

# Association of CYP3A5 genotypes with blood pressure and renal function in African families

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**Objective** Renal cytochrome P450 3A5 (CYP3A5) activity has been associated with blood pressure and salt sensitivity in humans. We determined whether CYP3A5 polymorphisms are associated with ambulatory blood pressure (ABP) and with glomerular filtration rate (GFR) in African families.

**Methods** Using a cross-sectional design, 375 individuals from 72 families, each with at least two hypertensive siblings, were recruited through a hypertension register in the Seychelles (Indian Ocean). We analyzed the association between the CYP3A5 alleles (\*1, \*3, \*6 and \*7) and ABP, GFR and renal sodium handling (fractional excretion of lithium), from pedigree data, allowing for other covariates and familial correlations.

**Results** CYP3A5\*1 carriers increased their daytime systolic and diastolic ABP with age (0.55 and 0.23 mmHg/year) more than non-carriers (0.21 and 0.04 mmHg/year). CYP3A5\*1 had a significant main effect on daytime systolic/diastolic ABP [regression coefficient (SE): -29.6 (10.0)/-8.2 (4.1) mmHg,  $P = 0.003/0.045$ , respectively] and this effect was modified by age (CYP3A5\*1  $\times$  age interactions,  $P = 0.017/0.018$ ). For night-time ABP, the effect of CYP3A5\*1 was modified by urinary sodium excretion, not by age. For renal function, CYP3A5\*1 carriers had a 7.6(3.8) ml/min lower GFR ( $P = 0.045$ ) than non-carriers. Proximal sodium reabsorption decreased with age in non-carriers, but not in CYP3A5\*1 carriers ( $P$  for interaction = 0.02).

## Introduction

The cytochrome P450 enzymes are classified into families and subfamilies based on the sequence of their amino acids [1]. Enzymes of the human CYP3A family are involved in the metabolism of endogenous substrates, such as steroids, and in the metabolism of many drugs. The genes of the CYP3A family (3A4, 3A43, 3A5 and 3A7) cluster on chromosome 7q21-7q22.1 [2] and show organ-specific patterns of expression [3-5]. Only the CYP3A5 gene is expressed in the human kidney [6,7].

The CYP3A5 enzyme catalyzes the conversion of cortisol to its 6 $\beta$ -hydroxy metabolite in the liver [3] and also accounts for most of the 6 $\beta$ -hydroxylase activity in mammals, including humans [8]. Renal CYP3A5 enzymatic

**Conclusions** These data demonstrate that CYP3A5 polymorphisms are associated with ambulatory BP, CYP3A5\*1 carriers showing a higher age- and sodium-related increase in ABP than non-carriers. The age effect may be due, in part, to the action of CYP3A5 on renal sodium handling. *J Hypertens* 24:923-929 © 2006 Lippincott Williams & Wilkins.

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activity has been shown to correlate with systolic blood pressure (BP) in the rat [9] and selective inhibitors of the CYP3A5 enzyme have been found to decrease the level of 6 $\beta$ -hydrocortisol and decrease BP in the rat [10]. These data suggest a possible link between the CYP3A5 gene and BP regulation, which could be mediated by an enhanced renal tubular sodium reabsorption through increased levels of 6 $\beta$ -hydrocortisol.

Several CYP3A5 alleles have been described ([www.imm.ki.se/CYPalleles/cyp3a5.htm](http://www.imm.ki.se/CYPalleles/cyp3a5.htm), accession date 13 May 2005) that show different levels of expression and are associated with different amounts of CYP3A5 enzyme [11,12]. Only CYP3A5\*1 carriers express large amounts of CYP3A5 messenger RNA (mRNA) and protein [11]. In Caucasians, a decreased CYP3A5 enzymatic activity

can be largely explained by the frequent presence of allele \*3, while in African Americans alleles \*6 and \*7 can be found in a significant proportion of subjects with low CYP3A5 activity [11,13]. It has been postulated that the *CYP3A5\*1* allele could confer a selective advantage in equatorial populations experiencing water shortage by increasing sodium retention [11]. Thompson *et al.* [14], who measured only alleles \*1 and \*3, have shown that the frequency of the *CYP3A5\*3* allele varies broadly across populations, which implies a complementary pattern between *CYP3A5\*1* and *CYP3A5\*3* alleles, and that this variation is positively correlated with geographical distance from the equator.

