# CYP17A1 Enzyme Activity Is Linked to Ambulatory Blood Pressure in a Family-Based Population Study

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# BACKGROUND

Genome-wide association studies have linked *CYP17A1* coding for the steroid hormone synthesizing enzyme  $17\alpha$ -hydroxylase (CYP17A1) to blood pressure (BP). We hypothesized that the genetic signal may translate into a correlation of ambulatory BP (ABP) with apparent CYP17A1 activity in a family-based population study and estimated the heritability of CYP17A1 activity.

# METHODS

In the Swiss Kidney Project on Genes in Hypertension, day and night urinary excretions of steroid hormone metabolites were measured in 518 participants (220 men, 298 women), randomly selected from the general population. CYP17A1 activity was assessed by 2 ratios of urinary steroid metabolites: one estimating the combined 17 $\alpha$ -hydroxylase/17,20-lyase activity (ratio 1) and the other predominantly 17 $\alpha$ -hydroxylase activity (ratio 2). A mixed linear model was used to investigate the association of ABP with log-transformed CYP17A1 activities exploring effect modification by urinary sodium excretion.

Arterial hypertension is a major public health threat due to its high prevalence and associated increased risk for cardiovascular disease.<sup>1</sup> Primary hypertension, also known as essential or idiopathic hypertension, accounts for about 95% of all cases of hypertension. As a complex trait, it is influenced by environmental (e.g., high salt intake) and genetic factors.<sup>2,3</sup> From genome-wide association studies, *CYP17A1* was identified as a sensitive locus, linked to blood pressure (BP) or arterial hypertension in the general population.<sup>4–6</sup> Functionally, this gene is translated into the cytochrome P450 (CYP) type II enzyme, 17 $\alpha$ -hydroxylase/17,20-lyase (CYP17A1). CYP17A1 is required for the production of

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#### RESULTS

Daytime ABP was positively associated with ratio 1 under conditions of high, but not low urinary sodium excretion (*P* interaction <0.05). Ratio 2 was not associated with ABP. Heritability estimates (SE) for day and night CYP17A1 activities were 0.39 (0.10) and 0.40 (0.09) for ratio 1, and 0.71 (0.09) and 0.55 (0.09) for ratio 2 (*P* values <0.001). CYP17A1 activities, assessed with ratio 1, were lower in older participants.

# CONCLUSIONS

Low apparent CYP17A1 activity (assessed with ratio 1) is associated with elevated daytime ABP when salt intake is high. CYP17A1 activity is heritable and diminished in the elderly. These observations highlight the modifying effect of salt intake on the association of CYP17A1 with BP.

*Keywords:* aging; blood pressure; CYP17A1; heritability; hypertension; salt; steroids.

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cortisol in the adrenal cortex and androgen precursors of sex hormones in both the adrenal glands and gonads.<sup>7</sup> Therefore, it would be of interest to investigate the association of BP and apparent CYP17A1 activity, by assessing precursor-to-product hormone metabolites ratios in the general population.

Until highlighted by genome-wide association studies, the association of CYP17A1 activity and BP was mainly investigated in patients with a  $17\alpha$ -hydroxylase deficiency (OMIM #202110), in whom a loss-of-function mutant of CYP17A1 leads to elevation of adrenocorticotropic hormone, with consecutive overproduction of mineralocorticoid active

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© American Journal of Hypertension, Ltd 2015. All rights reserved. For Permissions, please email: journals.permissions@oup.com hormones,<sup>8</sup> leading to salt sensitive hypertension.<sup>9</sup> As a consequence, arterial hypertension in 17a-hydroxylasedeficient patients is responsive to supplementation of glucocorticoids.7 Further information about the development of hypertension by an altered CYP17A1 activity came recently from men with advanced prostate cancer who were treated with an inhibitor of CYP17A1 (abiraterone) to stop androgen production in adrenal glands. As a side effect, some developed hypertension due to mineralcorticoid excess.<sup>10</sup> To prevent the overproduction by the stimulation with adrenocorticotropic hormone, patients were treated in further studies with glucocorticoids.<sup>11</sup> However in 2 large trials, hypertension was more frequently reported in the abiraterone plus prednisone than in the prednisone alone group, suggesting that increased BP may be unresponsive to exogenous glucocorticoid supplementation in such patients.<sup>12,13</sup>

