Relationship between left ventricular mass and the ACE *D/I* polymorphism varies according to sodium intake

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Background **In the European Project on Genes in Hypertension (EPOGH), we investigated to what extent left ventricular mass (LVM) in populations and families relates to the angiotensin-converting enzyme (ACE *D/I*) and aldosterone synthase (CYP11B2 −*344C/T*) polymorphisms and urinary sodium excretion.**

Methods **We recruited 219 nuclear families (382 parents and 436 offspring) randomly in Cracow (Poland), Novosibirsk (Russia) and Mirano (Italy). Echocardiographical LVM was indexed to body surface area, adjusted for covariables, and subjected to multivariate analyses using generalized estimating equations and quantitative transmission disequilibrium tests, in a population-based and family-based approach, respectively.**

Results **We found significant differences between the two Slavic centres and Mirano in left ventricular mass index (LVMI) (94.9 versus 80.3 g/m^2), sodium excretion (229 versus 186 mmol/day), and the prevalence of the ACE *D* allele (52.1 versus 58.5%). There was significant heterogeneity between Slavic and Italian subjects in the phenotype–genotype relationships with the ACE gene, but not with the aldosterone synthase gene. In the two Slavic centres, ACE *II* homozygosity was significantly associated with higher LVMI, in population-based as well as in family-based analyses. By contrast, in Mirano, LVMI was slightly higher in *DD* homozygotes ($P = 0.05$), but only in the population-based approach. LVMI increased with higher sodium excretion in ACE *II* homozygous offspring of both Slavic and Italian extraction (+4.2 ± 2.1 g/m^2 per 100 mmol; $P = 0.04$) and in Slavic (+2.6 ± 1.1 g/m^2 per 100 mmol; $P = 0.02$), but not Italian (−3.3 ± 3.2 g/m^2 per 100 mmol; $P = 0.29$) *D* allele carriers. We did not find any association between LVMI and the aldosterone synthase −*344C/T* polymorphism.**

Conclusions **The relationship between LVMI and the ACE *D/I* polymorphism differs across populations, possibly as a consequence of intermediate regulatory mechanisms responsive to varying levels of salt intake. *J Hypertens* 22:287–295 © 2004 Lippincott Williams & Wilkins.**

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Introduction

Left ventricular hypertrophy occurs in 15–20% of the general population and is an independent predictor of cardiovascular complications [1,2]. Left ventricular mass (LVM) depends on a complex interaction between genetic and environmental factors, and lifestyle. Known determinants of LVM include gender, age, body size, systolic blood pressure, smoking, alcohol

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consumption and various endocrine and paracrine factors [1,3,4]. In patients with essential hypertension, LVM may also increase with high dietary salt intake [5,6].

Numerous studies demonstrated that the renin–angiotensin–aldosterone system (RAAS), both system-wide and at the tissue level, regulates myocardial growth [7,8]. Key enzymes in the RAAS cascade are the angiotensin-converting enzyme (ACE) and aldosterone synthase. Many investigators therefore tested whether functional polymorphisms in the genes encoding these enzymes might influence LVM. However, there is growing awareness that complex multigenic disorders, such as left ventricular hypertrophy, should be studied within their ecogenetic context. In the European Project on Genes in Hypertension (EPOGH), we therefore investigated, in three countries, the extent to which LVM in populations and families relates to the ACE *D/I* and aldosterone synthase (CYP11B2) *–344C/T* polymorphisms and to urinary sodium excretion as an index of salt intake. Our analysis also accounted for other lifestyle factors.

Methods

General outline of the study

The primary goal of the EPOGH study was to investigate the complex relationship between blood pressure, analysed as a continuous or binary phenotype, and various candidate genes [9]. In addition to blood pressure, several other intermediate or associated phenotypes, such as LVM, were measured. The epidemiological methods used in EPOGH have been described previously [10]. The project was conducted according to the principles outlined in the Helsinki declaration for investigations in human subjects [11]. Each local Institutional Review Board approved the study. Participants gave informed written consent.