Recently Givens *et al.* [15], who measured only alleles \*1 and \*3, found an association between the *CYP3A5\*1* allele and both office systolic BP and creatinine clearance in a small group of healthy young African Americans. Furthermore, Ho *et al.* [16] found that the *CYP3A5\*1* allele was associated with hypertension in individuals of African descent but not in Caucasians. Unlike Givens *et al.* [15], Ho *et al.* [16] found no association of *CYP3A5* polymorphisms (alleles \*1 and \*3) with glomerular filtration rate (GFR) estimated by creatinine clearance.

In this report, we analyzed the association of *CYP3A5* polymorphisms (i.e. alleles \*1, \*3, \*6 and \*7) with ambulatory BP and renal function in a sample of families of African descent that included both normotensive and hypertensive individuals.

## 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. The study was approved by the Ethical Committees of the Ministry of Health in the Seychelles and of the Faculty of Medicine of the University of Lausanne (Switzerland). All participants provided written informed consent. Families were selected from an ongoing hypertension register that includes all patients with hypertension who attend primary health-care centers in the country. The selection process for families has been described previously [17]. Briefly, families were selected from the hypertension register if there were  $\geq 2$  full siblings with hypertension (i.e. average of three readings  $\geq 140/90$  mmHg). Seventy-six of the 135 screened families were found to be eligible, among which 375 individuals from 72 families of mean size  $4.89 \pm 2.93$  (with 1–3 generations that included 89 sibships of mean size  $4.01 \pm 1.99$ ) provided data for this analysis. Exclusion criteria were an age below 18 years, an inability to sign a written informed consent, and the presence of an acute medical condition (e.g. acute stroke or myocardial infarction within the past 6 months).

Antihypertensive therapy, if any, was stopped for 2 weeks before participants underwent BP measurement and blood and urine collections. Ambulatory BP was monitored using electronic Diasys devices (DIASYS Integra, Novacor SA, Rueil-Malmaison, France) placed on the left arm with an appropriately sized cuff. Additional methodological criteria have been described previously [18]. We used the average of 10 randomly selected daytime measures and 10 randomly selected night-time measures to have the same number of measures for each participant.

Participants were given plastic containers to collect urine for 24 h on their usual diet. Participants collected their urine on the same day as ambulatory BP monitoring was performed. Participants were examined in a study center after an overnight fast. Protocols began between 0700 and 0800 h in a quiet room with the subject lying on a bed for 1 h. Plasma renin activity (PRA) and aldosterone were measured using radioimmunoassays as previously described [19]. Urinary and plasma sodium and potassium concentrations were measured by flame photometry (IL-943, Instrumentation Laboratory, Milan, Italy). GFR was measured using two methods, namely a standard 2-h inulin clearance and a 24-h creatinine clearance [17]. Body surface area was calculated using the Dubois formula [20]. Fractional excretion of lithium (FELI) and fractional excretion of sodium (FENA) were obtained by dividing the lithium (or sodium) clearance by the inulin clearance. FELI is an indirect marker of proximal tubular sodium reabsorption [21].

## Genetic analyses

DNA was isolated using standard methods from blood drawn into K-EDTA tubes and stored at 4°C. The *CYP3A5\*3*, \*6 and \*7 alleles were determined by real-time polymerase chain reaction (PCR) with TaqMan<sup>®</sup>. Allelic discrimination assays were performed on an ABI Prism 7000 (Applied Biosystems, Rotkreuz, Switzerland) according to the manufacturer's instructions. The 25  $\mu$ l PCR mixture contained 12.5  $\mu$ l TaqMan Universal PCR master mix (2  $\times$  solution containing AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs, and optimized buffer), 700 nmol/l forward primer, 800 nmol/l reverse primer, 2  $\mu$ mol/l each probe, and 100 ng of DNA. After an activation step of AmpErase (50°C, 2 min) and of AmpliTaq Gold enzyme activation (95°C, 10 min), 35 PCR cycles were performed with 15 s at 92°C and 1 min at 61°C. For the *CYP3A5\*3* allele, the primers were 5'-CCA CCC AGC TTA ACG AAT GC-3' (forward) and 5'-GAA GGG TAA TGT GGT CCA AAC AG-3' (reverse), and the probes were 5'-TGT CTT TCA aTA TCT CT-3' (FAM, mutated allele) and 5'-TGT CTT TCA gTA TCT CT-3' (VIC, wild-type allele). For the *CYP3A5\*6* allele, the primers were 5'-GGC CTA CAG CAT GGA TGT GAT T-3' (forward) and 5'-AAA TAA TAG CCC ACA TAC TTA TTG AGA GAA AT-3' (reverse), and the probes were 5'-VIC-AGC

