CYP3A5 and ABCB1 Genes Influence Blood Pressure and Response to Treatment, and Their Effect Is Modified by Salt

Chin B. Eap, Murielle Bochud, Robert C. Elston, Pascal Bovet, Marc P. Maillard, Juerg Nussberger, Laurent Schild, Conrad Shamlaye, Michel Burnier

Abstract—The permeability–glycoprotein efflux-transporter encoded by the multidrug resistance 1 (ABCB1) gene and the cytochromes P450 3A4/5 encoded by the CYP3A4/5 genes are known to interact in the transport and metabolism of many drugs. Recent data have shown that the CYP3A5 genotypes influence blood pressure and that permeability–glycoprotein activity might influence the activity of the renin–angiotensin system. Hence, these 2 genes may contribute to blood pressure regulation in humans. We analyzed the association of variants of the ABCB1 and CYP3A5 genes with ambulatory blood pressure, plasma renin activity, plasma aldosterone, endogenous lithium clearance, and blood pressure response to treatment in 72 families (373 individuals; 55% women; mean age: 46 years) of East African descent. The ABCB1 and CYP3A5 genes interact with urinary sodium excretion in their effect on ambulatory blood pressure (daytime systolic: \( P = 0.05 \); nighttime systolic and diastolic: \( P < 0.01 \)), suggesting a gene–gene–environment interaction. The combined action of these genes is also associated with postproximal tubular sodium reabsorption, plasma renin activity, plasma aldosterone, and with an altered blood pressure response to the angiotensin-converting enzyme inhibitor lisinopril (\( P < 0.05 \)). This is the first reported association of the ABCB1 gene with blood pressure in humans and demonstration that genes encoding for proteins metabolizing and transporting drugs and endogenous substrates contribute to blood pressure regulation. (Hypertension. 2007;49:1-8.)

Key Words: blood pressure ■ genes ■ sodium ■ renin–angiotensin system ■ P glycoprotein

Cytchrome P450 3A (CYP3A) enzymes, which, in adults, are composed of CYP3A4 and CYP3A5, are involved in the metabolism of many drugs and endogenous substrates, such as steroids. CYP3A genes show organ-specific patterns of expression, and only CYP3A5 is expressed in the human kidney.1,2 Recent studies have found an association between the CYP3A5 gene and blood pressure (BP) in humans.3–5 It has been hypothesized that carriers of the CYP3A5*1 allele have an enhanced renal sodium reabsorption.3,6

The ATP-binding cassette, subfamily B, member 1 (ABCB1) or multidrug resistance 1 gene encodes the transmembrane permeability–glycoprotein (PGP), an efflux transporter expressed in the human kidney, (proximal tubules,7 mesangium, thick limbs of Henle’s loops, and collecting ducts8). In mice, PGP has been shown to transport aldosterone out of the brain9 and to play an important role in aldosterone plasma disposition.10 This may be of importance given that intracerebroventricular injection of aldosterone in Dahl salt-sensitive rats has been shown to increase BP.11 In Wistar rats, the increased BP resulting from intracerebroventricular injection of sodium is blocked by intracerebroventricular spironolactone injection.12 Taken together, these experimental findings in animals point toward a possible role of PGP in centrally mediated salt-sensitive hypertension.

Aldosterone may also be a physiological substrate of PGP in the human adrenal cortex.13 In addition, angiotensin II–stimulated aldosterone secretion is inhibited by PGP modulators in vitro.14 Studies in rats15 and humans16 suggest that the PGP inhibitor cyclosporine A influences the renin–angiotensin–aldosterone system. These experimental results suggest that PGP might play a role in the regulation of the renin–angiotensin–aldosterone system in humans. No study to date has, however, directly shown that the ABCB1 gene is associated with BP in humans.

PGP and CYP3A enzymes share many substrates in common,17 and their activity is regulated by the same nuclear receptors, that is, the constitutive androstane receptor and the pregnane X receptor.18–20 Although PGP and CYP3A enzymes have been evaluated for their roles in the transport and metabolism of drugs, little is known about their roles in human physiological processes.
In this report, based on the same families of East African descent as in the above-mentioned study, we have analyzed whether the 3435 C>T and 2677 G>T variants of the ABCB1 gene (either alone or in combination with the CYP3A5*1 allele) influence ambulatory BP and BP response to antihypertensive treatment, as well as plasma renin activity (PRA), plasma aldosterone, and endogenous lithium clearance.