Field work

Investigators from seven European countries (Belgium, Bulgaria, the Czech Republic, Italy, Poland, Romania and the Russian Federation) randomly recruited nuclear families of white Caucasian extraction, consisting of at least one parent and two siblings. Age ranged from 18 to 60 years. Three centres took part in the optional substudy on echocardiography, and together enrolled 976 subjects in Cracow (Poland, centre 1), Novosibirsk (the Russian Federation, centre 2) and Mirano (Italy, centre 3). The overall response rate was 61.3%. Subjects were excluded from analysis: (1) if they had declined the invitation for the echocardiographic examination ($n = 93$); (2) if the echocardiogram was of insufficient quality ($n = 25$); (3) if the subjects suffered from left ventricular dysfunction due to myocardial infarction ($n = 3$) or valvular diseases ($n = 4$); (4) if their DNA could not be amplified ($n = 22$); or (5) if their genotype could not be determined with certainty ($n = 6$). In addition, we detected five cases of inconsistency in Mendelian segregation. Thus, the number of subjects analysed statistically totalled 818.

The blood pressure phenotype was the average of five consecutive readings at one visit. After the subjects had rested in the sitting position for 10 min or longer, a trained observer measured blood pressure with a mercury sphygmomanometer [12]. Via a standardized questionnaire, the observers collected information on each subject's personal and familial 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, aldosterone and creatinine. If urinary volume or creatinine excretion were outside published limits [13], the urinary results were discarded. Serum ACE activity was determined by a spectrophotometric assay with furanacryloyl tripeptide as the substrate [14].

Echocardiographic measurements

In each centre, one experienced observer performed all echocardiograms, with the subject in the left decubitus position, using a commercially available ultrasonograph equipped with a 3.5-MHz transducer. M-mode echocardiograms of the left ventricle were obtained at end-expiration from the parasternal long-axis view under control of the two-dimensional image. The ultrasound beam was positioned just below the mitral valve at the level of the posterior chordae tendineae. Left ventricular internal diameter (LVID) and interventricular septal (IVST) and posterior wall thickness (PWT) were measured at end-diastole according to the recommendations of the American Society of Echocardiography, using the leading edge-to-leading edge convention [15]. For statistical analysis, the measurements of three cardiac cycles were averaged. Studies were recorded on videotapes. End-diastolic left ventricular dimensions were used to calculate LVM by an anatomically validated formula [16]. Mean wall thickness (MWT) was defined as (IVST + PWT)/2. Left ventricular mass index (LVMI) was defined as LVM/m^2 of body surface area, calculated as body weight $(kg)^{0.425} \times$ body height $(cm)^{0.725} \times 0.007184$. The intra-observer inter-session reproducibility coefficient for LVM, computed according to Bland and Altman's method [17], was 2.5% for centre 1, 2.0% for centre 2 and 2.6% for centre 3.

Determination of genotypes

Genomic DNA was extracted from peripheral blood. The ACE *D/I* polymorphism was detected, as described by Lindpaintner *et al.* [18]. All samples initially genotyped as *DD* underwent a second polymerase chain reaction (PCR) with insertion-specific primers [18]. For determination of the *–344C/T* aldosterone synthase (CYP11B2) gene variants, PCR and subsequent geno-

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typing were performed as described by Brand *et al.* [19].

Statistical analysis

We used the SAS software package, version 8.1 (SAS Institute, Cary, North Carolina, USA) for database management and most statistical analyses. Comparison of means and proportions relied on the standard normal z-test and the χ^2-statistic, respectively.

We performed both population-based and family-based association analyses. In the population-based approach, we tested the association of continuous traits with the genotypes of interest by use of generalized estimating equations (GEE). GEE allows adjusting for covariables as well as for the non-independence of observations within families [20]. In the GEE approach, we also tested for heterogeneity across populations using the appropriate interaction terms with the genotypes.

In the family-based analysis, we performed the transmission disequilibrium test (TDT) for quantitative traits using three different methods. First, we evaluated the within- and between-family components of phenotypic variance using the orthogonal model, as implemented by Abecasis *et al.* [21,22] in the QTDT software, version 2.3 (http://www.well.ox.ac.uk/asthma/QTDT). This algorithm enables the assessment of heterogeneity across populations [23]. To obtain estimates of probability less likely to be influenced by phenotypic distributions, we calculated empirical P-value from 1000 Monte-Carlo permutations [21]. Secondly, using the approach proposed by Allison [24], we regressed the quantitative phenotypes of the offspring on their genotypes, while controlling for parental genotypes. To allow for residual correlation among offspring, we implemented Allison's method using GEE. Finally, in the PROC LOGISTIC procedure of the SAS package, we modelled the probability of transmission of the allele of interest from each heterozygous parent as a function of the quantitative phenotype [25].