ACT AAg AAG TTC CTA AA-3' (wild-type allele), and 5'-FAM-AGA GCA CTA AaA AGT TCC TAA-3' (mutated allele). For the *CYP3A5*\*7 allele, the primers were 5'-CTC AGA TTA TCC AAT TCT GTT TCT TTC C-3' (forward), 5'-ATT GAT TTC AAC ATC TTT CTT GCA AGT-3' (reverse), and the probes were 5'-VIC-CAC CAC CTa CCT ATG AT-3' (wild-type allele), 5'-FAM-CAC CAC CTt ACC TAT GAT-3' (mutated allele).

### Statistical analyses

Differences between *CYP3A5*\*1 carriers and non-carriers were assessed using *t*-tests for continuous variables and  $\chi^2$  tests for categorical variables. One-way ANOVA was used to compare means across genotypes. Based on findings from descriptive analyses in our sample, we used a dominant mode of action on BP for allele \*1 over alleles \*3, \*6 and \*7, and an additive mode for allele\*3 with alleles \*6 and \*7, that is, among *CYP3A5*\*1 non-carriers. Using the ASSOC program in the S.A.G.E. [22] statistical software, we analyzed the association between BP and *CYP3A5* alleles from the family data. ASSOC analyzes from pedigree data the association between a continuous trait and one or more covariates, in the presence of familial correlations on the assumption that a power transformation of the trait leads to multivariate normality across pedigree members. We corrected for ascertainment as described previously [18]. We tested all two-way interactions between covariates with main effects significant at the 5% level and the *CYP3A5* alleles. Age, sex, body mass index (BMI), urinary sodium and potassium excretion, diabetes and smoking, as well as all two-way interactions significant at the 0.10 level, were included as covariates, in addition to the *CYP3A5* alleles. Age and sex were forced into all multivariable models. Similar analyses were conducted to determine whether the *CYP3A5* gene was a significant determinant of GFR, measured by inulin clearance and creatinine clearance [17], for the subgroup of 294 individuals who had data available on these variates. We used non-parametric trend tests across age (or urinary sodium) tertiles by *CYP3A5*\*1 carrier status. We conducted quantitative pedigree transmission disequilibrium tests (QTDT) [23]; this is implemented in the ASSOC program by means of a transmitted allele indicator. The QTDT is used to detect whether an association is due to linkage (or not) by comparing, within families, the effects of alleles that heterozygous parents transmit to their offspring to those of alleles they do not so transmit.

### Results

The frequency of the *CYP3A5*\*1 allele was 38.5% among founders (i.e. individuals for whom data from their parents were not available) and 43.7% among all participants. Other allele and genotype frequencies in the sample data are listed in Table 1. Participants' characteristics are shown in Table 2. The 24-h urinary

**Table 1 Sample allele and genotype frequencies**

Genotype	n (%)	Allele	n (%)
*1/*1	67 (17.9)	*1	328 (43.7)
*1/*3	126 (33.6)	*3	271 (36.1)
*1/*6	19 (5.1)	*6	69 (9.2)
*1/*7	49 (13.1)	*7	82 (10.9)
*3/*3	47 (12.5)	Total	750 (100)
*3/*6	28 (7.5)		
*3/*7	23 (6.1)		
*6/*6	6 (1.6)		
*6/*7	10 (2.7)		
*7/*7	0 (0.0)		
Total	375 (100)		

creatinine excretion (above 0.16 mmol/kg for all *CYP3A5* genotypes) and urine volume (above 1.75 litres for all *CYP3A5* genotypes) suggested adequate urine collection, on average. Among the 152 participants taken off treatment, 76 were on diuretics, 44 on beta-blockers, 73 on calcium-channel blockers and 47 on angiotensin-converting enzyme inhibitors. Sixty-six were on monotherapy and 86 on two or more drugs.