Methods

The study took place in the Seychelles islands, which are populated predominantly by individuals of East African descent. Participants were recruited between August 1999 and January 2002. Families were selected from the ongoing hypertension register that includes all of the participants with hypertension who attend primary health care centers there. The selection of families has been described previously.21 Briefly, families were selected if they were of African descent and if we could examine ≥2 full siblings with hypertension and 2 other first-degree relatives irrespective of their hypertension status. Seventy-six of the 135 screened families were found to be eligible, among whom 373 individuals from 72 families had data available for this analysis. The protocol of the study was submitted and accepted by the local ethical committees in Switzerland, as well as in the Seychelles islands.

Menopausal status, use of contraceptive pill, smoking, and alcohol consumption were obtained by trained health professionals using a standardized questionnaire. We considered as a smoker any participant who reported smoking ≥1 cigarette per day during the past month. Alcohol consumption, in grams per day, was included as a continuous variable in the analyses.

Antihypertensive therapy, if any, was stopped for 2 weeks before conducting ambulatory BP monitoring and measuring PRA and plasma aldosterone. Ambulatory BP monitoring was measured using validated Diasys devices (DIALYSYS Integra, Novacor SA, Rueil-Malmaison, France). Additional methodologic criteria have been described previously.23 We used the average of 10 randomly selected measures, separately for daytime and nighttime BP, to have a large number of measures for each participant. We showed previously that, in this sample, these phenotypes yielded heritability estimates similar to using all of the available ambulatory measures.23

PRA was measured using the antibody-trapping principle.24,25 Aldosterone was measured by a direct radioimmunoassay using a very sensitive and specific antiserum raised in a New Zealand white rabbit.26 The coefficients of variation for within-and among-assay precision were 0.04 to 0.13 for the PRA and aldosterone assays.25,26 Participants were given plastic containers to collect 24-hour urine under their usual diet on the same day that ambulatory BP monitoring was performed. For 24-hour collection, urine was collected separately for the day and the night, which were defined by the participants’ self-reported bedtimes and wake-up times. Urinary and plasma sodium and potassium concentrations were measured by flame photometry (IL-943, Instrumentation Laboratory). Endogenous trace lithium was measured by atomic absorption spectrophotometry.27 Glomerular filtration rate was measured using inulin clearance as described previously.21 After overnight fasting, clearance protocols began between 7:00 and 8:00 AM on the day after the 24-urine collection in a quiet room with the subject lying on a bed throughout the procedure, except for active voiding. Two intravenous catheters were inserted into antecubital veins, 1 for the infusion of inulin and the second into the contralateral forearm for blood drawing. Fasting blood samples were collected first. After an oral water load of 200 mL, a bolus and a following sustained infusion of inulin, which were adapted to the participant’s height, weight, and sex, were given to ensure a stable plasma concentration as described previously.21 Participants received 400 mL of oral water at time 60 minutes and 200 mL every hour thereafter. After a 2-hour equilibration period, two 1-hour inulin clearances were obtained to measure glomerular filtration rate. Based on these 2 consecutive clearances in all of the subjects, the reliability coefficient was 0.71 for glomerular filtration rate. Fractional excretion of endogenous lithium (FELi) and fractional excretion of sodium were obtained by dividing the lithium (or sodium) clearance by the inulin clearance, using measurements in timed urine collections during the inulin clearance procedure. Based on the 2 consecutive clearances in all of the subjects, reliability coefficients were 0.76 for FELi and 0.90 for fractional excretion of sodium. Fractional distal (ie, postproximal) sodium reabsorption was calculated by subtracting the ratio of the lithium to sodium clearances from 1 (ie, 1−(CLi/CLNa)). FELi is an indirect marker of proximal tubular sodium reabsorption; that is, a decrease in FELi indicates an increase in proximal tubular sodium reabsorption. Fasting blood glucose was the average of 2 measurements using a Glycocronic C reflectometer (Macherey-Nagel).

