Association Between the CYP3A5 Genotype and Blood Pressure

Herbert Ho, Amar Pinto, Stephen D. Hall, David A. Flockhart, Lang Li, Todd C. Skaar, Peter Cadman, Daniel T. O’Connor, Urban Wagner, Naomi S. Fineberg, Myron H. Weinberger

Abstract—We tested the hypothesis that the presence of a CYP3A5*1 allele is associated with increases in blood pressure in 2 studies of subjects with a total of 683 participants. The first study involving 271 subjects was part of a longitudinal study conducted at Indiana University Medical Center that consisted of 2 phases. The first phase studied the relationship of salt sensitivity with blood pressure, whereas the second phase, conducted ~26 years later, studied the relationship between blood pressure, carbohydrate intolerance, and vascular compliance in the same subjects. The second study was a cross-sectional evaluation of 412 normotensive and hypertensive subjects conducted at the University of California San Diego. The second study (Mantel–Haenszel χ² test; P=0.05) showed that a greater proportion of black participants with poor blood pressure control had CYP3A5*1/*1 genotype. Evaluation of the untreated blood pressure from phase 1 of the first study showed that the blacks with CYP3A5*3/*3 (146±35 mm Hg) had a higher systolic blood pressure than those with the *1/*3 (119±14.1 mm Hg; P=0.0006) and *1/*1 (125±17.4 mm Hg; P=0.009) genotypes. For blacks in study 2, the CYP3A5*1 allele was more common in hypertensives (Fisher exact test; P=0.025) than normotensives. In whites there was no association between CYP3A5 genotype and blood pressure in either study. We conclude that although untreated blood pressure may be higher in blacks with the CYP3A5*3/*3 genotype, the CYP3A5*1 allele may be associated with hypertension that is more refractory to treatment in this ethnic group.

Key Words: cytochrome p450 ■ blood pressure ■ genetics ■ polymorphism

Heart disease has been the most common cause of death in the United States since 1950.1–4 Hypertension and diabetes mellitus are major risk factors for coronary heart disease.5,6

A large number of candidate genes have been associated with hypertension.7 Some genes, such as the angiotensin-converting enzyme gene, have been associated with cardiovascular disease but not hypertension, whereas other genes, such as the β2-adrenergic receptor gene, have been implicated in the regulation of arterial pressure. Recently, the polymorphically expressed CYP3A5 has been associated with blood pressure (BP) in humans. In contrast to the functional CYP3A5*1 allele, the CYP3A5*3 variant has a mutation in intron 3 that leads to the production of an aberrant mRNA and ultimately a truncated protein.8,9 Givens et al10 reported that in a group of 25 blacks, the CYP3A5*1/*1 genotype exhibited higher systolic BP (SBP) and mean arterial BP and creatinine clearance when compared with the *1/*3 and *3/*3 genotypes.

The CYP3A enzymes are steroid 6β-hydroxylases that convert cortisol to 6β-hydroxycortisone11 and corticosterone to 6β-hydroxycorticosterone.12–14 CYP3A4 and CYP3A5 are abundantly expressed in the liver and small intestine, but in the kidney, CYP3A5 is predominant. Thus, the genetic polymorphism in CYP3A5 may manifest itself through an effect on endogenous cortisol metabolism in the kidney15 that may ultimately affect BP most probably through sodium and water retention. Our preliminary data indicate that the kidney is capable of cortisol 6β-hydroxylation but only in individuals who express CYP3A5.15,16 In animal12–14 and in vitro17–19 experiments, expression of CYP3A enzymes has been shown to correlate with sodium reabsorption17–19 and BP.12–14 However, the effects of 6β-hydroxycortisol or 6β-hydroxycorticosterone on sodium handling or BP in humans have not been examined.

It has been hypothesized that because CYP3A5 is present in the kidney, it is possible that individuals with ≥1 functional alleles may demonstrate increased BP, perhaps as a result of sodium and water retention. We evaluated the relationship of the CYP3A5 genotype with BP in 2 diverse and well-characterized populations of normal and hypertensive humans.

Methods

Subjects

We obtained DNA from participants in 2 studies completed previously. The first study was conducted in central Indiana and the...
subjects have been described in detail previously.\textsuperscript{20–23} This cohort consisted of subjects referred to the Hypertension Research Center of the Indiana University Medical Center for evaluation of hypertension and those recruited through advertisements. Phase 1 of the study was conducted in the early 1970s\textsuperscript{20} and phase 2\textsuperscript{21,22} an average of 26 years later. Only those who completed both phases were included in this analysis. Eighty three of 191 whites were determined to be salt sensitive, whereas 42 of 71 blacks were salt sensitive.