Figure 1 shows daytime ambulatory BP across age tertiles. There was a significant positive trend in BP with age in *CYP3A5*\*1 carriers ( $P < 0.001$ ) but not in non-carriers ( $P > 0.05$ ), for both systolic and diastolic daytime BP. Within each age tertile, the difference in mean daytime BP between *CYP3A5*\*1 carriers and non-carriers was not significant. There was a significant positive trend in BP with night-time urinary sodium in *CYP3A5*\*1 carriers ( $P < 0.001$ ) but not in non-carriers ( $P > 0.05$ ), for both systolic and diastolic night-time BP (data not shown). Within each night-time urinary sodium excretion tertile, the difference in mean night-time BP between *CYP3A5*\*1 carriers and non-carriers was not significant. These results suggest that daytime BP was associated more strongly with age, and night-time BP more strongly with urinary sodium excretion, in *CYP3A5*\*1 carriers than in non-carriers.

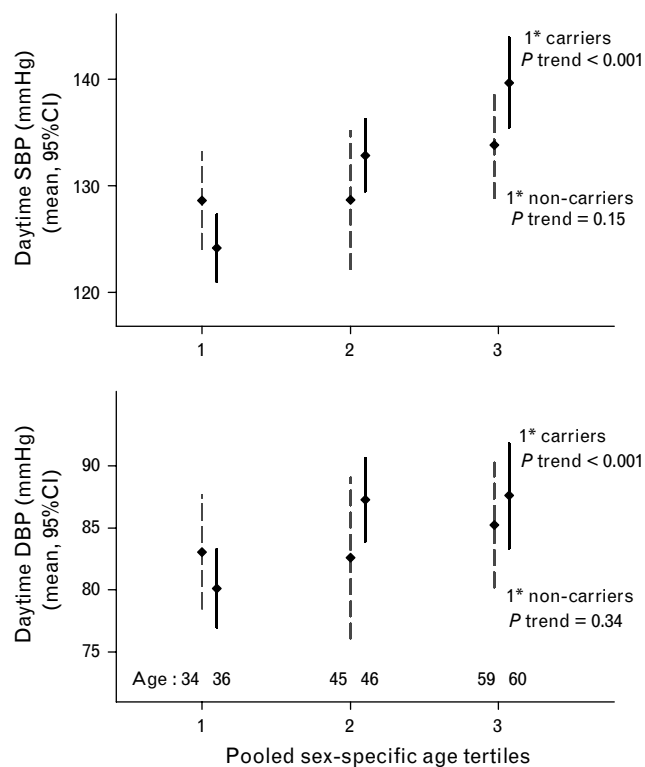
For daytime systolic BP (Table 3), multiple linear regression analysis showed a significant interaction between age and *CYP3A5*\*1 (regression coefficient  $\pm$  SE:  $0.801 \pm 0.217$ ,  $P = 0.0002$ ), and between age and *CYP3A5*\*3 ( $0.395 \pm 0.147$ ,  $P = 0.007$ ), and significant main effects of alleles \*1 ( $-29.556 \pm 10.014$ ,  $P = 0.003$ ) and \*3 ( $-14.145 \pm 6.665$ ,  $P = 0.034$ ). For daytime diastolic BP, there was a significant age  $\times$  *CYP3A5* interaction ( $0.203 \pm 0.086$ ,  $P = 0.018$ ) and a significant main effect for *CYP3A5*\*1 ( $-8.186 \pm 4.086$ ,  $P = 0.045$ ), but not for *CYP3A5*\*3. No significant interaction with age was observed for night-time BP. However, there was an interaction of BP with urinary sodium excretion, in particular in the subgroup of participants untreated at baseline (Table 3). These findings suggest that the associations between ambulatory BP and *CYP3A5* are not the same during the day and at night.