Single-Blind Randomized Crossover Trial

Fifty-four hypertensive participants (ie, having a daytime ambulatory BP >140/90 mm Hg, after a 2-week antihypertensive treatment washout, if any) from 37 families of the sample also participated in a single-blind randomized crossover trial.29 After a 2-week washout period, participants were assigned, in a random order, to two 4-week treatments, 1 with lisinopril (20 mg per day) and 1 with hydrochlorothiazide (25 mg per day), separated by a 2-week washout period. We conducted 24-hour ambulatory BP monitoring at the beginning and end of each treatment period. The mean (±SD) baseline systolic/diastolic BP levels were 143/94 (±98) for daytime and 127/85 (±12/10) for nighttime.

Genetic Analyses

DNA was isolated using standard methods from blood drawn into potassium-EDTA tubes and stored at 4°C. The ABCB1 genotypes (exon 21, 2677 G>T and exon 26, 3435 C>T) and the CYP3A5*1, *3, *6, and *7 alleles were determined by real-time PCR with TaqMan, as described previously.3,5,30 The markers did not significantly deviate from Hardy–Weinberg proportions (P>0.09) in founders, tested using the hwe function in Stata 9.0, and 2677 G>T and 3435 C>T were in strong linkage disequilibrium (Le-\text{w}ontin’s D’=0.90).

Statistical Analyses

Based on the findings from descriptive analyses in our sample and from the knowledge that only carriers of the CYP3A5*1 allele express a significant amount of the protein,31 we assumed a dominant mode of action on BP for the allele *1. We also assumed a dominant mode of action on BP for ABCB1 3435ST and 2677T alleles in our main analyses. We used the ASSOC program in S.A.G.E.32 to conduct multiple linear regression with the continuous ambulatory daytime and nighttime systolic and diastolic BP as dependent variables. We used as predictors age, sex, body mass index, urinary sodium and potassium excretion, plasma potassium, high-density lipoprotein cholesterol, triglycerides, fasting blood glucose, menopausal status, contraceptive pill use, reported alcohol consumption and smoking status, and, for each drug class reported, baseline antihypertensive treatment (before the 2-week washout) entered as dummy variables, that is, diuretics, β-blockers, calcium channel blockers, and angiotensin-converting enzyme (ACE) inhibitors. AS-SOC accounts for familial correlations by implementing maximum likelihood estimation of both familial components of variance and covariate coefficients. All of the models included age, sex, and ascertainment, as described previously.23 We first retained in the model all of the nongenetic covariates and their 2-way interactions significant at the 5% level. We then added the genetic covariates and also tested all of the 2-way interactions of variables in the model. Finally, we tested a single 3-way interaction, the one with urinary sodium excretion and the interaction between the ABCB1 3435ST (or 2677T) and CYP3A5*1 alleles. This last step was guided by the fact that there was a significant interaction between the 3435ST and the CYP3A5*1 alleles for daytime systolic BP, the CYP3A5 gene is a potential candidate gene for salt sensitivity,3,5 and in our sample the ABCB1 variants were associated with PRA and, to a lesser extent, with aldosterone. We went through the same process using PRA and plasma aldosterone as the dependent variables to assess their
association with the ABCB1 3435T (or 2677T) and CYP3A5*1 alleles. We analyzed the trend in age-, sex-, and ascertainment-adjusted fractional distal sodium reabsorption, FELi, aldosterone, PRA, and aldosterone across ABCB1 genotypes, with and without stratification for the CYP3A5*1 allele, using nonparametric trend tests (approximate P values).

For the participants in the treatment trial, we assessed by multiple linear regression the response to antihypertensive treatment, measured as the ambulatory BP difference between the end and the beginning of the treatment period. Because of the small sample size, we only tested the 2-way interaction between the CYP3A5*1 and the 3435T alleles, and not the 3-way interaction with urinary sodium in models adjusted for the confounding effects of age, sex, and aldosterone/renin ratio. We have already shown the absence of any significant carryover effect in this study.29

**Results**

Although 373 participants had complete data for daytime ambulatory BPs, PRA, and plasma aldosterone, only 317 had ≥10 nighttime BP measurements. Participants’ characteristics are presented in Table 1. Although none of the measured alleles showed a significant association with ambulatory BP on the unadjusted data, the CYP3A5*1 allele showed a significant interaction with age in its effect on ambulatory BP, as described previously.5 ABCB1 variants were not associated with ambulatory BP in analyses not accounting for CYP3A5*1 (data not shown).

