Association of peripheral and central arterial wave reflections with the *CYP11B2 −344C* allele and sodium excretion

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Objective **Angiotensin II and aldosterone, generated by the angiotensin-converting enzyme (ACE) and aldosterone synthase (CYP11B2), respectively, not only regulate sodium and water homeostasis, but also influence vascular remodeling in response to high blood pressure. In the European Project on Genes in Hypertension (EPOGH), we therefore investigated whether the *ACE I/D* and *CYP11B2 C-344T* polymorphisms influence early arterial wave reflections, a measure of vascular stiffness.**

Methods **We measured the peripheral and central augmentation index of systolic blood pressure by applanation tonometry at the level of the radial artery in 622 subjects (160 families and 64 unrelated individuals) randomly recruited from three European populations, whose average urinary sodium excretion ranged from 196 to 245 mmol/day. In multivariate analyses, with sodium excretion analyzed as a continuous variable, we explored the phenotype–genotype associations by means of generalized estimating equations and the quantitative transmission disequilibrium test.**

Results **The peripheral and central augmentation indexes were significantly higher in *CYP11B2 −344C* allele carriers than in *−344T* homozygotes. In offspring, early wave reflections increased with the transmission of the *−344C* allele. This effect of the *CYP11B2* polymorphism occurred in subjects with a higher than median urinary sodium excretion (210 mmol/day). The *ACE I/D* polymorphism did not influence augmentation of systolic blood pressure.**

Conclusions **The *CYP11B2 C-344T* polymorphism affects arterial stiffness. However, sodium intake seems to modulate this genetic effect.** *J Hypertens* **22:2311–2319 © 2004 Lippincott Williams & Wilkins.**

Journal of Hypertension 2004, **22**:2311–2319

Keywords: aldosterone synthase, angiotensin-converting enzyme, augmentation index, genetics, sodium

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Sponsorship: Nuclear families were recruited in the framework of the European Project on Genes in Hypertension, which was supported by the European Union (contract numbers IC15-CT98-0329-EPOGH and QLGI-CT-2000-01137-EURNETGEN). The study was also supported by research grant G.0424.03 from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium), by a special research grant (Onderzoekstoelage OT/99 per 28) from the Katholieke Universiteit Leuven (Leuven, Belgium), by research grants (OK 375 and OK 376) from the Czech Ministry of Education, and by the International Scientific and Technological Cooperation between Poland and Flanders (contract number BIL 00/18). E. B. participated in the research programme Innovationen des Therapiekonzeptes für das metabolische Syndrom – mit Fokus auf Hypertonie' (PTJ-BIO/0313040C), funded by the Bundesministerium für Bildung und Forschung (Berlin, Germany).

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Received 20 February 2004 **Revised** 3 June 2004
Accepted 15 July 2004

Introduction

The arterial pulse wave consists of a forward and reflected component. The amplitude and velocity of the reflected wave increase with cardiac contractility, a more proximal localization of the sites of reflection along the arterial tree and arterial stiffness. The augmentation index, derived from the pulse contour, reflects the degree to which reflected waves enhance

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systolic pressure [1]. Twin studies recently demonstrated that independent of blood pressure, heart rate, height, and age, the heritability of the aortic augmentation index was 37% [2].

Angiotensin II and aldosterone play key roles in the regulation of sodium and water homeostasis. In sodium deplete conditions, these hormones are upregulated, whereas the opposite occurs in the presence of a high salt intake. Circulating angiotensin II is produced by cleavage of angiotensin I by the angiotensin-converting enzyme (ACE) at the endothelial–luminal interface throughout the vasculature, in particular in the pulmonary circulation. Carriers of the *ACE* deletion polymorphism have constitutively increased plasma and tissue levels of ACE [3]. Aldosterone is generated in the adrenal gland by aldosterone synthase (CYP11B2). However, both angiotensin II and aldosterone are also locally produced in the arterial wall, influence vascular structure and function, and mediate vascular remodeling in response to pathological stimuli, such as a high blood pressure [4,5].

In the European Project on Genes in Hypertension (EPOGH), we investigated whether the *ACE I/D* and *CYP11B2 C-344T* polymorphisms impact on the peripheral and central augmentation indexes, as assessed by applanation tonometry at the level of the radial artery. Our analyses accounted for salt intake, estimated from the urinary excretion of sodium and for other host and environmental determinants of cardiovascular function.

Methods

Study population

The European Project on Genes in Hypertension (EPOGH) was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects. The Ethics Committee of each institution approved the protocol. Participants gave informed written consent.