**Table 2** Participants' characteristics by *CYP3A5* genotypes. Results are means (SD) unless stated otherwise

Genotypes	<i>CYP3A5</i> *1 carriers <sup>a</sup>				<i>CYP3A5</i> *1 non-carriers <sup>a</sup>			
	1/6 or 1/7	1/3	1/1	All	6/6,6/7	3/6,3/7	3/3	All
<i>n</i>	68	126	67	261	16	51	47	114
Sex (% females)	57	56	56	56	56	55	54	55
Off treatment (%)	40	42	31	39	50	50	32	39
Age (years)	47.7 (10.6)	46.9 (11.7)	49.4 (14.4)	46.8 (11.3)	40.5 (12.8)	47.1 (13.0)	45.1 (12.2)	45.3 (12.7)
Daytime SBP	130.6 (16.1)	133.5 (20.4)	130.8 (15.2)	132.1 (18.1)	129.4 (16.4)	129.1 (14.2)	132.3 (18.5)	130.5 (16.3)
Daytime DBP	83.8 (10.5)	85.9 (12.3)	84.7 (11.0)	85.0 (11.5)	82.8 (11.4)	82.3 (8.7)	85.6 (13.5)	83.7 (11.3)
Night-time SBP	117.3 (15.7)	119.1 (18.3)	117.8 (15.7)	118.3 (17.0)	117.3 (10.1)	119.2 (14.9)	119.0 (18.6)	118.8 (16.0)
Night-time DBP	74.6 (10.7)	76.5 (12.1)	75.6 (11.0)	75.8 (11.5)	73.2 (8.8)	76.2 (9.2)	77.3 (15.0)	76.3 (12.1)
In clear (ml/min)	108 (32)	117 (40)	117 (31)	115 (36)	119 (30)	120 (40)	130 (53)	124 (43)
Cr clear (ml/min)	102 (31)	120 (45)	108* (34)	112 (39)	110 (29)	118 (49)	131 (79)	126* (75)
Urine Na (mmol/24 h)	100 (56)	105 (51)	100 (53)	102 (53)	108 (59)	122 (58)	108 (54)	114 (57)
Urine K (mmol/24 h)	42 (16)	46 (19)	42 (24)	44 (20)	42 (22)	48 (22)	44 (15)	46 (20)

<sup>a</sup>*CYP3A5*\*1 carriers are individuals who carry at least one *CYP3A5*\*1 allele, while non-carriers carry no *CYP3A5*\*1 allele. SBP, systolic blood pressure; DBP, diastolic blood pressure in mmHg. In clear, inulin clearance; Cr clear, 24-h creatinine clearance. \* $P < 0.05$  for the comparison between *CYP3A5*\*1 carriers and non-carriers if in 'All' column, otherwise comparison across genotypes.

When GFR, measured using inulin clearance, was used as the dependent trait, multiple linear regression analysis showed a significant *CYP3A5*\*1 main effect (regression coefficient  $\pm$  SE =  $-7.552 \pm 3.767$ ,  $P = 0.045$ )

**Fig. 1**

Mean ambulatory daytime blood pressure by pooled sex-specific age tertiles. Solid dots represent mean daytime blood pressure values, dashed/solid vertical lines represent 95% confidence intervals for non-carriers/carriers of the *CYP3A5*\*1 allele, respectively. SBP, systolic blood pressure, DBP, diastolic blood pressure. Numbers below each tertile are mean age (in years).

(Table 3). The *CYP3A5*\*1 main effect on GFR was slightly reduced when adjusting for body surface area (regression coefficient  $\pm$  SE =  $-6.297 \pm 3.747$ ,  $P = 0.093$ ). The association between the *CYP3A5*\*1 allele and GFR was weaker when GFR was based on the 24-h creatinine clearance (Table 3) and adjustment for body surface area further decreased the *CYP3A5*\*1 effect (regression coefficient  $\pm$  SE =  $-3.108 \pm 4.160$ ,  $P = 0.455$ ).

For the relationship between FELI (as the dependent trait) and age, there was a significant interaction between the *CYP3A5*\*1 allele and age (Fig. 2). No such interaction was found between the *CYP3A5*\*3 allele and age. This indicates that *CYP3A5*\*1 non-carriers decreased their proximal tubular sodium reabsorption with age, while *CYP3A5*\*1 carriers did not.

