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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;49:1007-1014.)

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

Cytochrome 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.^{3–6} 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 intracerebroven-

tricular 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.

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In this report, based on the same families of East African descent as in the above-mentioned study,⁵ 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 patients 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 validated²² Diasys devices (DIASYS 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 the same 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-hour 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.²⁸ 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, $1 - (C_{Li}/C_{Na})$]. 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 Glycotronic C reflectometer (Machery-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 (\pm SD) baseline systolic/diastolic BP levels were 143/94 ($\pm 9/8$) for daytime and 127/85 ($\pm 12/10$) 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.^{5,30} The markers did not significantly deviate from Hardy-Weinberg proportions ($P > 0.09$) in founders, tested using the hwsnp function in Stata 9.0, and 2677 G>T and 3435 C>T were in strong linkage disequilibrium (Leuontin'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* 3435T 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. ASSOC 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* 3435T (or 2677T) and *CYP3A5*1* alleles. This last step was guided by the fact that there was a significant interaction between the 3435T 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 last columns of panels provide the corresponding results in each urinary sodium excretion tertile (*n*=20/19/19, 43/43/43, 19/19/18, and 44/43/43, respectively, for tertiles 1/2/3 in allelic combinations C0, C1, T0, and T1 for daytime BP). The interaction of the 3435T and *CYP3A5**1 alleles on ambulatory BP was not significant in the first and second tertiles but was strongly significant in the third tertile. These results suggest that the interaction between the 3435T and *CYP3A5**1 alleles on ambulatory BP is influenced by urinary sodium excretion. This was formally tested by means of a 3-way interaction in a multiple linear regression: there was a significant negative 3-way interaction between the *ABCB1* 3435T allele, the *CYP3A5**1 allele, and urinary sodium excretion for daytime systolic (*P*=0.05), nighttime systolic (*P*<0.001), and nighttime diastolic (*P*=0.004) ambulatory BP. Furthermore, each allele had a significant positive interaction with urinary sodium excretion (*P*≤0.05 for daytime systolic and nighttime systolic and diastolic BP). These results suggest that the main and interaction effects of these alleles on ambulatory BP are modified by dietary salt intake. The 3-way interactions and the 2-way interactions with urinary sodium excretion were stronger for nighttime than for daytime ambulatory BP. Results using the 2677T allele were similar but less significant.

The results for the 221 participants untreated at baseline were very similar to those obtained in the whole sample. In particular, the 3-way interactions were significant for both daytime and nighttime systolic BP (data not shown). Thus,

TABLE 1. Characteristics of the Patient Groups

Covariate	<i>ABCB1</i> 2677 G>T		<i>ABCB1</i> 3435 C>T		<i>CYP3A5</i> *1	
	GG (<i>n</i> =254)	GT+TT (<i>n</i> =119)	CC (<i>n</i> =187)	CT+TT (<i>n</i> =176)	No *1 (<i>n</i> =114)	*1 Carrier (<i>n</i> =259)
Age, y	46.0 (11.7)	47.0 (12.0)	45.8 (11.7)	46.8 (12.0)	45.5 (12.7)	46.7 (11.4)
Sex, M=0, F=1	0.57 (0.50)	0.52 (0.50)	0.58 (0.49)	0.52 (0.50)	0.56 (0.50)	0.55 (0.50)
BMI, kg/m ²	27.7 (5.4)	28.0 (4.8)	27.2 (5.3)	28.3 (5.0)	28.2 (5.5)	27.6 (5.1)
Treatment off, %*	0.39 (0.49)	0.45 (0.50)	0.41 (0.49)	0.40 (0.49)	0.44 (0.50)	0.39 (0.49)
Smoking, %	0.15 (0.35)	0.09 (0.29)	0.15 (0.36)	0.11 (0.31)	0.10 (0.30)	0.14 (0.35)
Alcohol, g/d	5.8 (13.6)	7.9 (16.2)	6.0 (15.0)	6.9 (14.0)	5.4 (8.6)	7.0 (16.5)
Day SBP, mm Hg	130.7 (17.4)	133.3 (17.4)	131.1 (17.5)	131.9 (17.4)	130.4 (15.9)	132.0 (18.0)
Day DBP, mm Hg	84.3 (11.6)	85.3 (11.2)	84.6 (11.7)	84.7 (11.3)	83.9 (11.3)	85.0 (11.6)
Night SBP, mm Hg	117.6 (16.6)	120.2 (16.7)	118.9 (16.9)	117.9 (16.4)	118.8 (15.7)	118.3 (17.1)
Night DBP, mm Hg	75.6 (11.9)	76.7 (11.7)	76.1 (12.1)	75.9 (11.6)	76.4 (12.2)	75.8 (11.7)
Plasma K, mmol/L	3.8 (0.3)	3.7 (0.3)	3.7 (0.3)	3.8 (0.3)	3.8 (0.3)	3.8 (0.3)
Urine Na, mmol/24 h	107 (54)	104 (54)	103 (53)	110 (55)	115 (56)	102 (52)†
Urine K, mmol/24 h	44 (20)	44 (18)	45 (20)	44 (18)	46 (19)	44 (20)
PA, pg/mL‡	50 (19)	57 (26)§	51 (18)	52 (26)	49 (21)	52 (21)
PRA, ng/mL/h¶	0.32 (0.44)	0.40 (0.45)§	0.30 (0.43)	0.42 (0.43)§	0.38 (0.42)	0.34 (0.42)