Three EPOGH centers opted to take part in vascular phenotyping. They randomly recruited nuclear families of Caucasian extraction, including offspring with a minimum age of 10 years in Belgium and 18 years in the two other countries. Overall, the response rate was 82%. Of 870 participants recruited in Cracow (Poland, $n = 302$), Hechtel-Eksel (Belgium, $n = 380$) and Pilsen (Czech Republic, $n = 188$), we discarded 21 from analysis because the recorded pulse wave was of insufficient quality and 60 because of missing genotypes. In addition, we detected six cases of inconsistency in Mendelian segregation. The Belgian sample included seven extended families spanning more than two generations. Because there is no generally agreed algorithm to construct the variance–covariance matrix for correlated data within extended pedigrees using generalized estimating equations (see below), we selected from each complex family the most informative nuclear unit with the largest number of phenotypes and genotypes. This procedure removed 161 Belgian subjects from our analyses. Thus, the overall number of participants analyzed statistically totaled 622.

Phenotypes and genotypes

After subjects had rested for 15 min, we recorded, during an 8-s period, the radial arterial waveform at the dominant arm by applanation tonometry. We used a high-fidelity SPC-301 micromanometer (Millar Instruments, Inc., Houston, Texas, USA) interfaced with a laptop computer running SphygmoCor software, version 6.31 (AtCor Medical Pty. Ltd., West Ryde, New South Wales, Australia). We discarded recordings when the systolic or diastolic variability of consecutive waveforms exceeded 5% or when the amplitude of the pulse wave signal was less than 80 mV. We calibrated the pulse wave by measuring blood pressure at the contralateral arm immediately before the recordings. From the radial signal, the SphygmoCor software calculates the aortic pulse wave by means of a validated and population-based generalized transfer function [6]. The radial augmentation index was defined as the ratio of the second to the first peak of the pressure wave expressed as a percentage. The aortic augmentation index was the difference between the second and first systolic peak given as a percentage of the aortic pulse pressure. For statistical analysis, we used the average of the peripheral and central waveforms over the 8-s measurement period.

The blood pressure phenotype was the average of five consecutive readings obtained at one home visit. Peripheral and central pulse pressures were defined as the difference between systolic and diastolic blood pressure derived from the brachial blood pressure measured at the subjects homes and from the aortic pulse wave, respectively. From the home readings, we calculated mean arterial pressure as the diastolic pressure plus one-third of peripheral pulse pressure. We administered a standardized questionnaire to obtain information on each subject's medical history, smoking and drinking habits and use of medications. The participants collected a 24-h urine sample in a wide-neck plastic container for the measurement of sodium, potassium, creatinine and aldosterone. For statistical analysis of the urinary phenotypes, we excluded 18 subjects because according to previously published criteria [7] the urine samples were judged to be under- or over-collected. Genomic DNA from white blood cells was amplified and genotyped for the *ACE I/D* and *CYP11B2 C-344T* polymorphisms, as previously described [8,9]. For the *ACE* gene, all samples initially genotyped as *DD* underwent a second polymerization chain reaction with insertion-specific primers.

Statistical methods

Database management and most statistical analyses were performed with SAS software version 8.1 (SAS Institute Inc., Cary, North Carolina, USA). Population means and proportions were compared by Tukey's multiple means test and the χ^2 statistic with Bonferroni's adjustment for multiple comparisons, respectively. If Shapiro–Wilk's test showed significant departure from normality, we analyzed logarithmically transformed variables. We searched for possible covariates of the augmentation indexes using stepwise multiple regression with the *P* value for independent variables to enter and stay in the model set at 0.10. We used the methods described by Kleinbaum *et al.* [10] to test the null hypothesis of no differences between the parameters of regression equations.

We performed population-based as well as family-based analyses. In the population-based approach, we tested association of continuous traits with the genotypes of interest by use of generalized estimating equations (GEE). This approach allows adjustment for covariates as well as for the non-independence of observations within families [11]. In GEE, we also tested for heterogeneity between populations, using appropriate interaction terms with the genotypes.

In the family-based analyses, we performed transmission disequilibrium tests for quantitative traits (QTDT) using two different methods. First, we evaluated the within- and between-family components of phenotypic variance, using the orthogonal model as implemented by Abecasis *et al.* in the QTDT software, version 2.3 (http://www.sph.umich.edu/csq/abecasis/QTDT) [12]. In addition, using the model proposed by Allison, we regressed the quantitative phenotypes of informative offspring on their genotypes, while controlling for parental genotypes [13]. To allow for residual correlation among offspring, we implemented Allison's model using GEE.

