The role of the cytochrome P450 3A5 enzyme for blood pressure regulation in the general Caucasian population

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Cytochrome P450 3A (CYP3A) enzymes are important for drug metabolism in gut and liver. The CYP3A5 isoenzyme is also expressed in the kidney and has been implicated in renal sodium reabsorption and blood pressure regulation. Its expression and activity is strongly linked to a polymorphism (i.e. 6986G>A). Thus, appreciable expression is found in carriers of the *CYP3A5*1* (6986A) but not in homozygous carriers of the *CYP3A5*3* (6986G) allele. We tested whether the presence of *CYP3A5*1* affects blood pressure in Caucasian individuals who were enrolled in the Prevention of REnal and Vascular ENd stage Disease (PREVEND) study. In addition, we evaluated whether the genetic effect of *CYP3A5*1* on blood pressure is modulated by sodium intake. *CYP3A5*1* was found in 13.3% (901 individuals) of the cohort (6777 individuals). Diastolic blood pressure was not affected by *CYP3A5*1*. Overall, systolic and pulse pressure were significantly lower in carriers of *CYP3A5*1*, both after univariate analysis adjusted for age ($P=0.012$ and $P=0.008$) and in logistic regression analysis ($P=0.015$ and $P=0.012$). The effect on systolic blood pressure was significantly modulated by sodium intake ($P=0.038$). In separate analysis according to gender, *CYP3A5*1* accounted for a significant age adjusted decrease in systolic blood pressure (−1.6 mmHg, $P=0.04$) and pulse pressure (−1.2 mmHg, $P=0.04$) in females but not in men. The present study demonstrates that the *CYP3A5*1* allele affects systolic blood pressure and pulse pressure in the general population. Its role in hypertensive disease and potential gender differences should be investigated in further studies. *Pharmacogenetics and Genomics* 15:831–837 © 2005 Lippincott Williams & Wilkins.

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Introduction

The cytochrome P450 3A (CYP3A) CYP3A4 and CYP3A5 isoenzymes are involved in the metabolism of many drugs (1,2). Both CYP3A4 and CYP3A5 are expressed in the liver and small intestine where expression shows a high degree of variability (1,2). Unlike CYP3A4, variable expression and enzyme activity of CYP3A5 is considerably influenced by genetic factors (1–4). Among several polymorphisms of *CYP3A5*, the frequent single nucleotide polymorphism 6986G > A showed the highest specificity and selectivity as a marker of the CYP3A5 polymorphism and this is therefore of primary clinical importance [1,2]. Homozygous or heterozygous carriers of 6986A (i.e. the *CYP3A5*1* allele) show a significant expression of CYP3A5 in liver, which is on average between 10- and 13-fold higher than in homozygous carriers of the 6986G (i.e. *CYP3A5*3*) [3–5]. Recently, it was demonstrated that the *CYP3A5*1* allele is also significantly associated with CYP3A5 expression in human kidney, whereas the CYP3A4 isoenzyme was not detected in renal microsomes [6]. Consequently, individuals that carry at least one *CYP3A5*1* allele are considered as expressors of CYP3A5 in both the kidney and extrarenal organs. According to previous reports, the *CYP3A5*1* allele is found in 10–25% of Caucasians and at higher frequencies in other ethnic groups [1,7]. Several studies have suggested that CYP3A5 is involved in blood pressure regulation [6,8,9], although the potential mechanisms and blood pressure effects are not well characterized. Animal studies in the spontaneously hypertensive rat (SHR) have suggested a link between overall increased renal CYP3A activity, increased 6β-hydroxysteroid formation and hypertension in this model [8]. Functional studies in epithelial kidney cells have indicated a role for CYP3A5 in 6β-hydroxysteroid formation, which may protect the mineralocorticoid receptor from activation by glucocorticoids and thus impair renal sodium reabsorption mediated by mineralocorticoid receptor [10]. In humans, the first small-scale pilot-study on *CYP3A5* was performed in African-Americans and demonstrated a significant association