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).

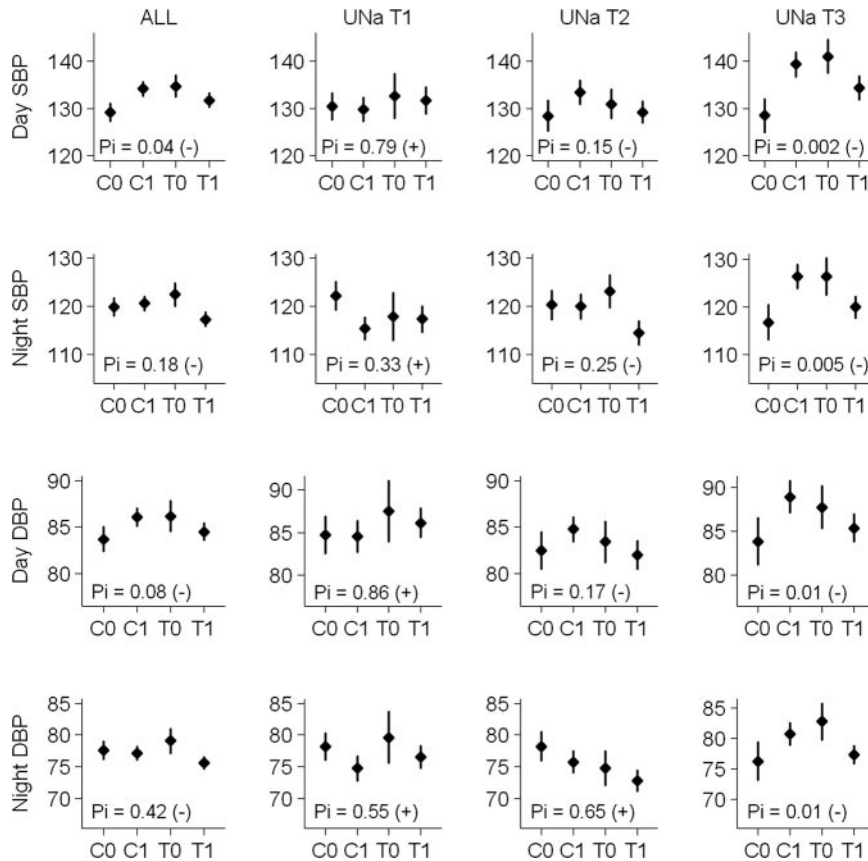


Figure 1. Age- and sex-adjusted ambulatory BP in groups defined by the *ABCB1* 3435 and *CYP3A5* alleles and by tertiles of urinary sodium excretion. UNa indicates urinary sodium. To compare the same individuals across rows, we used tertiles of nighttime urinary sodium excretion. The sign in parenthesis provides the direction of the interaction effect when both 3435T and *CYP3A5**1 alleles are present. C0 indicates subjects who do not carry the 3435T or *CYP3A5**1 alleles (n=58); T0, carry the 3435T allele but not the *CYP3A5**1 allele (n=56); C1, carry the *CYP3A5**1 allele but not the 3435T allele (n=129); T1, carry the 3435T and the *CYP3A5**1 alleles (n=130).