Results

Characteristics of participants

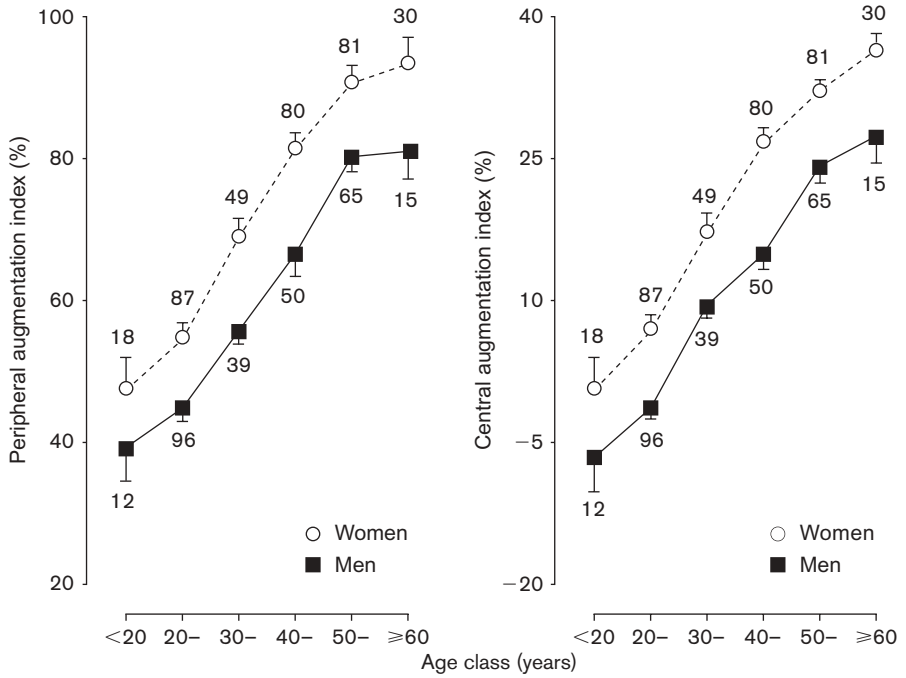
Table 1 gives the characteristics of the participants by country. Overall, the study population included 558 subjects from 160 nuclear families and 64 unrelated individuals. Mean (± SD) age of the 294 founders and 328 offspring was 51.7 ± 8.5 and 29.9 ± 10.6 years, respectively. The number of sibs per family amounted to one in 34 families, two in 102 families, and ranged from three to eight in 24 families. The Belgian participants were older than the subjects from the two other countries. Compared with Polish participants, fewer Belgians were on antihypertensive drug treatment. Czechs more frequently reported regular alcohol intake (≥ 5 g/day) than Belgians and Polish. Urinary sodium excretion was on average 50 and 36 mmol/day higher in Poland than in Belgium and Czechia, respectively. The urinary aldosterone excretion was lower in Poland than in the other two countries. Figure 1 shows the sex and age dependence of the peripheral and central augmentation indexes, which in the whole study population averaged 68.0 ± 23.1 and $16.4 \pm 17.1\%$, respectively. As shown in Figure 2, systolic pressure in

Table 1 Characteristics of the study participants by country

	Belgium	Czechia	Poland
No.	181	147	294
Anthropometrical characteristics			
Age (years)	46.8 ± 14.6	37.9 ± 13.5*	37.2 ± 13.9*
Female gender (%)	57.5	57.1	53.4
Height (cm)	167.4 ± 9.4	171.1 ± 9.1*	169.9 ± 8.7*
Weight (kg)	72.2 ± 14.5	76.7 ± 16.1*	73.3 ± 14.4
Hemodynamic measurements			
Systolic pressure (mmHg)†	125.0 ± 15.7***	123.8 ± 16.6***	128.8 ± 17.5
Diastolic pressure (mmHg)†	78.6 ± 11.4	77.8 ± 10.9	80.3 ± 12.2
Peripheral augmentation index (%)	72.7 ± 21.9	63.7 ± 23.7*	67.3 ± 23.0*
Central augmentation index (%)	21.2 ± 15.5	14.0 ± 17.9*	14.6 ± 17.1*
Pulse rate (beats/min)	62.8 ± 9.4***	67.0 ± 9.7*	73.3 ± 11.4**
Questionnaire data			
Antihypertensive treatment (%)	12.7	21.1	23.8*
Current smokers (%)	28.2	25.8	27.6
Regular alcohol intake (%)	27.1	45.6*	19.1[C]
Urinary excretion			
Volume (l/day)‡	1.64 ± 0.77***	1.93 ± 0.67*	1.45 ± 0.53**
Sodium (mmol/day)	196 ± 65***	210 ± 83	245 ± 87**
Potassium (mmol/day)	70 ± 27	62 ± 29*	65 ± 25
Aldosterone (nmol/day)	20.0 (17.4–22.9)***	17.4 (15.8–19.1)	10.7 (9.8–11.7)**
Creatinine (mmol/day)	10.7 ± 3.5***	13.3 ± 4.1*	12.1 ± 4.1**