The assessment of BPs during the salt sensitivity determination in phase 1 required the withdrawal of antihypertensive medications for \(\geq 2\) weeks,\textsuperscript{20,23} whereas during phase 2 of the study, subjects continued to take their respective medications. Subjects were classified as normotensive if BP readings were \(<140/90\) mm Hg during both studies and not receiving antihypertensive medications.\textsuperscript{20} Homeostasis Model Assessment (HOMA) was computed as the product of fasting plasma glucose (mmol/L) and insulin (\(\mu U/mL\)) concentrations divided by 22.5.\textsuperscript{24}

The second study was conducted in greater San Diego, Calif. Hypertensive subjects were recruited from the Hypertension Specialty Clinic and General Medical Clinic of the University of California at San Diego and the Veterans Affairs San Diego health care system. Normotensive subjects came from the hospital, university, and the community. The drug regimen for each hypertensive subject was also recorded. Both studies were approved by the institutional review boards of the respective institutions. Informed consent was obtained for all subjects.

Genotyping
CYP3A5*3 genotyping was performed by allele-specific real-time polymerase chain reaction using the method of Hiratsuka et al.\textsuperscript{25,26} with slight modifications.\textsuperscript{27}

Data Analysis
The 2 studies were analyzed separately. Whites and blacks were also analyzed separately. A \(\chi^2\) test was used to determine whether the distribution of genotypes was consistent with the Hardy–Weinberg equilibrium. Wilcoxon rank sum tests were used to compare the effects of the different genotypes (*1/*1, *1/*3, *3/*3) on the measured end points (vide supra) that have continuous scales and \(\chi^2\) test for end points with discrete scale. We considered a \(P\leq0.05\) to be statistically significant.

When subjects were not taking their antihypertensive medications (phase 1 of study 1), we compared the measured BP according to their CYP3A5 genotype. When they were taking their antihypertensive medications (study 2 and phase 2 of study 1), comparing measured BP would be inappropriate, and we adopted 2 approaches. First, we used the number of antihypertensive medications as a gauge of the severity of hypertension and compared the number of subjects with good BP control (SBP <140 mm Hg and DBP <90 mm Hg) with the poorly controlled ones. However, this was not a clinical trial, so we did not have control over the medications. For subjects taking \(\geq 3\) antihypertensive medications, each drug was titrated up to either the maximum recommended dose or maximum tolerable side effects before the next drug was added.

In the second approach, we accounted for the effects of the antihypertensive medications using the method described by Cui et al.,\textsuperscript{28} in which “stepped increments of 8/4 mm Hg, 14/10 mm Hg, and 20/16 mm Hg were added to measured SBP/DBP of treated subjects taking 1, 2, and 3 drug classes, respectively.” We modified this approach by using the same increment of 20/16 mm Hg for \(\geq 4\) drug classes.

Results
Demographic characteristics of the subjects in the 2 studies are presented in Table 1. Genotypic distribution of subjects and a comparison with published literature are given in Table 2. For study 1, there were no whites who exhibited the CYP3A5*1/*1 genotype. Both studies satisfied the Hardy–Weinberg equilibrium, including the subgroups of white hypertensives, white normotensives, black hypertensives, and black normotensives. There were no significant differences in the distribution of CYP3A5 genotypes between hypertensive and normotensive subjects for either study. However, if the CYP3A5*1/*1 and CYP3A5*1/*3 genotypes in study 2 were combined for blacks, there was a significantly higher proportion of the *1/*1 or *1/*3 group (90%) in hypertensives when compared with normotensives (81%; Fisher exact test; \(P=0.025\)). Using the \(\chi^2\) test, we found that gender was not significantly correlated with the CYP3A5 genotype for blacks or whites.