Results

Characteristics of the participants

Of the 818 participants, 282 were recruited in Cracow, 276 in Novosibirsk and 260 in Mirano. The characteristics of the study participants are summarized by centre in Table 1. Mean age of parents and offspring ($\pm$ SD) was 52.2 ± 5.1 years and 25.7 ± 4.9 years, respectively. Body mass index was similar across centres, and averaged 25.2 ± 4.2 kg/m^2 in men and 25.3 ± 5.3 kg/m^2 in women. Compared to Novosibirsk and Mirano, fewer Polish subjects reported regular alcohol intake ($\geqslant$ 5 g/day). Plasma ACE activity was, on average, 7% higher in Slavic subjects than in Italians. Urinary sodium excretion was, on average, 57 mmol/day higher in Cracow than in Mirano and intermediate in Novosibirsk. Urinary aldosterone excretion was higher in Novosibirsk than in the two other centres (Table 1). As shown in Table 2, LVM was significantly higher in Slavic participants than in Italians.

Genotypes

With the exceptions of the ACE *D/I* genotype in Mirano ($P = 0.02$), the within-centre frequencies of genotypes (Table 3) complied with Hardy–Weinberg equilibrium ($0.18 < P < 0.96$). The ACE *D* allele frequency was higher ($P < 0.05$) in Mirano ($n = 260$, 58.5%) than in Cracow ($n = 282$, 51.1%) and Novosi-

Table 1 Characteristics of participants by centre

Characteristic	Cracow ($n = 282$)	Novosibirsk ($n = 276$)	Mirano ($n = 260$)
Clinical characteristics			
Age (years)	35.1 ± 13.9	38.2 ± 14.1[C]	41.2 ± 13.9[C,N]
Female, *n* (%)	154 (54.5)	157 (56.9)	137 (52.7)
Height (cm)	169.5 ± 9.1	168.1 ± 9.3	167.1 ± 9.2[C]
Weight (kg)	72.6 ± 14.6	71.3 ± 14.1	70.6 ± 13.9
Pulse rate (beats/min)	73.3 ± 9.8	73.9 ± 7.7	73.2 ± 9.8
Systolic pressure (mmHg)[a]	125.9 ± 17.0	125.1 ± 19.1	124.7 ± 15.5
Diastolic pressure (mmHg)[a]	78.9 ± 11.4	80.6 ± 12.1	79.7 ± 9.6
Questionnaire data			
Current smokers, *n* (%)	70 (24.8)	83 (29.5)	62 (23.7)
Using ⩾5 g alcohol/day, *n* (%)	57 (20.2)	130 (46.3)[C]	110 (42.0)[C]
Treated with antihypertensive drugs, *n* (%)	54 (19.2)	38 (13.5)	41 (15.7)
Biochemical data			
ACE activity (U/l)	38.9 (20.0–72.4)	36.3 (20.0–63.1)	29.5 (17.0–52.5)[C,N]
Urinary volume (l/day)[b]	1.43 ± 0.51	1.33 ± 0.47	1.50 ± 0.57[N]
Creatinine excretion (mmol/day)	12.1 ± 4.0	11.0 ± 3.7[C]	10.7 ± 3.0[C]
Sodium excretion (mmol/day)	243 ± 83	213 ± 95[C]	186 ± 66[C,N]
Potassium excretion (mmol/day)	65 ± 24	60 ± 22[C]	63 ± 23
Aldosterone excretion (nmol/day)	10.7 (4.8–24.0)	18.6 (8.3–41.7)[C]	14.5 (6.9–29.5)[C,N]

ACE, angiotensin-converting enzyme. Values are arithmetic means ± SD or geometric means (10–90% percentile interval), or number of subjects (%). P-values for between-centre differences were adjusted for multiple comparisons, using Tukey's test: [C] $P < 0.05$ versus Cracow; [N] $P < 0.05$ versus Novosibirsk. [a] Average of five readings. [b] The number of subjects with 24-h urinary collection was 278 in Cracow, 245 in Novosibirsk and 243 in Mirano.