CYP17A1 activity catalyzes 2 enzymatic reactions: first, hydroxylation of pregnenolone and progesterone to 17a-hydroxypregnenolone and progesterone (17a-hydroxylase activity), respectively, and second, enhancement of side-chain cleavage of 17-hydroxylated steroids to generate dehydroepiandrosterone (DHEA) and androstenedione, the precursors of testosterone (17,20-lyase activity).<sup>14</sup> Comparing the precursor-to-product ratios of the steroid metabolites, CYP17A1 activity in humans can be assessed by calculating the ratios of the total urinary excretion of 17-hydroxylated steroids with or without sidechain cleavage (i.e., androsterone (An) and etiocholanolone (Et)) and the metabolites of cortisol (tetrahydrocortisone (THE), tetrahydrocortisol (THF) and 5a-tetrahydrocortisol (5a-THF)), with C-21 steroids without 17-hydroxyl groups (i.e., total urinary metabolites of corticosterone (tetrahydro-11-dehydrocorticosterone (THA), tetrahydrocorticosterone (THB), and 5α-tetrahydrocorticosterone (5α-THB)).<sup>15</sup>

The availability of steroid hormone profiles in a large cohort of participants with ambulatory BP (ABP) measurement of European descent offered us the possibility to explore the distribution of CYP17A1 activities in a contemporary population and investigate its association with ABP, while urinary sodium excretion served as proxies for dietary salt intake.<sup>16</sup> The family-based study design allowed assessment of the heritability of apparent CYP17A1 activities.

# **METHODS**

#### **Study population**

Swiss Kidney Project on Genes in Hypertension (SKIPOGH) is a family-based cross-sectional study exploring the role of genes and kidney hemodynamics in BP regulation and kidney function in the general population. A detailed description of the methods is provided elsewhere<sup>17,18</sup> and will be briefly described here. From December 2009 to March 2013, adult participants were recruited in 2 regions (Bern and Geneva) and in 1 city (Lausanne) of Switzerland. A random sample of the inhabitants were drawn using different strategies. Inclusion criteria were: (i) having a minimum age of 18 years; (ii) being of European ancestry; (iii) having at least 1 first degree family member willing to participate; and (iv) providing written, informed consent. Pregnant or

breastfeeding women were not included. The general participation rate was 25.6%.

The SKIPOGH study has been carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association, and has been approved by the Ethics Committees of each participating university hospital.

## **Measurements and definitions**

The study visit was performed in the morning after an overnight fast. Body weight was measured in kilograms to the nearest 100g using electronic scales (Seca, Hamburg, Germany). Height was measured to the nearest 05 mm using a Seca height gauge. Body mass index was calculated as weight (kilograms) divided by the height squared (square meters). BP was measured with a validated non-mercury manual auscultatory sphygmomanometer (A&D UM-101, A&D Company, Toshima Ku, Tokyo, Japan).<sup>19</sup> Each subjects conventional office BP was the mean of the 5 consecutive readings, and hypertension was defined as a mean office BP ≥140/90 mm Hg.<sup>20</sup> ABP was measured using Diasys Integra devices (Novacor, Rueil-Malmaison, France). Measurements were taken every 15 minutes during the day, and every 30 minutes during the night (from 10 PM to 7 AM). Participants were included in the analyses if they had at least 14 systolic BP (SBP) and diastolic BP (DBP) measurements during the day and at least 7 readings during the night in accordance with European Society of Hypertension recommendations.<sup>20</sup> During the measurement, a urine sample was saved separately for day- and nighttime covering 24 hours. To take potential incomplete urine collection into account, we excluded participants with a 24-hour urine volume below 300 ml and added urinary creatinine excretion per kilogram body weight as covariate in the analyses.<sup>21</sup> Renal function tests, as well as serum and urinary electrolytes, were analyzed by standard clinical laboratory methods in each center. Creatinine was measured using isotope dilution mass spectrometry-traceable methods. The Chronic Kidney Disease Epidemiology Collaboration formula was used to calculate the estimated glomerular filtration rate.<sup>22</sup>