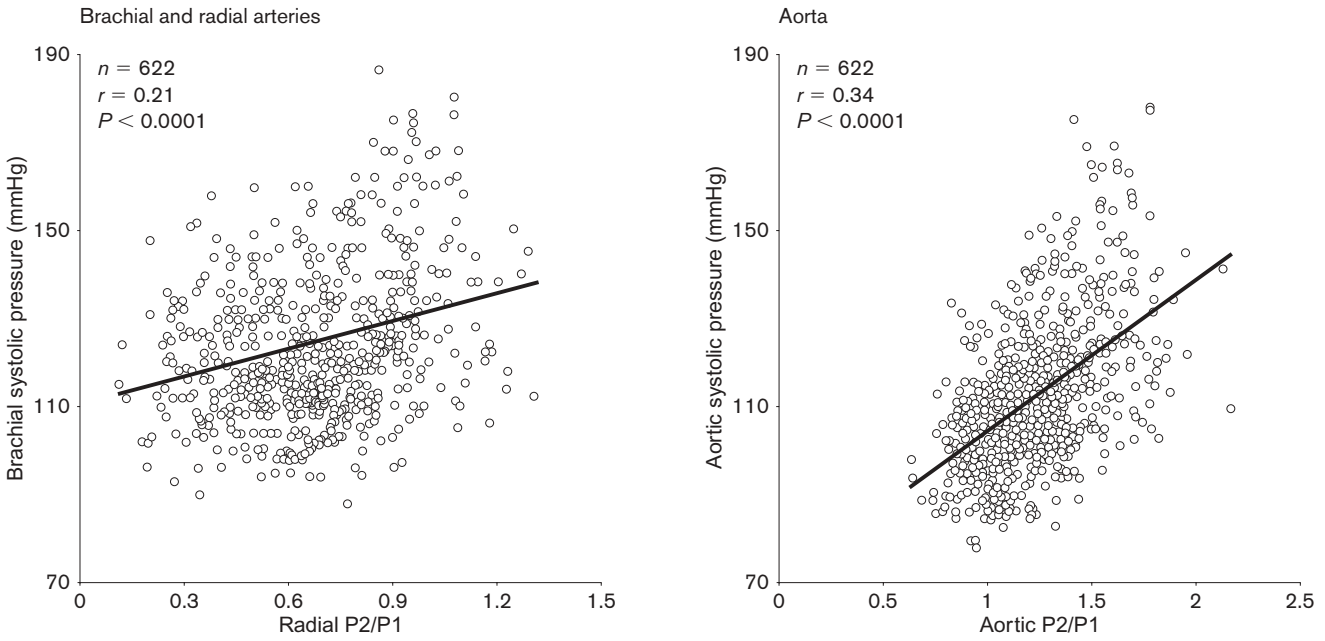
Values are arithmetic means ± SD, geometric means (95% confidence interval) or the percentage of subjects. *P* values for between-country differences were adjusted for multiple comparisons by Tukey's test (means) or Bonferroni's method (proportions): *$P \leq 0.05$ versus Belgium; **$P \leq 0.05$ versus Czechia; ***$P \leq 0.05$ versus Poland. †Average of five readings obtained at one home visit. ‡The number of subjects with 24 h collection was 181 in Belgium, 133 in Czechia and 290 in Poland.

Fig. 1



Peripheral and central augmentation indexes by sex and age class. Values are non-adjusted means ± SE. The number of subjects contributing to each mean is given.

Fig. 2



Local systolic pressure in the brachial and radial arteries and in the aorta in relation to systolic augmentation calculated as a the ratio of the second (P2) to the first (P1) systolic peak. The aortic measurements were extrapolated from the radial waveform by means of the SphygmoCor software.

the brachial artery and aorta significantly ($P < 0.0001$) increased with the ratio of the second to the first systolic peak. Furthermore, the slope of systolic blood pressure on this ratio was significantly ($P = 0.0002$) steeper in the aorta than in the radial artery.