We examined BP of the subjects in study 1, phase 1, after medications had been withheld for 2 weeks. Among blacks (Figure), those with *3/*3 had higher baseline SBP (\(P=0.023\) versus *1/*1; \(P=0.002\) versus *1/*3) and baseline diastolic BP (DBP; \(P<0.015\) versus *1/*3). The *3/*3 black group was also found to have a significantly higher SBP, DBP, and mean arterial BP after saline infusion and after receiving furosemide when compared with the other 2 genotypic groups (Figure). SBP and mean arterial BP for the *1/*3 individuals after furosemide administration were also significantly higher than those with the *1/*1 genotype. Although the *3/*3 group was \(\approx 7\) years older than the *1/*1 group and 8 years older than the *1/*2 group, this is not statistically significant. The conclusions regarding intergenotypic differences in BPs (baseline, after saline infusion, and after furosemide administration) remained after adjusting for age using the analysis of covariance. In whites, there were no differences in BP between those expressing the *1/*3 and *3/*3 genotypes (\(P>0.18\)).

In study 1, phase 1, there was a greater proportion of salt-sensitive subjects in the hypertensive group when compared with the normotensive group (0.60 versus 0.37 for whites and 0.80 versus 0.44 for blacks). The CYP3A5 genotype did not influence salt sensitivity status except for the white hypertensives where the *1/*3 group had a greater proportion of salt-sensitive subjects when compared with the *3/*3 group (\(\chi^2\) test \(P=0.01\) for white hypertensives; \(P=0.51\) for white normotensives; \(P=0.13\) for black normotensives; \(P=0.49\) for black hypertensives). Creatinine clearance and urine albumin excretion were not significantly different among genotypes for whites or blacks.

We also analyzed the BP measured while the subjects were on their respective antihypertensive medications. In study 2,
we grouped normotensive and hypertensive blacks with good control (SBP ≤ 140 mm Hg and DBP ≤ 90 mm Hg) with ≤ 2 antihypertensive medications and compared them with poorly controlled hypertensive blacks on ≥ 3 antihypertensive drugs. This comparison revealed that the poorly controlled group consisted of a higher proportion of the *1/*1 genotype than the subjects with good control (Table 3; Mantel–Haenszel $\chi^2$ test; $P<0.05$). No differences in genotype frequencies were noted between good and poor control for whites. For the second phase of study 1, there were no significant differences in genotype distribution between good and poor control subjects for blacks or whites.

For study 2 and phase 2 of study 1, the predicted BPs, obtained using the method described by Cui et al., were not significantly different between CYP3A5 genotypes in whites and blacks.

HOMA for whites in phase 1 of study 1 was significantly higher for the CYP3A5*1/*3 group (9.6±14.1) compared with the *3/*3 group (5.7±8.4; $P=0.05$). There were no differences in HOMA among the genotypes for blacks.

**Discussion**

CYPs are a superfamily of monoxygenases that are responsible for the oxidative metabolism of more than half the drugs currently available. In adults, the CYP3A subfamily consists of CYP3A4 that is abundantly expressed in the liver and small intestine of all individuals. CYP3A5 is also expressed abundantly in the liver and small intestine, but only in the 30% of whites and 70% of blacks who possess ≥ 1 CYP3A5*1 allele. CYP3A expression in the kidney is modest compared with the liver and is predominantly CYP3A5.15 There are several lines of evidence consistent with an association between CYP3A enzyme activity and BP or sodium retention. As early as 1975, it was shown that 6α-hydroxycortisol and 6β-hydroxycortisol were higher by an average of 48% in patients with essential hypertension compared with normotensive subjects.29 However, no causative association was established, and there was no distinction between 6α-hydroxycortisol and 6β-hydroxycortisol. More recently, Givens et al.10 reported that in a group of 25 blacks, those possessing the CYP3A5*1/*1 genotype exhibited higher SBP and mean arterial BP and creatinine clearance. A higher creatinine clearance could reflect volume expansion or glomerular hyperfiltration. Volume expansion could in turn trigger a cascade that eventually leads to hypertension.30 The results from study 1 showed that there was no relationship between CYP3A5 genotype and creatinine clearance for whites or blacks.