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,⁵ which confirmed previous findings associating the *CYP3A5* gene with BP in humans.^{3,6} 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 6 β -hydrocortisol.^{33,34} 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.

TABLE 2. Plasma Aldosterone, PRA, and Renal Tubular Sodium Handling by ABCB1 Genotypes and CYP3A5*1 Allele

Group	ABCB1 3435 C>T Genotypes				ABCB1 2677 G>T Genotypes			
	CC	CT	TT	P	GG	GT	TT	P
<i>CYP3A5*1</i> noncarriers								
N	58	40	16		80	26	8	
FDRNa, %	92.5	93.1	92.0	0.54	92.1	94.0	93.1	0.13
FELi, %*	18.2	17.1	16.4	0.46	17.6	18.7	12.6	0.99
PA, pg/mL	54	52	54	0.76	53	53	61	0.71
PRA, ng/mL/h	0.35	0.54	0.51	0.001	0.41	0.48	0.68	0.01
ARR	422	337	370	0.007	388	361	343	0.07
Day SBP	129.5	133.1	134.7	0.08	130.6	132.4	137.4	0.24
Day DBP	85.8	86.0	81.2	0.66	85.8	86.9	80.1	0.97
<i>CYP3A5*1</i> carriers								
N	129	107	23		174	78	7	
FDRNa, %	92.4	93.3	94.7	0.01	92.5	93.9	92.7	0.01
FELi, %*	16.1	18.0	19.2	0.48	16.0	18.7	17.4	0.12
PA, pg/mL	55	59	59	0.27	55	61	66	0.04
PRA, ng/mL/h	0.41	0.44	0.38	0.14	0.43	0.43	0.43	0.15
ARR	393	376	428	0.37	385	399	473	0.63
Day SBP	133.4	129.4	131.9	0.41	131.8	131.2	136.3	0.64
Day DBP	85.7	82.4	83.1	0.33	83.9	82.5	90.9	0.47
All								
N	187	147	39		254	104	15	
FDRNa, %	92.4	93.3	93.8	0.02	92.4	93.9	92.7	0.005
FELi, %*	16.7	17.6	18.4	0.94	16.5	18.7	15.3	0.30
PA, pg/mL	54	55	59	0.50	54	59	63	0.07
PRA, ng/mL/h	0.39	0.49	0.48	0.002	0.43	0.45	0.52	0.009
ARR	405	365	379	0.02	385	373	379	0.14
Day SBP	131.8	130.8	133.9	0.79	131.6	131.3	136.3	0.30
Day DBP	85.7	83.0	82.5	0.60	84.7	83.0	84.4	0.61

All of the results are medians of age- and sex-adjusted data. Approximate *P* values obtained using nonparametric tests for trend across *ABCB1* genotypes. FDRNa indicates fractional distal reabsorption of sodium; PA, plasma aldosterone; ARR, aldosterone/renin ratio; SBP, systolic BP; DBP, diastolic BP.

*FELi is an indirect marker of proximal sodium reabsorption.

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).³⁵ 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)³⁶ because of diminished mRNA stability for the T allele.³⁷ 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.³⁸ 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.³⁹ 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 animal⁹ and in vitro¹³ 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