Stepwise multiple regression demonstrated that the peripheral augmentation index significantly and independently increased with female gender (regression coefficient $\beta \pm$ SE; $9.16 \pm 1.58\%$; $P = 0.0001$), age ($0.81 \pm 0.04\%$ per year; $P = 0.0001$), mean arterial pressure ($0.38 \pm 0.05\%$ per mmHg; $P = 0.0001$), current smoking ($5.20 \pm 1.20\%$; $P = 0.0001$), whereas it decreased with body height ($-0.52 \pm 0.09\%$ per cm; $P = 0.0001$) and pulse rate ($-0.63 \pm 0.05\%$ per beat; $P = 0.0001$). Similarly, the central augmentation index increased with female gender ($6.80 \pm 1.17\%$; $P = 0.0001$), age ($0.61 \pm 0.03\%$ per year; $P = 0.0014$), mean arterial pressure ($0.26 \pm 0.04\%$ per mmHg; $P = 0.0001$), current smoking ($2.94 \pm 0.92\%$; $P = 0.0001$), whereas it decreased with body height ($-0.38 \pm 0.07\%$ per cm; $P = 0.0001$), pulse rate ($-0.50 \pm 0.04\%$ per beat; $P = 0.0001$) and current antihypertensive treatment ($-2.10 \pm 1.17\%$; $P = 0.07$). We adjusted all further analyses for the aforementioned covariates as well as for observer (one in Belgium, one in Czechia, and two in Poland). Further analyses also accounted for sodium intake estimated from the 24-h urinary excretion.

The within-country frequencies of genotypes (Table 2) complied with Hardy–Weinberg equilibrium ($0.36 \leqslant P \leqslant 0.94$). The genotype and allele frequencies were similar across countries for the *ACE* gene. The *CYP11B2 –344C* allele was more prevalent in Poland than Belgium.

Both before and after adjustment for urinary sodium and potassium, the 24-h urinary aldosterone excretion was not associated with the *CYP11B2 C-344T* polymorphism ($P \geqslant 0.16$). In all countries, aldosterone excretion adjusted for sex and age was closely correlated with urinary sodium and potassium. With additional adjustment for country, the overall partial correlation coefficients were -0.11 for sodium, 0.30 for potassium and -0.38 for the urinary sodium-to-potassium ratio ($P < 0.01$, for all).

Population-based association study

Because across centers there was no heterogeneity in the phenotype–genotype relations ($0.07 \leqslant P \leqslant 0.82$), we combined all countries. Furthermore, for none of the phenotype–genotype relations, we found significant interactions with gender ($0.54 \leqslant P \leqslant 0.94$) or generation (parents *versus* offspring; $0.39 \leqslant P \leqslant 0.80$).

GEE did not reveal any association between the augmentation indexes and the *ACE I/D* polymorphism. In the whole study population as well as in offspring, the peripheral and central augmentation indexes were significantly higher in the *CYP11B2 –344C* allele carriers than in the *–344TT* homozygotes with similar trends in founders (Table 3). Further analyses (Table 4) demonstrated that in founders central, but not peripheral, pulse pressure was also significantly higher in *CYP11B2–344C* allele carriers than in *–344TT* homozygotes. There was no interaction between the *ACE* and *CYP11B2* genotypes ($P > 0.22$). However, in untreated subjects ($n = 483$), we observed a significant interaction between the *CYP11B2* genotype and sodium excretion, analyzed as a continuous variable, in relation to the peripheral ($P = 0.029$) and central ($P = 0.013$) augmentation indexes. Figure 3 illustrates these interactions according to the country- and sex-specific median of sodium excretion (approximately 210 mmol/day).

Family-based association study

With the exception of the *ACE* gene in Czechia ($P < 0.01$), Abecasis' orthogonal model did not reveal population stratification in any country ($0.19 \leqslant P \leqslant 0.34$). In 216 informative offspring, it confirmed significant association between the peripheral augmentation index and the transmission of the *CYP11B2 –344C* allele (effect size, + 3.0%; $P = 0.028$) with a similar trend for the central augmentation index (effect size, + 2.2%; $P = 0.092$). Using Allison's approach, the

Table 2 Genotype and allele frequencies by country ordered according to the prevalence of the major allele

Gene	Country	Allele		Genotypes		
ACE		D	I	DD	DI	I I
	Czechia	158 (53.7)	136 (46.3)	40 (27.2)	78 (53.1)	29 (19.7)
	Poland	302 (51.4)	286 (48.6)	72 (24.5)	158 (53.7)	64 (21.8)
	Belgium	184 (50.8)	178 (49.2)	49 (27.1)	86 (47.5)	46 (25.4)
CYP11B2		T	C	TT	TC	CC
	Belgium	216 (59.7)	146 (40.3)	63 (34.8)	90 (49.7)	28 (15.5)
	Czechia	172 (58.5)	122 (41.5)	50 (34.0)	72 (49.0)	25 (17.0)
	Poland	297 (50.5)	291 (49.5)	70 (23.8)	157 (53.4)	67 (22.8)

Values indicate number of alleles or genotypes (%). Braces join countries with similar allele frequencies. ACE, angiotensin-converting enzyme.