1744-6872

between the *CYP3A5*1* allele and systolic but not diastolic blood pressure [6]. In a subsequent report, Ho *et al.* [11] showed that the *CYP3A5*1* allele was significantly more common in hypertensives than normotensives in African-American subjects but not in whites. In addition, they reported that *CYP3A5*1* was associated with salt-sensitivity in whites but not in black hypertensives [11]. Therefore, the present study hypothesised that the presence of the *CYP3A5*1* allele affects blood pressure in the general Caucasian population. To this end, the effect of the *CYP3A5*1* allele on systolic, diastolic blood pressure and pulse pressure was investigated in a large prospective population-based cohort of Caucasian individuals who were enrolled in the Prevention of REnal and Vascular ENd stage Disease (PREVEND) study [12]. In addition, the role of sodium intake on the effect of CYP3A5 on blood pressure phenotypes was evaluated, which was feasible in the PREVEND cohort because urinary sodium excretion was determined during two consecutive 24-h sampling periods [12].

Methods

Study population

The present investigation is part of the ongoing PREVEND study, conducted in the city of Groningen, The Netherlands. All inhabitants, who were aged 28–75 years ($n = 85\,421$), were asked to provide a morning urine sample and to fill out a short questionnaire on demographics and cardiovascular history. Subjects with insulin dependent diabetes mellitus or pregnant women were excluded from participation in this screening program. In total, 11 163 subjects were invited to the outpatient clinic, of which 8592 subjects completed the screening program. The study cohort was drawn from this screening program. Details of this protocol have been described elsewhere [12]. The screening program in the outpatient clinic consisted of two consecutive visits. At both visits, blood pressure was measured in the supine position over 10 min and 8 min, respectively, with an automatic DINAMAP XL Model 9300 series monitor (Critikon, Tampa, Florida, USA) [12].

In addition, subjects were asked to collect 24-h urine on two consecutive days during the week before the second visit. Measurements of urinary volume and albumin and creatinine concentrations were performed on each collection. At the second visit, blood was drawn after an overnight fast, for determination of serum creatinine, and cholesterol [12]. Laboratory measurements were performed as previously reported [12]. Non-Caucasian individuals ($n = 380$) and patients with suspected renal disease ($n = 56$), or those individuals who were being treated with antihypertensive drugs ($n = 1020$), were not included in the analysis. In addition, subjects who had missing data for any of the parameters used in the analyses were excluded. Thus, 6777 subjects were eligible for further study.

Definitions

Hypertension was defined as a systolic blood pressure of $\geq$ 140 mmHg or a diastolic blood pressure of $\geq$ 90 mmHg and blood pressure values given are the mean of the last two recordings of both days. Urinary albumin excretion and sodium excretion values are given as the mean of the two 24-h urine excretion periods, respectively. Microalbuminuria was defined as an urinary albumin excretion of 30–300 mg per 24 h averaged over the two consecutive 24-h urine collections. Body mass index was calculated as weight (kg) divided by square of height (m^2).