Although the number of informative individuals (i.e. offspring with at least one heterozygous parent for allele \*1) was small ( $n = 36$ ), the QTDT suggested association due to linkage of allele \*1 to ambulatory SBP ( $P = 0.06/0.15$  and  $P = 0.11/0.29$ , for daytime/night-time SBP and DBP, respectively). Because of the small number of informative individuals, we could not meaningfully assess interactions within QTDT analyses. Moreover, there was also evidence for association due to linkage of allele \*1 to GFR using inulin clearance ( $n = 22$ ,  $P = 0.05$ ), but not using creatinine clearance ( $P = 0.77$ ). These results suggest that, for each trait, the associations between *CYP3A5* and SBP, and between *CYP3A5* and GFR, are also due to linkage between the trait locus and the marker locus.

## Discussion

To our knowledge, the present study is the largest to date to analyze the association between the *CYP3A5* gene and ambulatory BP, and between the *CYP3A5* gene and GFR,

**Table 3 Multiple linear regression coefficients, standard errors and significance**

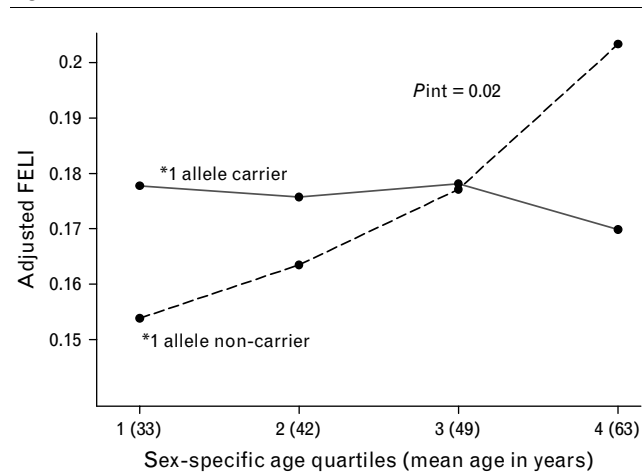
Dependent variable	Predictor variables	All participants (n = 375)			Untreated participants (n = 223)		
		Coeff.	SE	P value	Coeff.	SE	P value
Day SBP	<i>Cyp3a5</i> <sup>*1</sup> (dom)	-29.556	10.014	0.003	-22.084	13.642	0.105
	<i>Cyp3a5</i> <sup>*3</sup> (add)	-14.145	6.665	0.034	-10.853	8.247	0.188
	Age	-0.494	0.207	0.017	-0.331	0.315	0.293
	<b><i>Cyp3a5</i><sup>*1</sup> × age</b>	<b>0.801</b>	<b>0.217</b>	<b>0.0002</b>	<b>0.575</b>	<b>0.328</b>	<b>0.079</b>
	<b><i>Cyp3a5</i><sup>*3</sup> × age</b>	<b>0.395</b>	<b>0.147</b>	<b>0.007</b>	<b>0.281</b>	<b>0.197</b>	<b>0.153</b>
DBP	<i>Cyp3a5</i> <sup>*1</sup>	-8.186	4.086	0.045	-9.871	5.066	0.051
	Age	0.868	0.281	0.002	1.358	0.345	<0.0001
	Age <sup>2</sup>	-0.010	0.003	0.0006	-0.015	0.004	<0.0001
	<b><i>Cyp3a5</i><sup>*1</sup> × age</b>	<b>0.203</b>	<b>0.086</b>	<b>0.018</b>	<b>0.235</b>	<b>0.115</b>	<b>0.040</b>
Night SBP	<i>Cyp3a5</i> <sup>*1</sup>	-6.301	3.018	0.037	-9.473	3.710	0.011
	Urine Na (night)	0.039	0.043	0.354	0.003	0.055	0.963
	<b><i>Cyp3a5</i><sup>*1</sup> × Na</b>	<b>0.097</b>	<b>0.051</b>	<b>0.058</b>	<b>0.175</b>	<b>0.066</b>	<b>0.007</b>
DBP	<i>Cyp3a5</i> <sup>*1</sup>	-3.554	2.095	0.090	-5.759	2.680	0.032
	Urine Na (night)	0.027	0.030	0.358	-0.005	0.040	0.894
	<b><i>Cyp3a5</i><sup>*1</sup> × Na</b>	<b>0.042</b>	<b>0.036</b>	<b>0.232</b>	<b>0.100</b>	<b>0.047</b>	<b>0.034</b>
GFR In clear	<i>Cyp3a5</i> <sup>*1</sup>	-7.552	3.767	0.045	-8.412	4.580	0.066
	<i>Cyp3a5</i> <sup>*1</sup>	-4.493	4.196	0.284	-6.625	5.595	0.236