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Table 2 **Echocardiographical measurements by centre**

Variable	Cracow ($n = 282$)	Novosibirsk ($n = 276$)	Mirano ($n = 260$)
Left ventricular mass (g)	174.6 ± 49.5	173.3 ± 57.8	144.7 ± 41.1^{C,N}
Left ventricular mass/BSA (g/m^2)	94.7 ± 21.0	95.2 ± 26.5	80.3 ± 19.2^{C,N}
Left ventricular mass/height$^{2.7}$ (g/m$^{2.7}$)	42.1 ± 12.0	42.8 ± 14.6	36.2 ± 10.2^{C,N}
Left ventricular internal diameter (mm)	48.2 ± 4.1	49.1 ± 4.5^{C}	47.1 ± 4.7^{C,N}
Mean wall thickness (mm)	10.0 ± 1.6	9.7 ± 2.0	9.0 ± 1.5^{C,N}
Left ventricular fractional shortening (%)	39.7 ± 4.3	39.1 ± 5.5	40.5 ± 7.0^{N}

BSA, body surface area. Values are arithmetic means ± SD. *P*-values for between-centre differences were adjusted for multiple comparisons, using Tukey's test: $^{C}P < 0.05$ versus Cracow; $^{N}P < 0.05$ versus Novosibirsk.

Table 3 **Genotype and allele frequencies**

Gene	Genotype			Allele	
ACE	*DD*	*DI*	*II*	*D*	*I*
Cracow	72 (26.1)	144 (50.7)	66 (23.2)	288 (51.1)	276 (48.9)
Novosibirsk	77 (27.9)	138 (50.0)	61 (22.1)	292 (52.9)	260 (47.1)
Mirano	79 (30.4)	146 (56.2)	35 (13.4)	304 (58.5)	216 (41.5)*
Aldosterone synthase	*CC*	*CT*	*TT*	*C*	*T*
Cracow	59 (20.8)	152 (54.2)	71 (25.0)	270 (47.9)	294 (52.1)
Novosibirsk	79 (28.6)	134 (48.6)	63 (22.8)	292 (52.9)	260 (47.1)
Mirano	67 (25.6)	122 (47.2)	71 (27.2)	256 (49.2)	264 (50.8)

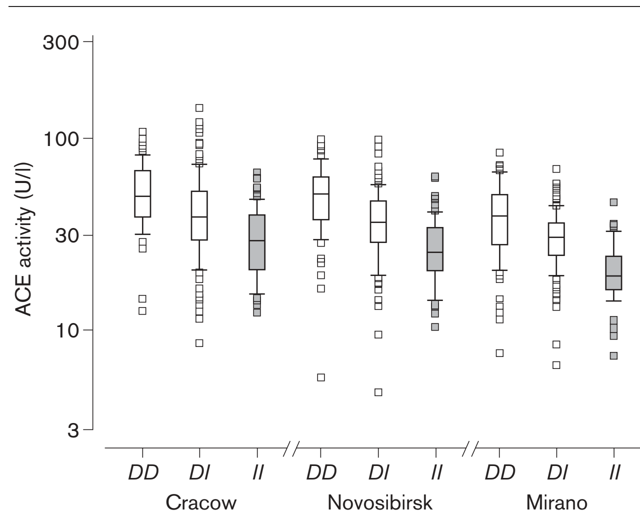
ACE, angiotensin-converting enzyme. *$P < 0.05$ versus both Slavic centres.

birsk ($n = 276$, 52.9%). Across centres (Fig. 1), plasma ACE activity was highest in *DD* subjects, lowest in *II* homozygotes, and intermediate in *DI* heterozygotes ($P < 0.0001$). Urinary aldosterone was not related to the *–344C/T* polymorphism ($P = 0.55$).

Population-based association study

We adjusted the left ventricular phenotypes for centre, sex, age, systolic blood pressure, body weight and height, use of antihypertensive drugs, and lifestyle factors, including smoking and alcohol consumption in excess of 5 g/day. Models with LVMI as the dependent variable were not adjusted for body weight and height.

Fig. 1



Box plots of angiotensin-converting enzyme (ACE) activity by centre and ACE *D/I* genotype. Statistics include median, interquartile range, 5th to 95th percentile interval, and outliers.

For LVMI ($P = 0.005$) and LVID ($P = 0.04$) in relation to the ACE *D/I* genotype, we found significant heterogeneity between Mirano and the two Slavic centres, but not between Cracow and Novosibirsk ($P > 0.21$). For the aldosterone synthase *–344C/T* polymorphism, we did not observe any heterogeneity in the phenotype–genotype relationships ($P > 0.70$). Thus, for analyses involving the ACE *D/I* polymorphism, we pooled the Polish and Russian participants and we analysed Italian subjects separately, whereas for analyses of the aldosterone synthase *–344C/T* genotype, we combined all subjects.