CYP3A5 genotype determination

DNA was extracted from blood samples according to standard protocols. Genotyping was performed by real-time polymerase chain reaction (PCR) as previously reported [13]. The ABI PRISM 7000 SDS instrument in conjunction with the ABI TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) was used to perform the assays [14]. Appropriate primers and fluorogenic probes were designed with Primer Express software (Applied Biosystems). Fluorogenic probes were synthesized by TIB Molbiol (Berlin, Germany) and primers were obtained from Proligo (Paris, France). Primers were selected according to the sequence available at GenBank accession number NG_000004 [15]. The following primers and probes were used for genetic determination of the *CYP3A*1* and *CYP3A5*3* alleles (nt 260167G >): (i) fluorogenic probes, 5′-VIC-TGTCT TTCAgTATCTCTT (18-mer, nt 260158–260175) and 5′-FAM-TGTCTTTCAaTATCTCTTC (19-mer, nt 260158–260176) and (ii) primers, ACCCAGCTTAAC GAATGCTCTACT (forward, 24-mer, nt 260098–260121) and GAAGGGTAATGTGGTCCAAACAG (reverse, 23-mer, nt 260178–260200). TaqMan assays were performed in 96-well plates (Perkin-Elmer, Applied Biosystems). Each well contained a final PCR-reaction volume of 20 µl with 50 ng of genomic DNA, 400 nM primers, 100 nM probes and 10 µl of TaqMan Universal PCR master mix (Perkin-Elmer, Applied Biosystems). Amplification was performed at 50°C for 2 min; 95°C for 10 min, followed by 40 cycles of 94°C for 15 s and 58°C for 1 min. Data were analysed using the ABI Prism 7000 SDS 1.0 Software (Perkin-Elmer, Applied Biosystems).

Genetic analysis

Data are presented according to the three genotypes, *CYP3A5*1/*1*, *CYP3A5*1/*3* and *CYP3A5*3/*3*. However, given the expectation that *CYP3A5*1* allele carriers have a higher CYP3A5 enzyme activity, the *CYP3A5*1/*1* and *CYP3A5*1/*3* groups were analysed as a single group against the *CYP3A5*3/*3* homozygotes. Thus, the influence of presence or absence of the *CYP3A5*1* allele on blood pressure was examined. The chi-square test was used to confirm Hardy–Weinberg equilibrium of the observed allele frequencies.

Overall statistics

Continuous data are reported as mean ± SD. In the case of skewed distribution, medians with 25th and 75th percentile are presented. Comparisons of different variables among the different genotypes were performed by Pearson chi-square analysis or analysis of variance (ANOVA) where appropriate. All *P*-values are two-tailed. $P < 0.05$ was considered statistically significant. In addition, separate analysis according to gender was performed. All calculations were performed using SPSS statistical software, version 12.1 (SPSS Inc., Chicago, Illinois, USA).

Regression modelling

Linear regression analyses were used to examine the effect of the *CYP3A5*1* allele on continuous blood pressure phenotypes, while adjusting for other confounders. In the crude overall analysis, only age was used as covariate. In further analyses, additional parameters known to influence blood pressure values were included (gender, body mass index, smoking status and urinary sodium excretion). Because age and urinary sodium excretion showed a significant quadratic relationship to blood pressure in exploratory uni- and multivariate analyses, squared values of these factors were implemented in subsequent linear regression model. Logistic regression analyses was used to examine the influence of the *CYP3A5*1* allele on the presence of hypertension. Genotype groups were entered in both types of regression analyses as binary dummy variables. To evaluate the effect of *CYP3A5*1* on systolic blood pressure under different urinary sodium conditions, at the same time as adjusting for other confounders, the coefficients and constants calculated by a linear regression model for systolic blood pressure with all confounders as covariates were used to obtain a function for systolic blood pressure in which only urinary sodium excretion and genotype was allowed to fluctuate. All other confounding parameters were set at their population mean.

Results

The distribution of the *CYP3A5*1/*1* (0.6%), *CYP3A5*1/*3* (12.7%) and *CYP3A5*3/*3* (86.7%) genotypes was in Hardy–Weinberg equilibrium. The allele frequencies of the *CYP3A5*1* and *CYP3A5*3* allele were 7% and 93%, respectively.

The characteristics of male and female individuals are summarized in Table 1 for the overall cohort and according to the three *CYP3A5* genotypes. No significant differences in mean age, frequency of hypertension, body mass index, smoking status, cholesterol and renal function parameters, including serum creatinine, creatinine clearance, urinary albumin excretion and frequency of microalbuminuria, was found between genotype groups. In male carriers of the *CYP3A5*1* allele, 24 h sodium excretion was lower compared to individuals carrying the *CYP3A5*3/*3* genotype ($P < 0.05$), whereas sodium excretion values were similar across genotype groups in female individuals.