All regression models allowed for familial correlations and included as predictors age, sex, ascertainment and *CYP3A5*. In addition, daytime SBP was adjusted for the presence of diabetes and urinary Na; daytime DBP was adjusted for age squared; night-time SBP was adjusted for urine Na and K, and DBP for urine Na and K and age squared. The models for participants untreated at baseline included the same predictor variables as the models for the whole sample. The models for inulin and creatinine clearance included as predictors age, sex, ascertainment, body mass index (BMI), diabetes and *CYP3A5*<sup>\*1</sup>. SBP/DBP, systolic/diastolic blood pressure; dom, dominant; add, additive; GFR, glomerular filtration rate; In clear, inulin clearance; Cr clear, creatinine clearance.

among individuals of African descent. Although the *CYP3A5* gene has already been associated with BP in humans [15,16], we provide new insight on this relationship, based on extensive genotypic and phenotypic characterization. In particular, we showed that age and urinary sodium excretion modify the association between

the *CYP3A5* alleles and BP (i.e. that this association is more marked in older persons for daytime BP and in persons with high urinary sodium excretion for night-time BP). Moreover, because we obtained similar results when restricting the analyses to those who were untreated at baseline, antihypertensive treatment did not appear to confound the observed associations in our study. However, this does not imply that antihypertensive treatment cannot be an important confounder in another setting.

**Fig. 2**



Plot of fractional excretion of lithium (FELI) by pooled sex-specific age quartiles in *CYP3A5*<sup>\*1</sup> carriers versus non-carriers. Dots are adjusted FELI, i.e. FELI values obtained from linear models in ASSOC (hence allowing for familial correlations) including as predictors fractional excretion of sodium (FENA), age, sex, body mass index and ascertainment. P for interaction between *CYP3A5*<sup>\*1</sup> and age.

We analyzed the *CYP3A5* alleles \*1, \*3, \*6 and \*7. Only *CYP3A5*<sup>\*1</sup> carriers produce high levels of *CYP3A5* mRNA [11] and express large amounts of *CYP3A5*. In addition, allele \*1 has been associated with the *CYP3A5* enzymatic activity in kidneys of persons of African descent [15]. We found that the *CYP3A5*<sup>\*1</sup> allele (43.7%) was more frequent than the \*3 allele (36.1%) in our population of African descent, in contrast to Caucasian populations, which is in agreement with previously published studies [11,24]. The *CYP3A5*<sup>\*6</sup> allele generates a splice variant mRNA in which exon 7 is deleted, which causes the encoded protein to be truncated at amino acid 184 [11]. The *CYP3A5*<sup>\*7</sup> allele creates an insertion, which terminates the open reading frame of *CYP3A5* at position 348 [13]. The *CYP3A5*<sup>\*3</sup> allele generates a cryptic splice site and alternative splicing which result in protein truncation and absence of *CYP3A5*. Because the splice site prediction score is less than one (0.9), small amounts of normally spliced *CYP3A5* mRNA and resulting protein can be

detected even in people homozygous for the *CYP3A5\*3* allele [11].

Although crude BP levels were similar between carriers and non-carriers of the *CYP3A5\*1* allele, a significant positive interaction between *CYP3A5\*1* and age was found for both daytime ambulatory systolic and diastolic BP. Our results indicate that age is an effect modifier of the association between the *CYP3A5\*1* allele and daytime ambulatory BP, that is only *CYP3A5\*1* carriers demonstrate an age-related significant BP increase. *CYP3A5\*1* carriers tended to have higher BP levels despite a tendency toward lower urinary sodium excretion (i.e. a lower salt intake). The low urinary sodium excretion is likely reflecting a diet based mainly on fish, vegetables and unsalted rice, and poor in processed food. In rats, Warrington *et al.* [25] observed a significant increase with age of the enzymatic activity of one CYP3A isoform in the kidney, while CYP3A activity decreased with age in the liver. As renal CYP3A5 enzymatic activity has been shown to correlate with BP in the rat [9], one could hypothesize that CYP3A5 enzymatic activity increases with age in human kidneys as well, consistent with our findings.