# Gas chromatography-mass spectrometry of steroid metabolites

Urinary steroid metabolites were extracted and analyzed by gas chromatography-mass spectrometry according to the method described by Shackleton.<sup>23</sup> Measured steroid metabolites were divided by urinary creatinine excretion. To assess the apparent CYP17A1 activity, the following precursor-to-product metabolite ratios were derived from the steroid measurements: ratio 1 (THA + THB + 5 $\alpha$ -THB)/(An + Et) and ratio 2 (THA + THB + 5 $\alpha$ -THB)/(THE + THF + 5 $\alpha$ -THF)<sup>24</sup> (Supplementary Figure 1). To more specifically target 17,20-lyase activity, we explored the distribution of the ratios of the total cortisol precursors to the androgen precursors and metabolites (pregnanediol (PD) + 17-hydroxyprogesterone (17HP) + pregnanetriol (PT)/(DHEA + An + Et)) and its relationship to ABP.

#### Statistical analyses

All the continuous variables with normal distribution (assessed graphically) are expressed as mean and ±SD and

as median and 25th to 75th interquartile ranges whenever distribution was skewed. Categorical variables are expressed as numbers and frequencies. Student's *t*-tests or Mann-Whitney *U*-tests, whenever appropriate, and chi-square tests were performed to compare baseline characteristics for continuous and categorical variables, respectively.

# Association analyses

Ratios 1 and 2 were log-transformed for statistical analysis. Univariate analyses were performed to examine the associations between either log-transformed ratio 1 or 2 with systolic and diastolic ABP during night and day. Pearson tests with P values were performed to obtain correlations for continuous variables. Statistical significance was considered for a 2-sided P < 0.05. For multivariable analyses, we used mixed linear models to analyze the association of systolic and diastolic ABP with log-transformed CYP17A1 ratios 1 and 2, taken one at a time, using separate models for day and night, while taking family correlations into account by way of a random family effect. We included age, sex, center, body mass index, urine flow rate, urinary potassium excretion, urinary creatinine excretion (24 hour per kilogram body weight), antihypertensive treatment, and estimated glomerular filtration rate as covariates in the models. We explored whether urinary sodium excretion modified the association of ABP with log-transformed CYP17A1 ratios by adding the appropriate interaction term in the model. For graphical illustration, we performed separate analyses for participants with urinary sodium excretion above median vs. those below the median. Statistical analyses were performed using STATA 12.0 (StataCorp, College Station, TX).

# Heritability analyses

We estimated heritability of CYP17A1 activity using the ASSOC program in the Statistical Analysis for Genetic Epidemiology (S.A.G.E.) package, version 6.3, as previously described.<sup>25</sup> To estimate heritability, ASSOC uses a linear regression, allowing for covariates to be entered in the model. Heritability estimates are expressed as h<sup>2</sup> values with SE. The main model included age and sex as covariates. Another model additionally included body mass index, 24-hour urinary sodium excretion, antihypertensive treatment, and estrogen covariate. The estrogen covariate (0/1) was coded as 1 for women having regular periods or taking oral contraceptive pill or for postmenopausal women taking hormonal replacement therapy.

# RESULTS

From December 2009 to March 2013, 1,128 participants from 271 nuclear families were included in the SKIPOGH study. Participants with missing or insufficient data for serum or urinary values, steroid metabolites, and 24-hour ABP were excluded, leaving 518 participants coming from 193 families (median size (interquartile range) = 3 (2;4), maximum size of 8) for the purpose of this analysis. The characteristics of the 298 women and 220 men are presented in Table 1. Urinary excretion of sodium, potassium, creatinine, and steroid hormone metabolites corrected for creatinine were higher in men than in women during both day and night (P < 0.001, except for pregnanediol and tetrahydroaldosterone, Table 2). However, urine flow rate was similar in men and women.