Figure 1 shows the age- and sex-adjusted ambulatory BP by groups of the 4 possible allelic combinations, C0, C1, T0, and T1. The first column of panels illustrates the interaction between the 3435T and the CYP3A5*1 alleles on ambulatory BP overall (n =58, 129, 56, and 130, respectively, in allelic combinations C0, C1, T0, and T1 for daytime BP), and the 3-way interactions were significant for both daytime and nighttime systolic BP (data not shown).

### Table 1. Characteristics of the Patient Groups

<table>
<thead>
<tr>
<th>Covariate</th>
<th>ABCB1 2677 G&gt;T</th>
<th>ABCB1 3435 C&gt;T</th>
<th>CYP3A5*1</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>(n=254)</td>
<td>(n=167)</td>
<td>(n=164)</td>
</tr>
<tr>
<td>Age, y</td>
<td>46.0 (11.7)</td>
<td>46.8 (12.0)</td>
<td>45.5 (12.7)</td>
</tr>
<tr>
<td>Sex, M=0, F=1</td>
<td>0.57 (0.50)</td>
<td>0.52 (0.50)</td>
<td>0.56 (0.50)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28.7 (5.4)</td>
<td>28.0 (4.8)</td>
<td>28.2 (5.5)</td>
</tr>
<tr>
<td>Treatment off, %</td>
<td>0.39 (0.49)</td>
<td>0.41 (0.49)</td>
<td>0.44 (0.49)</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>0.15 (0.35)</td>
<td>0.09 (0.29)</td>
<td>0.10 (0.30)</td>
</tr>
<tr>
<td>Alcohol, g/d</td>
<td>5.8 (13.6)</td>
<td>7.9 (16.2)</td>
<td>5.4 (8.6)</td>
</tr>
<tr>
<td>Day SBP, mm Hg</td>
<td>130.7 (17.4)</td>
<td>131.1 (17.5)</td>
<td>130.4 (15.9)</td>
</tr>
<tr>
<td>Day DBP, mm Hg</td>
<td>84.3 (11.6)</td>
<td>84.6 (11.7)</td>
<td>83.9 (11.3)</td>
</tr>
<tr>
<td>Night SBP, mm Hg</td>
<td>117.6 (16.6)</td>
<td>118.9 (16.9)</td>
<td>118.8 (15.7)</td>
</tr>
<tr>
<td>Night DBP, mm Hg</td>
<td>75.6 (11.9)</td>
<td>76.1 (12.1)</td>
<td>76.4 (12.2)</td>
</tr>
<tr>
<td>Plasma K, mmol/L</td>
<td>3.8 (0.3)</td>
<td>3.7 (0.3)</td>
<td>3.8 (0.3)</td>
</tr>
<tr>
<td>Urine Na, mmol/24 h</td>
<td>107 (64)</td>
<td>104 (54)</td>
<td>110 (53)</td>
</tr>
<tr>
<td>PA, pg/mL</td>
<td>50 (19)</td>
<td>57 (20)</td>
<td>51 (18)</td>
</tr>
<tr>
<td>PRA, ng/mL/h</td>
<td>0.32 (0.44)</td>
<td>0.40 (0.45)</td>
<td>0.38 (0.42)</td>
</tr>
</tbody>
</table>

Results are means (SD) unless specified otherwise. BMI indicates body mass index; M, male; F, female; SBP, systolic BP; DBP, diastolic BP; PA, plasma aldosterone.

* Treatment off: antihypertensive therapy, if any, was stopped for 2 weeks before measuring BP.

† P<0.01 versus No. 1.

‡ PA indicates plasma aldosterone with results in medians (interquartile ranges).

§ P<0.10 to test for the association of the trait with the T allele (3435 C>T and 2677 G>T) or *1 allele (CYP3A5).