In the overall crude linear regression analysis, including both males and females after adjustment for age, the presence of the *CYP3A5*1* allele was associated with both lower systolic blood pressure (−1.53 mmHg, $P = 0.012$) and lower pulse pressure (−1.13 mmHg, $P = 0.008$), whereas diastolic blood pressure was not affected by the *CYP3A5*1* allele (−0.4 mmHg, $P = 0.20$). This effect of the *CYP3A5*1* allele on blood pressure phenotypes was confirmed after logistic regression analysis and adjustment for additional confounders. When logistic regression analysis was performed in men and women separately, the *CYP3A5*1* allele affected systolic blood pressure and pulse pressure only in women (Table 2). However, no significant interaction between genotype and gender in relation to systolic blood pressure or pulse pressure was observed. Moreover, the effect on blood pressure in females was independent from menopausal status or use of oestrogen replacement therapy.

As expected in a Caucasian population, the number of individuals carrying the homozygous *CYP3A5*1/CYP3A5*1* genotype was too small to perform a conclusive separate analysis in this group. Nevertheless, as shown in Table 2, the lowest mean systolic blood pressure and pulse pressure values in both men ($n = 23$) and women ($n = 20$) were found in the homozygous *CYP3A5*1/CYP3A5*1* individuals. This supports the finding of a blood pressure lowering effect of *CYP3A5*1* and opens the possibility that a gene-dosage effect might be of relevance.

The overall prevalence of hypertension in the study population, which included only hypertensive without drug treatment, was 22% and was significantly higher in males compared to females (28.5% versus 16.3%, $P < 0.001$, Pearson Chi-square analysis). The adjusted odds ratios in logistic regression analysis for hypertension among men (0.90) and women (0.87) for the presence of the *CYP3A5*1* were similar and not significant ($P = 0.37$ and $P = 0.28$, respectively). To account for possible stratification due to population selection, allele frequencies in excluded subjects on anti-hypertensive treatment were compared with the study population, but no significant differences were found.

The ecogenetic interaction between genotype and urinary sodium excretion was also tested in relation to blood pressure. No interaction between genotype and sodium excretion was detected when the linear effect of sodium excretion on blood pressure was assessed ($P = 0.55$). The linear regression model showed a better

Table 1 Overall characteristics of male and female subjects and according to *CYP3A5* genotypes in the PREVEND study

Characteristic	Total	*CYP3A5* genotype		
		**1/*1*	**1/*3*	**3/*3*
Men	3344	23	414	2907
Age (years)	49.0 ± 12.6	51.9 ± 11.6	49.5 ± 13.0	48.1 ± 11.0
Hypertension, *n* (%)	954 (28.5)	6 (26.1)	118 (28.5)	830 (28.5)
Body mass index (kg/m^2)	26.0 ± 3.6	24.8 ± 2.6	26.0 ± 3.4	26.0 ± 3.6
Smoking, *n* (%)	1311 (39.2)	11 (47.8)	156 (37.7)	1144 (39.4)
Cholesterol (mmol/l)	5.7 ± 1.1	5.6 ± 1.2	5.6 ± 1.1	5.7 ± 1.1
Median urinary albumin excretion (25–75th percentiles) (mg per 24 h)	10.1 (6.8–19.4)	7.5 (5.7–14.1)	9.9 (6.6–18.8)	10.1 (6.8–19.6)
Microalbuminuria (%)	14.9	9.1	13.1	13.5
Serum creatinine concentration (μmol/l)	90.9 ± 20.8	90.5 ± 12.6	90.0 ± 12.4	91.0 ± 21.8
Creatinine clearance (ml/min per 1.73 m^2)	95.9 ± 20.5	92.1 ± 19.8	95.7 ± 20.3	96.0 ± 20.5
Urinary sodium excretion (mmol per 24 h)	158.4 ± 52.4	148.2 ± 47.4	151.9 ± 50.8	159.4 ± 52.6[a]
Women	3433	20	444	2969
Age (years)	46.9 ± 11.8	48.1 ± 11.0	46.4 ± 11.8	47.0 ± 11.9
Hypertension, *n* (%)	560 (16.3)	2 (10)	67 (15.1)	491 (16.5)
Body mass index (kg/m^2)	25.4 ± 4.4	25.3 ± 5.1	25.4 ± 4.5	25.5 ± 4.4
Smoking, *n* (%)	1370 (39.9%)	11 (55)	190 (42.8)	1169 (39.4)
Cholesterol (mmol/l)	5.7 ± 1.2	5.7 ± 1.2	5.5 ± 1.1	5.6 ± 1.2
Median urinary albumin excretion (25–75th percentiles) (mg per 24 h)	8.3 (5.8–13.4)	8.5 (6.3–16.3)	8.0 (5.8–14.7)	8.2 (5.8–13.3)
Microalbuminuria (%)	8.5	10	10.1	8.3
Serum creatinine concentration (μmol/l)	75.3 ± 10.6	74.6 ± 9.7	75.5 ± 9.3	75.4 ± 10.8
Creatinine clearance (ml/min per 1.73 m^2)	91.4 ± 19.6	86.8 ± 15.6	92.9 ± 20.6	91.2 ± 19.5
Urinary sodium excretion (mmol per 24 h)	125.4 ± 42.7	117.2 ± 44.5	126.7 ± 44.3	125.2 ± 42.4