Age did not significantly interact with the relation between night-time ambulatory BP and *CYP3A5\*1*. However, there was an interaction between *CYP3A5\*1* and urinary sodium excretion for night-time systolic and diastolic BP, which was stronger in the subgroup of participants untreated at baseline. These results suggest that *CYP3A5\*1* carriers tend to increase their night-time BP with increasing urinary sodium excretion (i.e. increasing dietary salt intake) more than *CYP3A5\*1* non-carriers. The reason why age modified the relation between daytime BP and *CYP3A5\*1*, while urinary sodium excretion modified the relation between night-time BP and *CYP3A5\*1*, may be the confounding effect of posture-related neuro-hormonal changes. Several studies have found a positive correlation between BP and urinary sodium excretion during the night but not during the day [26–28]. Therefore, considering that (1) salt sensitivity is related to the increase of BP with age [29] and (2) *CYP3A5\*1* carriers tended to have a higher age-associated BP increase despite lower urinary sodium excretion, both daytime and night-time results consistently suggest that *CYP3A5\*1* carriers could be more salt-sensitive than non-carriers.

We also found differences in renal sodium handling between *CYP3A5\*1* carriers and non-carriers. While FENa increased with age in subjects not carrying the *CYP3A5\*1* allele (i.e. the proximal tubular sodium reabsorption decreased with age), this relation was not found in carriers of the *CYP3A5\*1* allele. Given that proximal sodium reabsorption may be a determinant of the BP response to salt [21], this result strengthens

the view that the *CYP3A5\*1* allele is associated with salt sensitivity, as previously demonstrated by Ho *et al.* [16].

Unlike allele \*1, which had a clearly dominant effect, allele \*3 appeared to act additively on BP in non-carriers of the *CYP3A5\*1* allele. Noticeably, individuals who carried two copies of the \*3 allele had a pattern of BP increase with age similar to that of carriers of the \*1 allele in our study. This observation might reflect the fact that, although only *CYP3A5\*1* carriers express large amounts of CYP3A5 mRNA and protein, homozygous *CYP3A5\*3* carriers also produce small amounts of normally spliced CYP3A5 mRNA and resulting protein [11]. This result is consistent with the finding by Ho *et al.* [16] that persons of African descent with the \*3/\*3 genotype and who were 7–8 years older than the \*1/\*1 or \*1/\*3 genotype groups, had higher systolic BP than those carrying the \*1/\*3 or the \*1/\*1 genotypes.

Our study has some limitations. Its cross-sectional nature inherently cannot distinguish between cause and consequence. It will be important to further analyze the effect of carrying the \*1 allele on BP, salt-sensitivity and renal function in longitudinal studies. The small number of individuals informative for association due to linkage (because most pedigrees in our study had one or two missing parental genotypes) did not allow us to assess an interaction of age or urinary sodium excretion for the relation between BP and the *CYP3A5* alleles using the QTDT. Also, we have not measured other genes of the *CYP3A* family, and cannot comment on their role. It is, however, unlikely that the closely located *CYP3A4* gene could account for our findings, since a previous study found no expression of this gene in human kidney microsomes [15]. Nevertheless, we cannot exclude the possibility that an allele at another locus, in strong linkage disequilibrium with the *CYP3A5\*1* allele, could account for our observed associations.

In conclusion, age and urinary sodium excretion were significant effect modifiers of the association between the *CYP3A5\*1* allele and ambulatory BP. These findings stress the importance of accounting for these covariates. *CYP3A5\*1* carriers had a higher age-associated daytime BP increase than non-carriers, despite a tendency toward lower urinary sodium excretion. Considering that *CYP3A5\*1* carriers also showed an increased proximal tubular sodium reabsorption with age as compared to non-carriers, our data, consistent with findings by others, support the hypothesis that *CYP3A5\*1* carriers are more salt-sensitive than non-carriers. Given that enzymes of the CYP3A family are involved in the metabolism of many drugs, such as calcium-channel blockers [30], this protein might also be involved in drug interactions, which emphasizes the need to explore the relation of this gene with BP further. Additional studies are needed to

further investigate the association of CYP3A5\*1 with renal function.

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