Table 3 Peripheral and central augmentation indexes by genotypes in nuclear families

Gene	Augmentation index		*n*	Adjusted mean ± SE†			
ACE			DD/DI/I I	DD	DI	II	*P**
	Peripheral	All	161/322/139	68.4 ± 1.0	68.1 ± 0.8	67.9 ± 1.3	0.79
		Founders	79/156/59	80.4 ± 1.5	81.5 ± 1.1	81.1 ± 1.9	0.58
		Offspring	82/166/80	57.3 ± 1.3	56.2 ± 1.1	55.6 ± 1.5	0.39
	Central	All	161/322/139	17.1 ± 0.8	16.1 ± 0.6	16.5 ± 1.0	0.35
		Founders	79/156/59	25.9 ± 1.0	25.7 ± 0.7	25.6 ± 1.3	0.85
		Offspring	82/166/80	9.3 ± 1.2	7.6 ± 0.9	7.7 ± 1.2	0.24
CYP11B2			TT/TC/CC	TT	TC	CC	*P***
	Peripheral	All	183/319/120	66.1 ± 1.1	68.8 ± 0.9	69.5 ± 1.1	0.027
		Founders	84/155/55	79.3 ± 1.5	81.3 ± 1.2	83.3 ± 1.4	0.16
		Offspring	99/164/65	53.1 ± 1.4	57.9 ± 1.0	57.4 ± 1.6	0.004
	Central	All	183/319/120	15.1 ± 0.8	16.8 ± 0.6	17.6 ± 0.9	0.050
		Founders	84/155/55	24.4 ± 1.0	25.8 ± 0.7	27.6 ± 1.1	0.12
		Offspring	99/164/65	6.2 ± 1.2	9.1 ± 0.8	8.4 ± 1.5	0.049

†Adjustments included: observer, sex, age, body height, pulse rate, mean arterial pressure, current smoking and antihypertensive treatment. **P* value for comparison between *ACE DD* versus *DI + II*. ***P* value for comparison between *CYP11B2 TT* versus *TC + CC*. ACE, angiotensin-converting enzyme.

Table 4 Peripheral and central pulse pressure in relation to the *CYP11B2* genotype

Pulse pressure		*n* (CC/TC/TT)	Adjusted mean ± SE† TT	TC	CC	*P**
Peripheral	All	161/322/139	47.9	47.5	46.8	0.53
	Founders	79/156/59	49.6	49.4	48.8	0.84
	Offspring	82/166/80	46.1	44.9	45.7	0.45
Central	All	161/322/139	31.9	33.4	32.9	0.06
	Founders	79/156/59	34.8	38.4	36.4	0.01
	Offspring	82/166/80	29.2	28.8	29.5	0.75

†Pulse pressure was adjusted for sex, age, body height, pulse rate, current smoking and antihypertensive treatment. Additional adjustments included observer and mean arterial pressure for the central pulse pressure and country for the peripheral pulse pressure. **P* value for comparison between *CYP11B2 TT* versus *TC + CC*.

number of informative offspring decreased to 135, but the test statistic remained significant for the association between the peripheral augmentation index and the *CYP11B2* polymorphism with a similar tendency for the central augmentation index (Table 5). None of the QTDT models provided any evidence for association between the pulse wave phenotypes and transmission of the *ACE D* allele.