# Distribution of CYP17A1 activities in the general adult population

The distribution of CYP17A1 activities based upon 2 different ratios of urinary steroid hormone metabolites are shown in Figure 1. Higher precursor-to-product metabolites ratios indicate a lower activity of the enzyme, while lower ratios denote higher enzyme activity. Both ratios showed a unimodal distribution. Ratio 1 had a second peak at higher values, which indicates participants with lower apparent CYP17A1 activities.

# Association of CYP17A1 ratios with ambulatory blood pressure

Due to their asymmetric unimodal distribution, the CYP17A1 ratios were log-transformed for association analyses with ABP. In univariable mixed linear regression analyses, including all participants (n = 518), SBP and DBP tended to be higher during day and night in participants with lower apparent CPY17A1 activity specified by ratio 1 (Supplementary Figure 2), although not reaching statistical significance. There was no association of ABP

#### Table 1. Characteristics of participants

Variables	Men	Women
Numbers	220	298
Age, years	48 (17.7)	48.5 (17.4)
Use of contraceptive pill, numbers (%)		61 (23)
Menopause, numbers (%)		150 (54)
On antihypertensive treatment, numbers (%)	42 (19)	37 (12)
BMI, kg/m <sup>2</sup>	25.7 (3.9)	23.7 (4)
Serum Na, mmol/l	141 (3)	141 (2)
Serum K, mmol/l	4.2 (0.3)	4.1 (0.4)
eGFR, ml/min/1.73 m <sup>2</sup>	97.3 (18.9)	93.9 (16.6)
Number of day measures	52 (8)	52 (10)
Day SBP, mm Hg	127.8 (13.7)	120.1 (14.7)
Day DBP, mm Hg	83.3 (9.9)	78.7 (9.1)
Number of night measures	17 (4)	17 (5)
Night SBP, mm Hg	111.1 (14.7)	103.9 (13.3)
Night DBP, mm Hg	71.1 (8.3)	65.7 (7.1)

Data are mean and SD unless otherwise specified.

Abbreviations: BMI, body mass index; Na, sodium; K, potassium; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate.