Presented are means ± SD, median (25–75th percentile) or percentages. Pearson chi-square test did not reveal any significant differences between *CYP3A5* genotype groups. [a]ANOVA test between-group differences, $P < 0.05$. PREVEND, Prevention of Renal and Vascular End-Stage Disease.

Table 2 Effect of *CYP3A5* genotype on systolic, diastolic and pulse pressure in the PREVEND study

	B	*P*	Population mean		
	**1/*1 + *1/*3 versus *3/*3*	**1/*1 + *1/*3 versus *3/*3*	**1/*1*	**1/*3*	**3/*3*
Systolic blood pressure					
Men			129.3 ± 19.5	131.7 ± 16.7	132.2 ± 17.8
Age	1.0	0.21			
Fully adjusted	1.0	0.21			
Women			119.1 ± 14.3	120.0 ± 17.5	121.9 ± 19.1
Age	1.6	0.04			
Fully adjusted	1.6	0.04			
Diastolic blood pressure					
Men			77.0 ± 9.5	76.2 ± 9.1	76.1 ± 9.4
Age	0	0.95			
Fully adjusted	0	0.97			
Women			72.7 ± 8.2	69.3 ± 8.7	70.4 ± 8.7
Age	0.7	0.08			
Fully adjusted	0.7	0.07			
Pulse pressure					
Men			52.2 ± 14.5	55.5 ± 11.6	56.1 ± 12.5
Age	1.1	0.07			
Fully adjusted	1.0	0.08			
Women			46.4 ± 12.0	50.6 ± 12.2	51.6 ± 13.7
Age	1.2	0.04			
Fully adjusted	1.1	0.05			

B indicates the blood pressure effect in mmHg obtained after linear regression analysis after adjustment for either age or after full adjustment by comparing carriers of the *CYP3A5*1* allele (i.e. *CYP3A5*1/CYP3A5*1* and *CYP3A5*1/CYP3A5*3* genotype groups) with non-carriers (i.e. *CYP3A5*3/CYP3A5*3* genotype group). *P* indicates the significance of the contribution of the genotype to the model. Arithmetic population mean ± SD of the pressure in mmHg per genotype group is shown; $n = 6777$.

fit when sodium excretion was analysed as a quadratic variable in relation to blood pressure phenotypes. In this analysis, a significant interaction between genotype and squared urinary sodium excretion was observed in the multivariate analysis ($P = 0.01$). *CYP3A5*1* allele carriers demonstrated consistently lower systolic blood pressures in the range of sodium excretion between 100 and 200 mmol per 24 h compared to homozygous *CYP3A5*3/*3* individuals, with a maximum reduction of 2 mmHg ($P = 0.038$), whereas this difference in systolic blood pressure between genotype groups disappeared in the more extreme ranges of sodium excretion below 100 mmol per 24 h and above 200 mmol per 24 h.