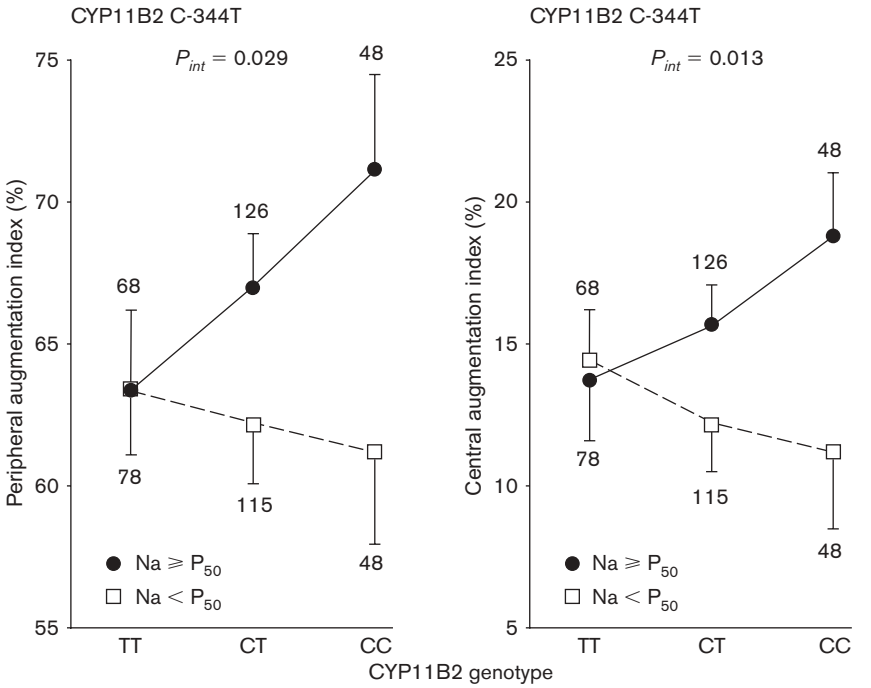
Discussion

The main finding of our study was that in both population-based and family-based analyses, early arterial wave reflections increased with the presence or transmission of the *CYP11B2 –344C* allele. This genetic effect was more pronounced in subjects whose sodium excretion exceeded 210 mmol/day. The frequency of the *–344T* allele was higher in Belgians (60%) than Polish (51%). However, this difference in allele frequency did not impact on our results. Indeed, we neither observed heterogeneity in the phenotype–genotype relation across countries nor stratification within any population. Previous studies reported *CYP11B2 –344T* allele frequencies of 45% [14] in Caucasians and 64% [15] in Japanese.

Aldosterone synthase is a cytochrome P450 enzyme and catalyzes the terminal steps in aldosterone biosyntheses. Chromosome 8 harbors the human gene. The *C-344T* polymorphism is located in an enhancer element, which is also present in the genes of other adrenal hydroxylases [16] and interacts with the regulatory protein steroidogenic factor (SF-1) [17]. Several *in vitro* studies addressed the possible functional role of *CYP11B2* [17–20]. Preliminary gel-shift experiments suggested that on a molar basis the *C* compared with the *T* containing element bound SF-1 about four times more effectively [18]. Subsequent transfection studies of human adrenocortical cells showed that the *C-344T* locus is not essential for basal or regulated expression of human *CYP11B2*, apparently because the human gene contains an additional SF-1 binding site closer to the start of transcription [17,19]. However, these experiments do not exclude a functional role of the *C-344T* polymorphism, because *CYP11B2* is expressed in cardiac [21] and vascular [5,21] tissue and because its expression might be differentially regulated in steroidogenic and non-steroidogenic tissues [20].

Within and across populations, we did not find any

Fig. 3



Peripheral and central augmentation indexes by *CYP11B2* genotype and median sodium excretion (210 mmol/day) in 483 untreated subjects. Values are adjusted means ± SE. The significance of the genotype-by-sodium interaction (P_{int}) was derived from a generalized estimating equations (GEE) model, which included sodium excretion as a continuous variable and which accounted for clustering within families and significant covariates (Table 3).

Table 5 QTDT analyses of peripheral and central augmentation indexes

Gene	Number of offspring informative/all	Peripheral augmentation index			Central augmentation index		
		β (%)	χ^2	*P*	β (%)	χ^2	*P*
ACE							
Orthogonal model	212/328	+ 1.7	1.51	0.22	+ 1.8	2.21	0.14
Allison's model	137/328	+ 2.4	0.77	0.37	+ 3.0	1.70	0.19
CYP11B2							
Orthogonal model	216/328	+ 3.0	4.84	0.028	+ 2.2	2.83	0.092
Allison's model	135/328	+ 7.2	4.61	0.031	+ 4.3	2.94	0.086

The orthogonal model accounted for between- and within-family components of phenotypic variability. The parameter estimate (β) for the within-family variability component indicates the direction and size of the association when the *ACE D* allele or the *CYP11B2 −344C* allele were transmitted. Adjustments were similar as in Table 3. ACE, angiotensin-converting enzyme.

relation between the urinary aldosterone excretion and the *CYP11B2 C-344T* polymorphism. Other studies on the putative association between plasma or urinary aldosterone and genetic variation in the *CYP11B2* gene produced inconsistent results. Indeed, they showed higher plasma levels of aldosterone associated with the *–344C* [22] or *–344T* allele [23], higher urinary aldosterone excretion in the presence of the *–344T* allele [24,25] and divergent results for blood pressure analyzed as a binary or continuous phenotype [14,15,22,24–26]. In the light of our present findings, the contradiction in the literature is not surprising, because few studies accounted for sodium intake, which is an important determinant of aldosterone production. We noticed a major interaction between the *CYP11B2 C-344T* polymorphism and sodium excretion in relation to a vascular phenotype.