In view of the potential association between CYP3A5 expression and elevated BP, we sought evidence for such an association in a large group of subjects that had taken part previously in 2 well-documented studies. Part of our analysis

<table>
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<th>Study 2†</th>
<th>Published Literature9</th>
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<td></td>
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<td>*3/*3</td>
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<td>Total</td>
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<td>136 (100%)</td>
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Black

<table>
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<th>Study 2†</th>
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<tr>
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<tr>
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<td>15 (50.0%)</td>
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<td>2 (4.8%)</td>
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<tr>
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<td>42 (100%)</td>
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</table>

*Study 1 was done at the Indiana University and was described previously.20–23
†Study 2 was done at the University of California San Diego.
of these studies supported the association of the CYP3A5*1 allele with increased BP, in agreement with the findings of Givens et al.10 First, study 2 showed that in blacks, there was a significantly greater proportion of those with the CYP3A5*1 allele in the hypertensive group (90%) than in the normotensive group (81%; P=0.025). Additionally, we found that in study 2 (Table 3; P=0.05), the CYP3A5*1 allele is associated with poor BP control, although the size of these subgroup analyses was small. A significant part of our analyses also contradicted the association of the CYP3A5*1 allele with increased BP. Phase 1 of study 1 showed that baseline SBP and mean arterial BP of blacks were higher for the group with the CYP3A5*3/*3 genotype when compared with the other 2, and some relationship persisted even after salihe loading and furosemide administration (Figure). Although the age difference between the genotypic groups were not statistically significant, they may be clinically significant considering that the lowest mean age was 53 years (*1/*3 group).

In an attempt to adjust or correct for the effect of antihypertensive medications in study 2 and phase 2 of study 1, we used the method described by Cui et al. These analyses did not show any significant differences for both studies. One reason why these incremental adjustments in BP did not help is because our subjects were unrelated and different from those of Cui et al, whose subjects were related and came from 767 nuclear families. The fact that CYP3A metabolizes calcium channel blockers and not the other antihypertensive agents may also be a contributing factor. Of course it is possible that the CYP3A5 genotype is not related to BP. All the BP comparisons involving whites showed no association with the CYP3A5 genotype.

The seemingly different findings in studies 1 and 2 could be explained by the different demographic characteristics (Table 1). Study 1 had almost equal numbers of each gender but twice as many normotensives as hypertensives. Study 2 had approximately the same number of hypertensives and normotensives but 3x as many men as women.

It is interesting to note that for blacks in whom the CYP3A5 genotype showed a difference in BP, there was no difference in the proportions of salt-sensitive and salt-resistant subjects among the genotypes. In contrast, we found that there was a greater proportion of salt-sensitive subjects among the CYP3A5*1/*3 white hypertensives when compared with their *3/*3 counterparts.

As far as insulin sensitivity is concerned, although the euglycemic insulin clamp technique is still the gold standard in its assessment,24-33 HOMA correlates well with clamp-derived insulin sensitivity.24 In phase 1 of study 1, whites carrying the CYP3A5*1/*3 genotype had a significantly higher HOMA was (indicating a lower insulin sensitivity or greater insulin resistance) than those carrying the *3/*3 genotype, even after adjustment for age and body mass index. Because CYP3A5 metabolizes cortisol, and cortisol reduces insulin sensitivity, we would expect those expressing this enzyme (those carrying ≥1 CYP3A5*1 allele) to be more insulin sensitive. However, our results showed the opposite. We are not aware of any information that would explain this phenomenon. It is also difficult to explain why there was no difference for blacks.

One limitation in study 1 was that only those subjects who were able to complete a follow-up study (ie, they were still alive and still living within accessible distances to the university at the time of follow-up) and who consented to DNA analysis were included. Antihypertensive medications were also confounding variables (for both studies) because different subjects take different numbers and classes of these drugs. Finally, association studies were never intended to show causal relationships.

**Perspectives**

This study provided an alternative view of the effect of CYP3A5 genotype on BP. If this were later verified by additional research, we would have 1 more gene that could be predictive of hypertension, and the possibility that the CYP3A5 genotype has an effect on antihypertensive response could broaden our therapeutic options. The CYP3A5 genotype was also associated with insulin sensitivity as indicated by the HOMA. Because the genotype indicates the presence or absence of the CYP3A5 enzyme, it is possible that in the future we may be able to use CYP3A inducers and inhibitors to control BP and improve insulin sensitivity.

**Conclusion**

We conclude that although untreated BP may be higher in the CYP3A5*3/*3 blacks, the CYP3A5*1 allele may be associated with hypertension that is more refractory to treatment in this ethnic group. Also, the CYP3A5*1 allele seem to be associated with a salt-sensitive status in white hypertensives. This may be related to the inadequate conversion of cortisol to 6α-hydroxycortisol or through its interaction with a yet to be identified receptor. Whites carrying the CYP3A5*1/*3 genotype had a greater insulin resistance than those carrying the *3/*3 genotype through an unknown mechanism. We hope that future studies will be able to elucidate the mechanism(s).

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