	Variables	Men	Women	P-value
	Creatinine excretion, mg/kg BW/24 h	22.3 (19;24.6)	18 (14.8;20.5)	<0.001
Day	Urine duration, min	960 (900;1005)	945 (870;990)	<0.01
	Urine volume, ml	1,171 (836;1657)	1191 (843;1596)	0.51
	Urine flow rate, ml/min	1.22 (0.88;1.75)	1.29 (0.91;1.73)	0.38
	Sodium excretion, mmol	109 (81;146)	83 (60;111)	<0.001
	Potassium excretion, mmol	54.4 (41.3;67.6)	42 (32.1;54.2)	<0.001
	Tetrahydrodehydrocorticosterone, µg/creat	4.83 (3.17;6.91)	3.39 (2.11;5.45)	<0.001
	Tetrahydrocorticosterone, µg/creat	100.5 (70;137.7)	70.2 (48.2;97.7)	<0.001
	5α-Tetrahydrocorticosterone, µg/creat	241 (172;328)	114 (80;169)	<0.001
	Tetrahydro-11-dehydrocorticosterone, µg/creat	71.5 (49.9;95.4)	50.1 (35;68.6)	<0.001
	Tetrahydroaldosterone, µg/creat	13.1 (7.8;21.7)	12 (7.3;19.7)	0.48
	Pregnanediol, µg/creat	132 (97;201)	126 (73;232)	0.29
	17-Hydroxypregnanolone, μg/creat	115.1 (76.4;170.7)	31 (20.6;59.8)	<0.001
	Pregnanetriol, µg/creat	442 (333;601)	203 (122;297)	<0.001
	Tetrahydrosubstance S, µg/creat	47.9 (34.3;63.6)	35.8 (24.7;47.2)	<0.001
	Tetrahydrocortisol, µg/creat	1351 (1051;1722)	827 (579;1054)	<0.001
	5α-Tetrahydrocortisol, μg/creat	1,076 (774;1466)	383 (247;585)	<0.001
	Tetrahydrocortisone, µg/creat	2,262 (1810;2864)	1253 (956;1863)	<0.001
	Dehydroepiandrosterone, µg/creat	101.4 (37.2;400.2)	32.8 (14.8;95)	<0.001
	Androsterone, µg/creat	1,185 (811;1736)	402 (229;734)	<0.001
	Etiocholanolone, µg/creat	1,093 (659;1530)	561 (306;869)	<0.001
Night	Urine duration, min	480 (435;515)	492.5 (450;540)	<0.05
	Urine volume, ml	500 (357;700)	500 (300;683)	0.5
	Urine flow rate, ml/min	1.06 (0.72;1.49)	1.02 (0.63;1.39)	0.48
	Sodium excretion, mmol	50.1 (34.2;66.5)	34.3 (23.2;50.7)	<0.001
	Potassium excretion, mmol	15.1 (10.6;21.3)	11.3 (8.4;16.1)	<0.001
	Tetrahydrodehydrocorticosterone, µg/creat	1.94 (1.4;2.96)	1.37 (0.89;2.44)	<0.001
	Tetrahydrocorticosterone, µg/creat	39.7 (27.7;63.5)	29.4 (19.4;41.7)	<0.001
	5α-Tetrahydrocorticosterone, μg/creat	72.4 (51.9;101)	33.9 (21.7;54)	<0.001
	Tetrahydro-11-dehydrocorticosterone, µg/creat	25 (17.4;36)	18.1 (12.2;26.5)	<0.001
	Tetrahydroaldosterone, µg/creat	5.25 (3.29;8.95)	4.8 (2.77;8.51)	0.33
	Pregnanediol, μg/creat	65.3 (45.2;93.3)	58.9 (36.7;98)	0.29
	17-Hydroxypregnanolone, μg/creat	55.9 (33.2;83.2)	12.3 (8;25.1)	<0.001
	Pregnanetriol, µg/creat	228 (159;307)	101 (61;150)	<0.001
	Tetrahydrosubstance S, μg/creat	18 (12.9;25.1)	14 (9.6;19.6)	<0.001
	Tetrahydrocortisol, µg/creat	423 (297;554)	248 (179;355)	<0.001
	5α-Tetrahydrocortisol, μg/creat	344 (236;482)	120 (79;185)	<0.001
	Tetrahydrocortisone, µg/creat	734 (540;995)	440 (296;618)	<0.001
	Dehydroepiandrosterone, µg/creat	38.5 (13.1;131.8)	13.8 (6.4;32)	<0.001
	Androsterone, µg/creat	577 (366;864)	210 (105;339)	<0.001
	Etiocholanolone, µg/creat	533 (345;780)	281 (163;449)	<0.001

**Table 2.** Descriptive data for day and night urine for sodium, potassium, urinary volume, and creatinine excretion as well as steroid hormone metabolites (corrected for creatinine)

Data are median and interquartile range (IQR). Abbreviation: BW, body weight.



**Figure 1.** Apparent CYP17A1 activities separated by gender, day- or nighttime and ratio 1 or 2. Dashed lines represent women, black lines represent men. Abbreviations: THA, tetrahydrocorticosterone; THB, tetrahydrocorticosterone; 5α-THB, 5α-tetrahydrocorticosterone; THE, tetrahydrocorticosterone; THE, tetrahydrocorticosterone; THE, tetrahydrocortisol; 5α-THF, 5α-tetrahydrocortisol; An, androsterone; Et, etiocholanolone.