Arterial stiffness and the velocity and amplitude of reflected arterial waves are the main determinants of the peripheral and central augmentation indexes. In keeping with current physiological concepts [27], we found that the augmentation of systolic pressure was significantly higher in the aorta compared with the

brachial artery. Wave reflections occur at sites of changes of arterial impedance along the arterial tree, such as branching points or atherosclerotic plaques. However, in the present study, we adjusted the augmentation indexes for body height and pulse rate, so that to a large extent these phenotypes probably reflected vascular stiffness rather than changes in the localization of the reflection points. In keeping with our present observations, some researchers reported a positive association of the *CYP11B2 –344C* allele with arterial stiffness [22] or with the prevalence [15] or incidence [26] of hypertension. However, the physiological and molecular pathways via which this polymorphism might impact on vascular stiffness remain to be elucidated.

Blacher and coworkers noticed in hypertensive patients ($r = -0.497$; $P < 0.01$), but not normotensive subjects, a close and inverse correlation between systemic arterial compliance and the plasma aldosterone concentration [28]. The absence of a direct relation between vascular stiffness and plasma aldosterone in our study ($P > 0.25$) and other reports [26,28] might be explained by the local generation of aldosterone in the endothelium or in vascular smooth muscle cells [5]. Indeed, in rats, the concentration of aldosterone in cardiac tissue was 17-fold higher than in plasma [21].

We observed a significant interaction between the *CYP11B2 C-344T* polymorphism and sodium excretion, analyzed as a continuous variable, in relation to the peripheral and central augmentation of systolic blood pressure. Previous studies demonstrated that in humans a high salt intake is associated with increased arterial stiffness and vascular and cardiac hypertrophy [29]. Furthermore, in normotensive Wistar–Kyoto rats, a high salt diet (0.9% NaCl) stimulated the expression of mRNA for CYP11B2 in the myocardium [30]. Sodium loading activated local aldosterone synthesis with elevated tissue levels of the steroid and produced cardiac hypertrophy in the absence of a noticeable increase in blood pressure [30]. Similarly, stroke-prone spontaneously hypertensive rats fed 0.9% NaCl in drinking water, compared with the control group given tap water, had increased expression of mRNA for CYP11B2 in the arterial wall, but lower levels of circulating aldosterone [31]. Thus, an excessive salt intake might contribute to increased arterial stiffness by inappropriately sustaining the expression of the *CYP11B2* gene in the arterial wall, despite a decrease of the angiotensin II and aldosterone levels in the blood.

Because plasma and tissue levels of ACE increase with the number of copies of the *ACE D* allele [3] and because angiotensin II is a potent vasoconstrictor and stimulates vascular growth, the hypothesis that the *ACE* deletion polymorphism might be associated with increased vascular stiffness is plausible. However, in keeping with the contradictory results of previous studies [32,33], we did not observe such a relation. Our null findings with respect to the *ACE I/D* polymorphism might be due to the plasticity of the renin–angiotensin system, as exemplified by the multiple pathways via which angiotensin II can be generated, or to the activation by angiotensin II of counter-regulatory hormonal or paracrine mechanisms [4].

The present study has to be interpreted within the context of its limitations and strengths. One 24-h urine collection is insufficient to characterize an individual's habitual sodium intake, but it does accurately reflect the average salt consumption of groups of subjects [34]. We investigated the interaction between the *CYP11B2* polymorphism and sodium excretion in relation to augmentation of systolic pressure only in untreated subjects, because many antihypertensive drugs, in particular diuretics, influence renal sodium handling. There was consistency between the population-based and family-based statistical approaches and between the results involving the central and peripheral augmentation indexes. The latter was obtained directly from the radial pulse wave and did not involve extrapolation based on the transfer function implemented in the SphygmoCor software.

In conclusion, our study suggests that a common variant of the *CYP11B2* gene, located in the promoter area, may be involved in the pathophysiology of arterial stiffness. However, sodium intake seems to modulate this genetic effect.

Acknowledgements

This study would not have been possible without the voluntary collaboration of the participants. Professor Michael F. O'Rourke (Saint Vincent's Hospital, Darlinghurst, Sydney, Australia) helped with the interpretation of the SphygmoCor data. The authors acknowledge the expert assistance of Rina Bollen, Lieve Gijsbers, Sylvia Van Hulle and Marie-Jeanne Jehoul (Study Coordinating Centre, University of Leuven, Leuven, Belgium).

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