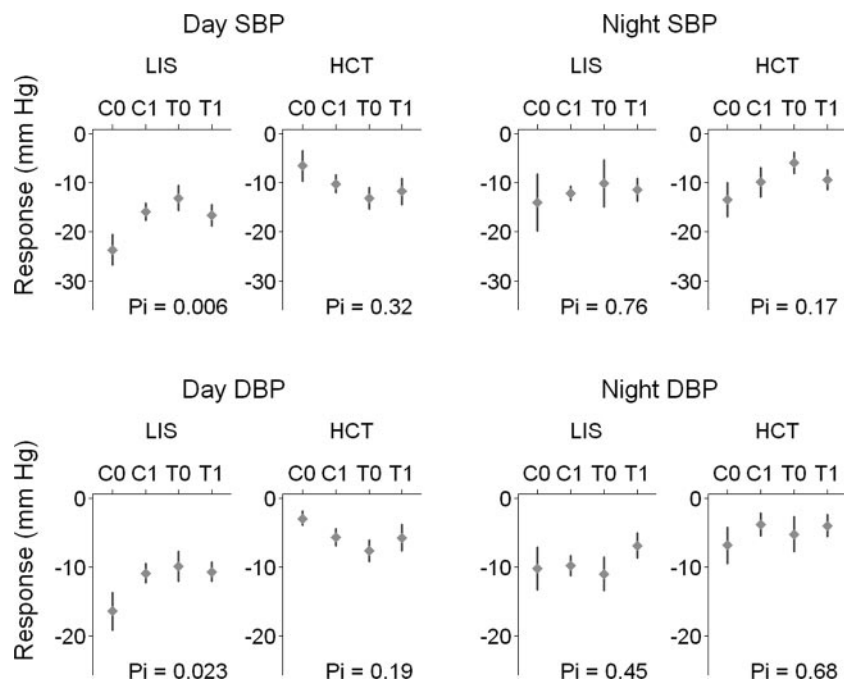


Figure 2. Ambulatory BP response to lisinopril (LIS) and hydrochlorothiazide (HCT) for the 3435T and CYP3A5*1 alleles. P int indicates P value for interaction between 3435T and CYP3A5*1 from multiple linear regression models adjusted for age, sex, and aldosterone/renin ratio. SBP/DBP indicates systolic/diastolic BP. For definition of C0, C1, T0, and T1 see legend of Figure 1.

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,⁴⁰ 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 hypertension⁴¹ and has been shown to stimulate *ABCB1* gene expression.⁴² Digoxin, an ouabain-like substance, is a well-known PGP substrate.⁴³ 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.⁴⁴ 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,¹⁷ and their activity is regulated by the same nuclear receptors.^{18–20} 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.

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Disclosures

None.

