP, mmHg</td>
<td>136.0±7.9</td>
<td>121.6±8.4</td>
<td>116.7±11.0</td>
<td>0.0007</td>
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<tr>
<td>DBP, mmHg</td>
<td>71.4±5.1</td>
<td>68.1±10.1</td>
<td>63.3±6.5</td>
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<tr>
<td>MAP, mmHg</td>
<td>92.9±3.8</td>
<td>85.9±9.0</td>
<td>81.6±6.5</td>
<td>0.0075</td>
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<tr>
<td>PP, mmHg</td>
<td>64.6±10.3</td>
<td>53.4±7.0</td>
<td>53.4±10.6</td>
<td>0.0309</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>78.9±15.3</td>
<td>73.9±9.0</td>
<td>70.6±8.3</td>
<td>0.3633</td>
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<tr>
<td>SBP-HR, mmHg/min</td>
<td>10,743.1±2,253.3</td>
<td>8,948.6±1,006.2</td>
<td>8,189.6±810.8</td>
<td>0.0088</td>
</tr>
<tr>
<td>JNC-VI stratum (N/H)</td>
<td>27/2</td>
<td>27/2</td>
<td>27/2</td>
<td></td>
</tr>
<tr>
<td>CrCl, ml/min</td>
<td>147.2±30.9</td>
<td>106.9±23.7</td>
<td>110.4±10.5</td>
<td>0.0035</td>
</tr>
</tbody>
</table>

Females

|                  |       |       |       |       |
| n                | 4     | 6     | 5     |       |
| Age, yr          | 20.5±2.8 | 20.8±2.6 | 25.8±9.6 | 0.3426 |
| SBP, mmHg        | 130.8±6.2 | 117.5±3.9 | 112.2±9.1 | 0.0039 |
| MAP, mmHg        | 91.9±3.8 | 82.5±2.9 | 80.2±7.6 | 0.0127 |
| CrCl, ml/min     | 156.9±16.6 | 114.7±24.3 | 111.0±10.4 | 0.0059 |

Males

|                  |       |       |       |       |
| n                | 5     | 3     | 2     |       |
| Age, yr          | 27.6±14.0 | 33.3±9.1 | 23.0±7.1 | 0.6439 |
| SBP, mmHg        | 140.2±6.8 | 129.7±9.8 | 128.0±5.7 | 0.1355 |
| CrCl, ml/min     | 139.5±39.2 | 91.4±15.4 | 109.0±14.9 | 0.1661 |

Values are means ± SD; n, no. of subjects. CYP3A5, cytochrome P-450 3A5; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; CrCl, creatinine clearance; JNC-VI, combined BP stratum from the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; N, optimal or normal (SBP <130 and DBP <85 mmHg); H, high-normal or higher (SBP ≥130 or DBP ≥85 mmHg).
CYP3A5 GENOTYPE, CYP3A ACTIVITY, AND BP

0.25 pmol/mg, the limit of quantitation (LOQ; Fig. 1A). Midazolam (MDZ) 1'-hydroxylized was measured after a 15-min incubation of 200 µg protein with 8 µM MDZ; microsomes with undetectable activity were given the LOQ value of 0.04 pmol·min⁻¹·mg⁻¹.

We genotyped 89 unrelated volunteers for the CYP3A5 A6986G single-nucleotide polymorphism using previously described methods (9); all subjects were self-identified African-Americans. Among the subset of this group consisting of 25 healthy individuals (age range: 18–52 yr) who volunteered to be screened for a pharmacogenetic study, we tested the ancillary hypothesis of CYP3A5 genotype association with BP and CrCl. Nurses blind to subject genotypes and medical histories performed and recorded all clinical measurements. Studies were approved by the University of North Carolina Committee on the Protection of Human Research Subjects.

RESULTS

The average CYP3A5 content (1.98 pmol/mg, n = 5) of microsomes from *1/*3 kidneys exceeded the average of those from *3/*3 kidneys (n = 16, Wilcoxon P = 0.0001), which was at or below the LOQ (Fig. 1A). None of the kidneys was *1/*1. The closely related CYP3A4 was not detected with a CYP3A4-specific antibody in any of the microsomal preparations. Mean micromolar CYP3A activity, reflected by MDZ 1'-hydroxylation, was 18-fold higher in *1/*3 kidney microsomes (8.04 pmol·min⁻¹·mg⁻¹) than in those from *3/*3 kidneys (0.43 pmol·min⁻¹·mg⁻¹, Wilcoxon P = 0.0137; Fig. 1B). Analysis of DNA from the *1/*3 outlier, indicated by arrows in Fig. 1, revealed an inactivating frameshift mutation (27131/27132insT) that produces an inactivating allele known as *7 (6). Removal of this outlier slightly lowered P values.

CYP3A5*1 allele frequency among 89 African-Americans was 0.7. Among the 25-individual subset (Table 1), CYP3A5 genotype associated with seated systolic BP (SBP), mean arterial pressure, the product of SBP and heart rate (HR) (SBP × HR, an indicator of left-ventricular oxygen consumption), and Cockcroft-Gault CrCl. The *1/*1 group averaged the highest value for each measure. Average *1/*1 SBP exceeded that of the *3/*3 group by 19.3 mmHg (Fig. 2), and a gene-dose effect was apparent. CYP3A5 genotype associated significantly with combined BP strata (P = 0.0048; Table 1) from the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High BP (N = optimal or normal [SBP <130 and diastolic BP (DBP) <85 mmHg]; H = high-normal or higher [SBP ≥130 or DBP ≥85 mmHg]) (7).

Sex-specific analysis detected CYP3A5 genotype associations with SBP, mean arterial pressure, and estimated CrCl among females (Table 1); similar trends were noted among the smaller sample of males, but these did not reach statistical significance. In multiple regression analyses, sex and genotype accounted for 70% of the variability in SBP. Compared with non-*1/*1 females, *1/*1 males showed a >20-fold increased risk of high normal or higher BP (P = 0.0002).

Subject age, averaging ∼25 yr, did not vary between genotype groups. Age and body mass index effects on BP were nonsignificant. Genotype association with body mass index, DBP, pulse pressure, and HR did not reach statistical significance.

DISCUSSION

CYP3A5*1 allele associates with CYP3A5 expression in human kidney, as previously reported for liver and intestine. However, in the liver and intestine, there is also substantial CYP3A4 expression (9, 10). As a result, hepatic and intestinal aggregate CYP3A activities associate only weakly with the CYP3A5*1 allele. In contrast, CYP3A4 was not detected in renal microsomes, and mean CYP3A activity differed markedly between CYP3A5 genotype groups.

Our preliminary finding of CYP3A5 genotype association with resting BP among healthy adults may be consistent with a role for CYP3A enzymes in BP control. Young adults with supernormal BP have an increased long-term risk of death due to cardiovascular and coronary heart disease (12); the identification of common genetic polymorphisms relevant to BP control is thus an important line of investigation. We are presently testing our hypothesis of a CYP3A5 genotype-BP correlation in a larger sample of adults. If shown true, our conjecture might portend a role for CYP3A5 inhibition in the treatment of some forms of hypertension.

The CYP3A5*1 allele frequency among our cohort of African-Americans agrees roughly with a previous report (6). This frequency exceeds those among all other ethnic populations studied to date (6, 9). Interethnic differences in the prevalence of sodium sensitivity (17) parallel those of hypertension (2), with African-Americans having the highest global prevalence of each. A possible link between CYP3A5 activity and the high prevalence of sodium-sensitive hypertension among African-Americans may merit further study.

DISCLOSURES

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REFERENCES