with ratio 2 (Supplementary Figure 3) nor with estimated 17,20-lyase activity (data not shown). As CYP17A1 inhibition is associated with an excessive mineralocorticoid signaling, the impact of salt intake—using urinary sodium excretion as a surrogate marker-on ABP in relation to CYP17A1 activity was addressed. To graphically illustrate the effect modification, men and women were separated into low and high sodium excretion subgroups. Day SBP and DBP were associated positively with ratio 1 (hence negatively with CYP17A1 activity) in participants with high sodium excretion (P for interaction between log-CYP17A1 ratio 1 and salt strata = 0.033 for day SBP and =0.004 for day DBP), whereas no such positive association was found in participants with low sodium excretion (Figure 2). We also used sex-specific tertiles of sodium excretion, instead of low and high sodium excretion strata, which leads to similar observations and illustrates the dose-response modifying effect of sodium excretion (Supplementary Figure 4). Nighttime SBP showed the same pattern; however, the corresponding interaction was not statistically significant. We found no significant effect modification of sex for its effect on the association of ABP with CYP17A1 activity (data not shown). There was no association of ABP with ratio 2 (Supplementary Figure 5) nor with estimated 17,20-lyase activity (data not shown) upon dichotomizing the participants based on urinary sodium excretion.

#### Heritability estimates of CYP17A1 ratios

Unadjusted heritability estimates showed only a significant heritability for ratio 2 (Table 3). Adjustment for age and sex showed a significant adjusted heritability for ratio 1 (0.36 for day and 0.38 for night, P < 0.001) and for ratio 2 (0.58 for day and 0.52 for night, P < 0.001). Further adjustment for body mass index, 24-hour urinary sodium excretion, antihypertensive treatment, and estrogen status slightly modified heritability estimates, which remained approximately 0.40 for ratio 1 and between 0.55–0.71 for ratio 2 (all P < 0.001).

# Association of CYP17A1 ratios with age

We observed a significant positive association of day and night CYP17A1 ratio 1 with age (P < 0.001) and a significant negative association of day and night CYP17A1 ratio 2 with age (P < 0.001) (Figure 3).

### Different urinary steroid profile patterns for ratios 1 and 2

Because ratios 1 and 2 showed different results, the pattern of the ratios of urinary steroid metabolites between participants in the highest and those in the lowest quintiles of ratio 1 or ratio 2 during the day are presented (Figure 4). This figure indicates which urinary metabolites predominate in participants with a lower CYP17A1 activity assessed with ratio 1 or



**Figure 2.** Association of day and night ABP with log transformed ratio 1 by salt intake strata. Sex-specific medians for daytime and nighttime urinary sodium excretion were used to separate low (below median) vs. high (above median) salt intake. The black lines indicate the high sodium excretion group, the gray lines the low sodium excretion group. For daytime, the median urinary Na excretion was 146 mmol in men and 107 mmol in women for high intakes and 80 mmol in men and 58 mmol in women for low intakes. For nighttime, the median urinary Na excretion was 65 mmol for men and 51 mmol for women for high intakes and 32 mmol in men and 23 mmol in women for low intakes. Data are adjusted for age, sex, center, body mass index, urine flow rate (day or night), urinary potassium excretion (day or night), 24-hour urinary creatinine excretion per kilogram body weight, antihypertensive treatment, and estimated glomerular filtration rate (GFR) based on the Chronic Kidney Disease Epidemiology Collaboration equation (systolic blood pressure (SBP), diastolic blood pressure (DBP), *P* for interaction (*P*<sub>int</sub>)).

Table 3.	Heritability estimates of log-transformed CYP17A1
activity ra	tios 1 and 2 and day and night, respectively

Variables	Model	h²	P-value
CYP17A1 ratio 1, day	Unadjusted	0.10 (0.09)	0.133
	Model 1	0.36 (0.10)	<0.001
	Model 2	0.39 (0.10)	<0.001
CYP17A1 ratio 1, night	Unadjusted	0.11 (0.09)	0.102
	Model 1	0.38 (0.09)	<0.001
	Model 2	0.40 (0.09)	<0.001
CYP17A1 ratio 2, day	Unadjusted	0.58 (0.10)	<0.001
	Model 1	0.75 (0.09)	<0.001
	Model 2	0.71 (0.09)	<0.001
CYP17A1 ratio 2, night	Unadjusted	0.52 (0.09)	<0.001
	Model 1	0.63 (0.09)	<0.001
	Model 2	0.55 (0.09)	<0.001

Data are narrow sense  $h^2$  estimates in percentage ± SE.