Discussion

In the present study, we analysed the relevance of *CYP3A5*1* for blood pressure levels in the largest population reported so far. We demonstrated that approximately 13% of the Caucasian population carry

the *CYP3A5*1* allele and can therefore be considered as expressors of CYP3A5 [1,2]. Blood pressure analysis showed that carriers of the *CYP3A5*1* allele exhibit significantly lower systolic blood pressure and pulse pressure values compared to homozygous *CYP3A5*3/*3* individuals (i.e. reduced expressors of CYP3A5) [1,2].

Previous studies have suggested a role of cortisol as an intermediate phenotype in blood pressure control and the pathogenesis of essential hypertension [16,17]. Although the abnormal regulation and/or metabolism of cortisol has been implicated in blood pressure regulation and hypertension for some time, the mechanisms leading to cortisol-induced blood pressure increases are still not fully understood [18,19]. It is well established that the intrarenal conversion of active cortisol into inactive cortisone by the renal isoform of 11β-HSD (i.e. 11β-HSD type 2; 11β-HSD2) is crucial in preventing cortisol-induced sodium retention and blood pressure increases by protecting the non-selective mineralocorticoid receptor from occupation by cortisol [18,20]. Decreased activity of 11β-HSD2 in the kidney either as an inherited syndrome (apparent mineralocorticoid excess syndrome; AME) [18,21] or acquired following licorice consumption, allows cortisol access to the mineralocorticoid receptor, resulting in hypertension [18–20]. It appears possible that the presence of a second enzyme (i.e. CYP3A5, in carriers of the *CYP3A5*1* allele) provides an additional pathway to protect mineralocorticoid receptor from occupation by cortisol [22]. This could result in lower blood pressures and/or diminished blood pressure increases in response to high-salt intake. This notion is supported by studies performed *in vitro* showing that 6β-hydroxylation of glucocorticoids mediated by CYP3A enzyme activity may indeed protect the mineralocorticoid receptor from activation by glucocorticoids [22]. By contrast, studies in the SHR model suggest that excessive intrarenal conversion of cortisol to 6β-hydroxycorticosterone by CYP3A isoenzymes may mediate increased tubular reabsorption of sodium, and thus lead to blood pressure increases and salt-sensitive hypertension [8]. However, the CYP3A5 isoenzyme expression patterns in the kidney differ between the rat and humans [23,24] and it is difficult to compare overall CYP3A enzyme activity and function between the rat and human kidney. Moreover, the potential role of the rat homologs of CYP3A isoenzymes expressed in the rat kidney for the genetic basis of hypertension in SHR has not been supported [25,26].

Thus, the findings obtained in the present study are at variance with the data obtained in SHR and support the hypothesis that CYP3A5 expression is capable of contributing to lower blood pressure levels in humans. The effect of *CYP3A5*1* on blood pressure was significant when the overall cohort was analysed; however, in the separate analysis according to gender, it was revealed that the significant effect of *CYP3A5*1* was attributable to females only. Although no significant interaction between gender and *CYP3A5* genotype was found in our statistical analysis, this finding nevertheless indicates a potential gender specific effect on blood pressure, as has previously been described for autosomal loci in humans [27,28] and experimental hypertension in the rat [29]. The mechanism of this potential sex-specific effect on blood pressure mediated by *CYP3A5*1* is unclear. Our finding obtained in females was independent of menopausal status or the use of oestrogen replacement therapy. However, previous experimental studies conducted in genetically hypertensive rats have demonstrated that blood pressure effects mediated by ecogenetic interactions between autosomal gene loci and sodium intake are indeed modified by gender [30]. The observed interaction between sodium intake, as estimated by urinary sodium excretion values over a period of 48 h in our study, and *CYP3A5* genotype modulating systolic blood pressure, and not diastolic pressure, is compatible with the finding that systolic blood pressure is more sensitive to variation in dietary sodium content [31], which might be particularly important in women [32]. On the other hand, our finding demonstrating that the significant association between *CYP3A5*1* and systolic blood pressure and pulse pressure was confined to women in the sub-group analysis should not be overemphasized because we did not observe a significant interaction between gender and genotype and cannot exclude the possibility that this is a false positive result.