References

- Haehner BD, Gorski JC, Vandenbranden M, Wrighton SA, Janardan SK, Watkins PB, Hall SD. Bimodal distribution of renal cytochrome P450 3A activity in humans. *Mol Pharmacol*. 1996;50:52–59.
- Koch I, Weil R, Wolbold R, Brockmoller J, Hustert E, Burk O, Nuessler A, Neuhaus P, Eichelbaum M, Zanger U, Wojnowski L. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos*. 2002;30:1108–1114.
- Ho H, Pinto A, Hall SD, Flockhart DA, Li L, Skaar TC, Cadman P, O'Connor DT, Wagner U, Fineberg NS, Weinberger MH. Association between the CYP3A5 genotype and blood pressure. *Hypertension*. 2005;45:1–5.
- Fromm MF, Schmidt BM, Pahl A, Jacobi J, Schmieder RE. CYP3A5 genotype is associated with elevated blood pressure. *Pharmacogenet Genomics*. 2005;15:737–741.
- Bochud M, Eap CB, Elston RC, Bovet P, Maillard M, Schild L, Shamlaye C, Burnier M. Association of CYP3A5 genotypes with blood pressure and renal function in African families. *J Hypertens*. 2006;24:923–929.
- Givens RC, Lin YS, Dowling AL, Thummel KE, Lamba JK, Schuetz EG, Stewart PW, Watkins PB. CYP3A5 genotype predicts renal CYP3A activity and blood pressure in healthy adults. *J Appl Physiol*. 2003;95:1297–1300.
- Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A*. 1987;84:7735–7738.
- Ernest S, Rajaraman S, Megyesi J, Bello-Reuss EN. Expression of MDR1 (multidrug resistance) gene and its protein in normal human kidney. *Nephron*. 1997;77:284–289.
- Uhr M, Holsboer F, Muller MB. Penetration of endogenous steroid hormones corticosterone, cortisol, aldosterone and progesterone into the brain is enhanced in mice deficient for both mdr1a and mdr1b P-glycoproteins. *J Neuroendocrinol*. 2002;14:753–759.
- Parker RB, Yates CR, Laizure SC, Weber KT. P-glycoprotein modulates aldosterone plasma disposition and tissue uptake. *J Cardiovasc Pharmacol*. 2006;47:55–59.
- Huang BS, Wang H, Leenen FHH. Chronic central infusion of aldosterone leads to sympathetic hyperreactivity and hypertension in Dahl S but not Dahl R rats. *Am J Physiol Heart Circ Physiol*. 2005;288:H517–H524.
- Huang BS, Cheung WJ, Wang H, Tan J, White RA, Leenen FH. Activation of brain renin-angiotensin-aldosterone system by central sodium in Wistar rats. *Am J Physiol Heart Circ Physiol*. 2006;291:H1109–H1117.
- Ueda K, Okamura N, Hirai M, Tanigawara Y, Saeki T, Kioka N, Komano T, Hori R. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J Biol Chem*. 1992;267:24248–24252.
- Bello-Reuss E, Ernest S, Holland OB, Hellmich MR. Role of multidrug resistance P-glycoprotein in the secretion of aldosterone by human adrenal NCI-H295 cells. *Am J Physiol Cell Physiol*. 2000;278:C1256–C1265.
- Stern N, Lustig S, Petrasko D, Jensen G, Eggena P, Lee DB, Tuck ML. Cyclosporin A-induced hyperreninemic hypoaldosteronism. A model of adrenal resistance to angiotensin II. *Hypertension*. 1987;9:III31–III35.
- Adu D, Turney J, Michael J, McMaster P. Hyperkalaemia in cyclosporin-treated renal allograft recipients. *Lancet*. 1983;2:370–372.
- Schuetz EG, Beck WT, Schuetz JD. Modulators and substrates of P-glycoprotein and cytochrome P4503A coordinately up-regulate these proteins in human colon carcinoma cells. *Mol Pharmacol*. 1996;49:311–318.
- Burk O, Arnold KA, Geick A, Tegude H, Eichelbaum M. A role for constitutive androstane receptor in the regulation of human intestinal MDR1 expression. *Biol Chem*. 2005;386:503–513.
- Burk O, Koch I, Raucy J, Hustert E, Eichelbaum M, Brockmoller J, Zanger UM, Wojnowski L. The induction of cytochrome P450 3A5 (CYP3A5) in the human liver and intestine is mediated by the xenobiotic sensors pregnane x receptor (PXR) and constitutively activated receptor (CAR). *J Biol Chem*. 2004;279:38379–38385.
- Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem*. 2001;276:14581–14587.
- Bochud M, Elston RC, Maillard M, Bovet P, Schild L, Burnier M. Heritability of renal function in hypertensive families of African descent in the Seychelles (Indian Ocean). *Kidney Int*. 2005;67:61–69.
- O'Brien E, Coats A, Owens P, Petrie J, Padfield PL, Littler W, de Swiet M, Mee F. Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British Hypertension Society. *BMJ*. 2000;320:1128–1134.
- Bochud M, Bovet P, Elston RC, Paccaud F, Falconnet C, Shamlaye C, Burnier M. High heritability of ambulatory blood pressure in families of East African descent. *Hypertension*. 2005;45:445–450.
- Poulsen K, Jorgensen J. An easy radioimmunoassay microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J Clin Endocrinol Metab*. 1974;39:816–825.
- Nussberger J, Fasanella d'Amore T, Porchet M, Waeber B, Brunner DB, Brunner HR, Kler L, Brown AN, Francis RJ. Repeated administration of the converting enzyme inhibitor cilazapril to normal volunteers. *J Cardiovasc Pharmacol*. 1987;9:39–44.
- Nussberger J, Waeber B, Brunner HR, Burris J, Vetter W. Highly sensitive microassay for aldosterone in unextracted plasma: comparison with two other methods. *J Lab Clin Med*. 1984;104:789–796.
- Magnin JL, Decosterd LA, Centeno C, Burnier M, Diezi J, Biollaz J. Determination of trace lithium in biological fluids using graphite furnace atomic absorption spectrophotometry: variability of urine matrices circumvented by cation exchange solid phase extraction. *Pharm Acta Helv*. 1996;71:237–246.
- Buclin T, Pechère-Bertchi A, Séchaud R, Décosterd LA, Munafo A, Burnier M, Biollaz J. Sinistrin clearance for determination of glomerular filtration rate: a reappraisal of various approaches using a new analytical method. *J Clin Pharmacol*. 1997;37:679–692.
- Falconnet C, Bochud M, Bovet P, Maillard M, Burnier M. Gender difference in the response to an angiotensin-converting enzyme inhibitor and a diuretic in hypertensive patients of African descent. *J Hypertens*. 2004;22:1213–1220.
- Eap CB, Fellay J, Buclin T, Bleiber G, Golay KP, Brocard M, Baumann P, Telenti A. CYP3A activity measured by the midazolam test is not related to 3435 C>T polymorphism in the multiple drug resistance transporter gene. *Pharmacogenetics*. 2004;14:255–260.
- Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan R, Strom S, Thummel K, Boguski MS, Schuetz E. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet*. 2001;27:383–391.
- S.A.G.E. (Statistical Analysis for Genetic Epidemiology) [computer program]. Version 5.1.1. Available at: <http://darwin.c.wru.edu/sage/index.php>; 2005. Accessed January 30, 2007.
- Ghosh SS, Basu AK, Ghosh S, Hagley R, Kramer L, Schuetz J, Grogan WM, Guzelian P, Watlington CO. Renal and hepatic family 3A cytochromes P450 (CYP3A) in spontaneously hypertensive rats. *Biochem Pharmacol*. 1995;50:49–54.
- Watlington CO, Kramer LB, Schuetz EG, Zilai J, Grogan WM, Guzelian P, Gizek F, Schoolwerth AC. Corticosterone 6 beta-hydroxylation correlates with blood pressure in spontaneously hypertensive rats. *Am J Physiol*. 1992;262:F927–F931.
- Marzolini C, Paus E, Buclin T, Kim RB. Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther*. 2004;75:13–33.
- Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, John A, Cascorbi I, Gerloff T, Roots I, Eichelbaum M, Brinkmann U. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A*. 2000;97:3473–3478.

37. Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics*. 2005;15:693–704.
38. Chowbay B, Zhou S, Lee EJ. An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and drug transporters: experience in Singapore. *Drug Metab Rev*. 2005;37:327–378.
39. Ameyaw MM, Regateiro F, Li T, Liu X, Tariq M, Mobarek A, Thornton N, Folayan GO, Githang'a J, Indalo A, Ofori-Adjei D, Price-Evans DA, McLeod HL. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics*. 2001;11:217–221.
40. Hauser IA, Schaeffeler E, Gauer S, Scheuermann EH, Wegner B, Gossmann J, Ackermann H, Seidl C, Hocher B, Zanger UM, Geiger H, Eichelbaum M, Schwab M. ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. *J Am Soc Nephrol*. 2005;16:1501–1511.
41. Hamlyn JM, Hamilton BP, Manunta P. Endogenous ouabain, sodium balance and blood pressure: a review and a hypothesis. *J Hypertens*. 1996;14:151–167.
42. Brouillard F, Tondelier D, Edelman A, Baudouin-Legros M. Drug resistance induced by ouabain via the stimulation of MDR1 gene expression in human carcinomatous pulmonary cells. *Cancer Res*. 2001;61:1693–1698.
43. Rautio J, Humphreys JE, Webster LO, Balakrishnan A, Keogh JP, Kunta JR, Serabjit-Singh CJ, Polli JW. In vitro p-glycoprotein inhibition assays for assessment of clinical drug interaction potential of new drug candidates: a recommendation for probe substrates. *Drug Metab Dispos*. 2006;34:786–792.
44. Lamba J, Strom S, Venkataramanan R, Thummel KE, Lin YS, Liu W, Cheng C, Lamba V, Watkins PB, Schuetz E. MDR1 genotype is associated with hepatic cytochrome P450 3A4 basal and induction phenotype. *Clin Pharmacol Ther*. 2006;79:325–338.