Model 1: adjusted for age and sex; Model 2: adjusted for age, sex, study center, body mass index, 24-hour urinary sodium excretion, antihypertensive treatment, and estrogen status.

ratio 2, respectively. During the day, participants in the highest quintile of ratio 1 or 2 showed a higher excretion of mineralocorticoid active hormones than the participants in the lowest quintile, but the pattern for the other steroid metabolites was different. Participants with a higher CYP17A1 ratio 1 had a trend to a higher excretion of glucocorticoids, a lower excretion of 17a-hydroxylated glucocorticoid precursors and a much lower excretion of androgen precursors and androgen metabolites. Therefore, in the SKIPOGH population, ratio 1 seems to indicate a higher mineralocorticoid production with a maintained cortisol availability. This first pattern of diminished apparent CYP17A1 activity was associated with higher BP under high sodium intake. In contrast, participants with a higher CYP17A1 ratio 2 had a trend to a lower excretion of glucocorticoids and a higher excretion of 17a-hydroxylated glucocorticoid precursors, androgen precursors and androgen metabolites, thus indicating an increased production of mineralocorticoid hormones with a decreased glucocorticoid availability. This pattern was not associated with higher BP.

### DISCUSSION

We provide evidence that a decreased CYP17A1 activity (assessed with ratio 1) is associated with increased systolic



Figure 3. Apparent CYP17A1 activities—assessed with ratios 1 and 2—separated by gender and day- and nighttime. Open triangles represent women and black circles represent men. Dashed lines are the regression lines of participating women and solid lines the ones of men.

and diastolic daytime ABP, when salt intake is high. Our data support the heritability of CYP17A1 activity and highlight the genetic role of CYP17A1 in BP control in the adult population. To our knowledge, this is also the first study to report the distribution of CYP17A1 activities—assessed with 2 different ratios—in the general adult population.

The association of ratios 1 and 2 to ABP is different, however. In patients affected with 17a-hydroxylase deficiency, lack of CYP17A1 activity is characterized by hypertension and elevated CYP17A1 ratios 1 and 2, whereas in our study ABP was associated with CYP17A1 ratio 1, but not with ratio 2. This suggests that the assessment of apparent CYP17A1 activity with 2 different ratios is not the same in patients with 17a-hydroxylase deficiency, compared to the general population. In 17a-hydroxylase-deficient patients, glucocorticoid deficiency leads to an increased secretion of adrenocorticotropic hormone, which stimulates the synthesis of mineralocorticoid hormones. In contrast, participants with an elevated CYP17A1 ratio 1, in our study, had a preserved production of glucocorticoid hormones, with a decreased secretion of 17a-hydroxylated glucocorticoid precursors and side-chain cleaved androgens. Therefore, participants with a diminished CYP17A1 activity (assessed with ratio 1) seem to have a diminished  $17\alpha$ -hydroxylase and 17,20-lyase activity with an increased secretion of mineralocorticoid hormone that is not triggered by glucocorticoid deficiency. When our participants were assessed with ratio 2, participants with an elevated CYP17A1 ratio 2 had

a decreased excretion of glucocorticoid hormones, but a preserved excretion of  $17\alpha$ -hydroxylated glucocorticoid precursors and androgen metabolites, indicating a decrease in glucocorticoid production without a concomitantly decrease in  $17\alpha$ -hydroxylated glucocorticoid precursors and sidechain cleaved androgens. From this point of view, it seems likely that a higher CYP17A1 ratio 2 reflects a deficiency of glucocorticoid hormones that triggers adrenocorticotropic hormone secretion. However, this pattern of increased secretion of mineralocorticoid hormones had no effect on ABP in our population.

Our observational results are supported by results from interventional studies in men with advanced prostate cancer. In these patients, decreased and rogen synthesis and increased mineralcorticoid hormone excretion was observed upon treatment with abiraterone, a CYP17A1-inhibitor. Under abiraterone treatment, a proportion of these men developed hypertension.<sup>10</sup> To overcome this side effect, these patients were additionally treated with prednisone.<sup>11-13</sup> However, in 2 large prospective randomized studies, mineralocorticoid side effects were more commonly reported in the abiraterone and prednisone treated groups, rather than the prednisone therapy groups, suggesting that prednisone only partially prevents the symptoms of mineralocorticoid excess induced by abiraterone.<sup>12,13</sup> This latter situation is in line with our observation of combined mineralocorticoid excess and maintained glucocorticoid availability in the presence of low CYP17A1 activity (assessed with a high ratio 1).