¶ PRA indicates plasma renin activity with results in medians (interquartile ranges).
our results do not merely reflect the action of these genes on antihypertensive drug metabolism and transport. The 3435 CT and TT genotypes were associated with an increased postproximal reabsorption of sodium (ie, an increased fractional distal sodium reabsorption), as compared with the CC genotype, but the trend was clear and significant only in CYP3A5*1 carriers (Table 2). In multivariable linear regression models (results not shown), the CYP3A5*1 allele tended to be associated positively with plasma aldosterone (P = 0.06), and the ABCB1 3435T (P = 0.03) and 2677T (P = 0.09) alleles tended to be associated positively with PRA, without significant interaction between ABCB1 and CYP3A5.

These results suggest that these genetic variants are associated with postproximal tubular sodium reabsorption and the renin–angiotensin–aldosterone system.

The 3435T and the CYP3A5*1 alleles interacted in their effect on ambulatory BP response to the ACE inhibitor lisinopril during the daytime but not the nighttime, as shown in Figure 2 (n = 8, 20, 11, and 15 for the allelic combinations C0, C1, T0, and T1, respectively). No such association was found for hydrochlorothiazide. These results suggest that the CYP3A5*1 and 3435T alleles decrease the BP response to ACE inhibition, but not to a diuretic, which provides additional evidence that the interaction between the CYP3A5 and ABCB1 genes on BP is mediated through the activity of the renin–angiotensin–aldosterone system.

**Discussion**

The present data describe a significant interaction between variants in CYP3A5 and ABCB1, 2 genes encoding for drug and hormone metabolizing and transporting proteins, in their effect on ambulatory BP. This interaction is modified by urinary sodium excretion. Especially in subjects with a high urinary sodium excretion, the CYP3A5*1 allele is associated with a higher BP among those who do not carry the 3435T allele, and the ABCB1 3435T allele is associated with a higher BP among those who do not carry the CYP3A5*1 allele, whereas subjects carrying both alleles have a lower BP than those carrying either allele. This suggests that the ABCB1 3435T and CYP3A5*1 alleles have an antagonistic effect on BP with increasing salt intake. To our knowledge, this is the first reported association of the ABCB1 gene with BP in humans and the first time that these 2 genes have been shown to interact in their effect on BP. This key finding is of the utmost importance because, if confirmed in other settings, it would point toward the existence of a new pathway for BP regulation in humans.

We have recently reported an association between the CYP3A5 gene and ambulatory BP in the Seychelles population, which confirmed previous findings associating the CYP3A5 gene with BP in humans. Animal data suggest that the link between the CYP3A5 gene and BP regulation could be mediated by an enhanced renal tubular sodium reabsorption through increased levels of 6β-hydrocortisol.

In addition, the present data show that the BP response to an ACE inhibitor is blunted significantly in CYP3A5*1 carriers. This could be explained by a sodium retaining effect, which is known to reduce the antihypertensive efficacy of ACE inhibitors.
The 2 single nucleotide polymorphisms of the ABCB1 gene are in strong linkage disequilibrium: 3435 C>T in exon 26 (synonymous) and 2677 G>T in exon 21 (nonsynonymous), which leads to a change of amino acid from alanine to serine (Ala893Ser). The 3435 C>T variant has been associated with variable expression of the PGP in the duodenum (TT homozygotes expressed less than half of the amount of PGP expressed by CC homozygotes) because of diminished mRNA stability for the T allele. The 3435 C>T variant, therefore, seems to be a functional synonymous single nucleotide polymorphism.

The frequency of the CYP3A5*1 allele varies from 45% in subjects of African descent to 8% to 15% in whites and 23% to 40% in Asians. The frequency of the ABCB1 3435T allele varies from 16% to 27% in subjects of African descent to 48% to 57% in whites and 41% to 66% in Asians. Given the large interethnic difference in allele frequencies, it is important to explore these associations in other ethnic groups.