Our finding that *CYP3A5*1* might confer a blood pressure-lowering effect is at variance with the first study in a small group of African-American individuals demonstrating higher systolic and diastolic blood pressure in carriers of the *CYP3A5*1* allele [6]. In this report, only 25 healthy and normotensive adults, including nine homozygous *CYP3A5*1/*1* individuals, were studied and the observed increase in systolic blood pressure associated with the presence of the *CYP3A5*1* allele was largely attributable to these same homozygous individuals [6]. In a subsequent report by Ho *et al.* [11], significant higher blood pressures were reported in blacks carrying the homozygous *CYP3A5*3/*3* genotype, which would be compatible with our findings. In the study by Ho *et al.* [11] two independent populations of black and white individuals with either normotension or hypertension, including both genders, were analysed. However, no consistent findings regarding the role of *CYP3A5*1* for blood pressure regulation and hypertension were obtained [11]. In one population from the study by Ho *et al.* [11], *CYP3A5*1* was significantly more common in hypertensives than in normotensive blacks, whereas no association with hypertension was found in whites. Moreover, the *CYP3A5*1* allele had no overall influence on salt sensitivity status in normotensive and hypertensive individuals, except for in white hypertensives where the proportion of salt-sensitive subjects was higher in *CYP3A5*1* carriers. More recently, in a population of elderly individuals age $\geq$ 75 years from

Finland, the proportion of individuals with the *CYP3A5*1/*3* genotype was significantly higher in hypertensives compared to normotensive elderly subjects, suggesting a role of *CYP3A5*1* for the development of hypertension in elderly subjects [33]. Thus, taken together, there are data to support a blood pressure-increasing and -lowering role for *CYP3A5*1*.

In this regard, the relevance of CYP3A5 for blood pressure may vary between different ethnic populations due to significant differences in allele frequencies of *CYP3A5*1* [1,2] and the variable impact of environmental factors such as salt intake on blood pressure [34]. Hence, the allele frequencies of *CYP3A5*1* are considerably higher in African-Americans (45–73%) [2] compared to Caucasians (7% in the present study) and the prevalence of salt-sensitive blood pressure regulation is also higher in African-Americans [34]. In addition, the sample size of association studies needs to be taken into account [35,36]. A strength of the present study is the large sample size of 6777 individuals compared to previous reports, which included 25, 678 and 373 individuals, respectively [6,11,33]. Nevertheless, a note of caution is warranted before our findings can be extrapolated to other populations, especially because the observed effect of *CYP3A5*1* was small and the presence of *CYP3A5*1* did not significantly decrease the odds ratio for hypertension in the PREVEND study population. At first glance, and from a single gene perspective, the significance of this genetic variation for clinical practice also appears to be very small. However, the genetic basis of hypertension is assumed to involve the combined effects of multiple genes, each with a small effect [37,38]. In this regard, our findings suggest that *CYP3A5* is one of those genes. On the population level, small effects can be of epidemiological importance because death from both ischaemic heart disease and stroke increases progressively and linearly from systolic blood pressure levels as low as 115 mmHg [39]. Thus, additional epidemiological and clinical studies are warranted to further evaluate the role of *CYP3A5*1* for blood pressure regulation in the general population as well as hypertensive disease in both females and males.

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