**Figure 4.** Pattern of the urinary steroid metabolites between participants in the highest and the lowest quintiles of ratio 1 or ratio 2 during the day. Abbreviations: THDOC, tetrahydrodeoxycorticosterone; THA, tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; 5α-THB, 5α-tetrahydrocorticosterone; THALDO, tetrahydroaldosterone; PD, pregnanediol; 17HP, 17-hydroxyprogesterone metabolites; PT, pregnanetriol; THF, tetrahydrocortisol; 5α-THF, 5α-tetrahydrocortisol; THE, tetrahydrocortisone; DHEA, dehydroepiandrosterone; An, androsterone; Et, etiocholanolone. THDOC, THB, α-THB, THA, and THALDO are mineralocorticoid active hormones, PD, 17HP, and PT are glucocorticoid precursors, THS, THF, 5α-THF and THE are metabolites of glucocorticoids, DHEA is an androgen precursors, and An and Et are androgen metabolites.

Our novel findings of the close association of BP control with CYP17A1 activity are in line with recent associations of the CYP17A1 gene locus with BP in the adult population.<sup>4-6</sup> In the International Consortium for Blood Pressure analysis, including data on 200,000 individuals of European descent, the single nucleotide polymorphism located within the CYP17A1 gene had the strongest effect size of all genome-wide signals for BP.6 This locus was further associated with BP in East-Asians,<sup>26</sup> Japanese,<sup>27</sup> Han Chinese,<sup>28</sup> and She Chinese.<sup>29</sup> The size of the effect we observe sharply contrasts with that observed in the genetic association studies (i.e., 1 mm Hg per allele).<sup>6</sup> We found a systolic and diastolic daytime ABP difference of 10 and 7 mm Hg between extremes of CYP17A1 activity, when assessed with ratio 1, under conditions of high sodium intake. If confirmed in other studies and in experimental settings, these results may have public health relevance.

We observed CYP17A1 activities to be substantially heritable in the general population, which is compatible with a genetic continuum between rare monogenic arterial hypertension and essential hypertension. Even in the rare monogenic form of hypertension with loss-of function mutations in the *CYP17A1* gene, BP is highly sensitive to salt intake,<sup>9</sup> which highlights the importance of environmental factors. The lower heritability estimate and larger variance of ratio 1 as compared to ratio 2 suggest that environmental factors are more susceptible to impact on ratio 1. Furthermore, the lower heritability of ratio 1 may result, in part, from the fact that it captures a more complex enzymatic activity than ratio 2. Similarly, the substantial heritability of CYP17A1 activities could participate in the clinical observation that a family history of high BP predisposes other family members to arterial hypertension.<sup>2,3</sup>

This study also revealed a lower CYP17A1 activity assessed with ratio 1—in older participants. It is known that DHEA and androstenedione levels decrease with age in men and women between the age groups of 20- to 30 years old and 50- to 60 years old, with smaller changes observed after the age of 60 years.<sup>30</sup> The decreased androgen levels are most likely due to a decreased CYP17A1 activity. Given this observation, it seems obvious, but until now not formally shown, that the decline in androgen synthesis leads also to an increased secretion of steroid precursors with mineralocorticoid properties exposing older individuals to a higher risk of increases in BP in the presence of excess salt intake.<sup>31</sup>

In summary, we identified individuals of European ancestry with a diminished CYP17A1 activity who might profit from reduced salt intake, such as currently recommended.<sup>32,33</sup> Further interventional trials should investigate the extent blood pressure could be lowered in participants with lower estimated CYP17A1 activity.

#### SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal* of *Hypertension* (http://ajh.oxfordjournals.org).

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### DISCLOSURE

The authors declared no conflict of interest.

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