The association between the ABCB1 gene and BP does not necessarily mean that there is a cause–effect relationship. However, our data provide some insights on the potential mechanism(s) whereby PGP, the product of this gene, might affect BP. Previous animal and in vitro data have suggested that PGP might play a role in the transport of aldosterone. Our data also provide some indications suggesting that this gene may be related to the sodium/renin–angiotensin–aldosterone system interaction. Indeed, in our population, the ABCB1 3435T and 2677T alleles are associated with an elevated PRA and aldosterone. The CYP3A5*1 allele seems to modify these associations in the sense that the 3435T and 2677T alleles are associated with aldosterone only in CYP3A5*1 carriers and with PRA only in CYP3A5*1 noncarriers. In addition, we found that CYP3A5*1 and ABCB1 3435T alleles interact on the ambulatory BP response to lisinopril but not hydrochlorothiazide. Because lisinopril is excreted unchanged, the effect of CYP3A5 cannot be mediated by the metabolism of...
this drug, and, to our knowledge, there are presently no indications that this drug could be a PGP substrate. These experimental results, therefore, further strengthen the hypothesis that the ABCB1 and CYP3A5 genes interact on ambulatory BP by modulating the activity of the renin–angiotensin system and, hence, sodium excretion. The CYP3A5 and ABCB1 genes encode proteins involved in the metabolism and transport of drugs and endogenous substrates that might affect BP regulation. The 3435T allele has been strongly associated with cyclosporine-induced nephrotoxicity, which is typically associated with hypertension. The association between the ABCB1 3435T allele and BP may, therefore, be caused by altered transport of an unknown xenobiotic and/or endogenous compound. For instance, ouabain is known to induce hypertension and has been shown to stimulate ABCB1 gene expression. Digoxin, an ouabain-like substance, is a well-known PGP substrate. Therefore, another mechanism by which the ABCB1 gene could influence BP is through the transport of endogenous ouabain-like substances. Lastly, it has been shown recently that ABCB1 genotypes likely influence basal CYP3A4 expression in the liver and intestine by limiting the intracellular concentration of an endogenous regulator. Through a similar mechanism, ABCB1 genotypes could also influence CYP3A5 expression (in CYP3A5 expressors) in the kidneys, but, to our knowledge, this has never been demonstrated.

We did not adjust for multiple testing because we only tested for a single 3-way interaction that was guided by a priori knowledge of both genes, and it is highly unlikely that a false-positive result would have also led to the observed associations with postproximal tubular sodium reabsorption, PRA, and aldosterone and to a selective gene–gene interaction with the BP response to an ACE inhibitor. The a priori probability of finding an interaction between the CYP3A5 and ABCB1 genes was further enhanced by the fact that the proteins encoded by these genes share many substrates in common, and their activity is regulated by the same nuclear receptors. Because it would be very difficult and very costly to conduct a sodium load challenge on such a large number of participants, we conducted multiple linear regression analyses in which urinary sodium excretion was included in the analysis as a covariate. The low FELi could raise the suspicion that lithium is reabsorbed, in part, distally. Although we cannot exclude minor distal lithium reabsorption in subjects of African descent, we can reasonably consider that lithium is reabsorbed mainly proximally in humans and, therefore, represents a good marker of proximal tubular sodium reabsorption.

**Perspectives**

We report a gene–gene–environment interaction on ambulatory BP in subjects of African descent that involves the CYP3A5 and ABCB1 genes and urinary sodium excretion. Our data support the hypothesis that these genes influence BP through the renin–angiotensin–aldosterone system. Although the CYP3A5 and ABCB1 genes are known to interact in their effect on drug metabolism and transport, this is, to our knowledge, the first reported association of the ABCB1 gene with BP in humans. These results underscore the importance of accounting for gene–gene interactions and the key role of sodium as an effect modifier in BP genetics. If confirmed in other settings, these results would stimulate further research on a new pathway for BP regulation and have important implications regarding BP response to treatment.

**Acknowledgments**

We thank Murielle Brocard and Nathalie Cochard for performing the DNA analyses, the Ministry of Health of the Republic of Seychelles for their support of this epidemiological research, and Air Seychelles and SkyChef for their logistic support in transporting equipment and samples.
Sources of Funding

The study benefited from a grant from the Swiss National Science Foundation (TANDEM No 31–51115.97) and was also supported in part by a US Public Health Service resource grant (RR03655) from the National Center for Research Resources and research grant GM28356 from the National Institute of General Medical Sciences.

Disclosures

None.

References


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Hypertension. published online March 19, 2007;

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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