In the two Slavic centres, LVMI was higher in ACE *II* homozygotes than in *D* allele carriers, both in the whole population and in unrelated founders (Table 4), due to the higher MWT in *II* homozygotes (Table 4). By contrast, in all Italian subjects, LVMI was slightly higher in *DD* homozygotes than in the other ACE genotypes, because of the higher LVID (Table 4). However, in neither all subjects nor in founders of the three centres combined were the left ventricular phenotypes associated with the aldosterone synthase *–344C/T* polymorphism ($0.39 < P < 0.97$).

Family-based association study

Our study population ($n = 818$) included 382 parents and 436 offspring. The number of offspring per family amounted to one in 25 families, two in 171 families and three in 23 families.

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Table 4 Left ventricular phenotypes by ACE *D/I* genotypes

Phenotype		Genotype			P
		DD, statistics (SE)	*DI*, statistics (SE)	*II*, statistics (SE)	
Slavic					
Number	All	149	282	127	
	Founders	65	123	58	
LVMI (g/m^2)	All	93.0 (1.55)	94.3 (1.23)	99.1 (1.81)	0.01
	Founders	103.5 (2.43)	105.8 (2.13)	113.0 (2.67)	0.007
LVID (mm)	All	48.7 (0.34)	48.7 (0.27)	48.7 (0.32)	0.95
	Founders	49.2 (0.56)	48.7 (0.40)	48.7 (0.42)	0.51
MWT (mm)	All	9.7 (0.10)	9.9 (0.07)	10.1 (0.10)	0.01
	Founders	10.6 (0.13)	10.8 (0.12)	11.4 (0.17)	0.001
Mirano					
Number	All	79	146	35	
	Founders	44	73	19	
LVMI (g/m^2)	All	84.3 (2.27)	79.5 (1.36)	78.5 (2.14)	0.05
	Founders	91.2 (3.00)	86.9 (2.05)	87.6 (3.82)	0.29
LVID (mm)	All	48.3 (0.53)	46.8 (0.40)	46.4 (0.59)	0.02
	Founders	48.3 (0.75)	46.6 (0.52)	46.8 (0.91)	0.12
MWT (mm)	All	9.0 (0.13)	9.0 (0.10)	9.0 (0.13)	0.92
	Founders	9.7 (0.17)	9.7 (0.14)	9.8 (0.18)	0.55

LVMI, left ventricular mass index; LVID, left ventricular internal diameter; MWT, mean wall thickness. Analyses were adjusted for centre (Slavic subjects), sex, age, systolic blood pressure, antihypertensive treatment, smoking and alcohol intake. In addition, LVID and MWT were also adjusted for body weight and height. *P*-values for comparison across genotypes were derived by generalized estimating equations (GEE).

For LVMI in relation to both genotypes, the orthogonal model did not demonstrate population stratification in any centre ($P > 0.08$). However, for LVMI in relation to the ACE *D/I* polymorphism, the orthogonal model revealed significant heterogeneity between the Slavic and Italian populations ($\chi^2 = 36.3$, df 11, $P < 0.01$).

In Slavic offspring, both the orthogonal ($P = 0.023$; empirical $P = 0.021$) and the logistic ($P = 0.034$) models disclosed significant association between LVMI and the ACE *D/I* polymorphism. Furthermore, as shown in Figure 2, the probability of the *I* allele being transmitted to offspring increased with higher LVMI in the two Slavic populations, but not in the Italians. Using Allison's approach in Slavic subjects, the number of informative offspring decreased to 142, but the test statistic for association between LVMI and the ACE *D/I* polymorphism remained borderline significant ($\chi^2 = 5.63$, $P = 0.06$). None of the TDT models provided any evidence for association between the left ventricular phenotypes and the *–344C/T* polymorphism in the aldosterone synthase gene (Table 5).

LVMI and sodium excretion

Because of the between-centre differences in LVMI, urinary sodium excretion and the frequency of the ACE *D/I* genotype, we searched in untreated offspring for possible interaction between these measurements. Independent of the ACE genotype and other covariables, there was positive relationship between LVMI and urinary sodium excretion in Slavic offspring ($\beta = 0.026 \pm 0.0098$; $P = 0.01$), but not in Italians ($\beta = -0.008 \pm 0.0188$; $P = 0.67$). Furthermore, in Slavic as well as Italian ACE *II* homozygotes, LVMI significantly increased with higher sodium excretion, on average by 4.2 g/m^2 per 100 mmol (Fig. 3). In ACE *D* allele carriers, LVMI also increased with higher sodium excretion in Slavic offspring, but not in Italians. The interaction between ACE *D/I* genotype and urinary sodium excretion was significant in Italian offspring ($P = 0.03$).

Discussion

The main finding of the present study was that the relationship between LVMI and the ACE *D/I* polymorphism differed across populations, possibly as a consequence of the intermediate regulatory mechanisms responsive to varying levels of salt intake. Indeed, LVMI, urinary sodium excretion and plasma ACE activity were higher in Slavic than Italian participants, whereas the frequency of the *D* allele was significantly higher in Italians. The heterogeneity between the Slavic and Italian subjects in the phenotype–genotype relationship with the ACE *D/I* genotype was statistically significant. Thus, our present findings are in agreement with the concept that phenotype–genotype associations differ according to epidemiological context, including genetic background and lifestyle [26]. On the other hand, observations collected in a general population should not be extrapolated to patients with left ventricular hypertrophy as a consequence of a chronic disorder such as hypertension.

LVMI and MWT increased with the ACE *I* allele in Slavic subjects, whereas in Italians LVMI and LVID were slightly higher in *DD* homozygotes. At first sight, these findings may seem contradictory. However, the

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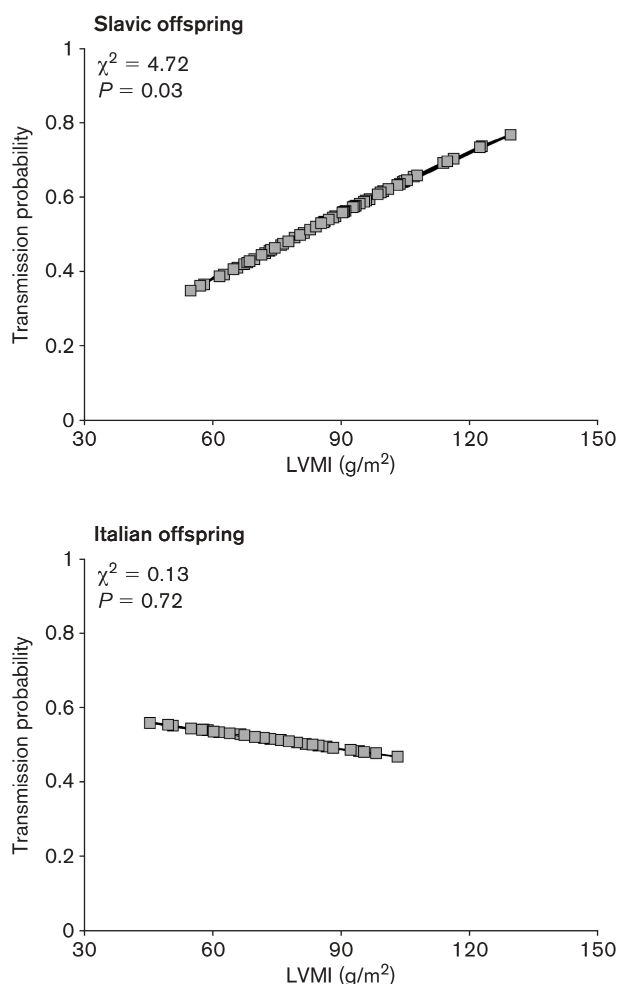
Fig. 2



Probability of *I*-allele transmission of the angiotensin-converting enzyme (ACE) gene as a function of left ventricular mass index (LVMI). The analyses were adjusted for centre (Slavic subjects), sex, age, systolic blood pressure, antihypertensive treatment, smoking and alcohol intake.

association with the ACE *DD* genotype in Italians was weak and appeared only in population-based analyses, and not in the TDT approach, which allows for population stratification and admixture. In *II* homozygous offspring of both Slavic and Italian extraction, LVMI increased with higher sodium excretion. In Slavic, but not Italian, siblings carrying the *D* allele, there was a positive relationship between LVMI and urinary sodium excretion. The interaction between the ACE genotype and urinary sodium excretion was significant in Mirano. These findings, although based on small numbers, suggest that the relationship between LVMI and the ACE *D/I* polymorphism might be modulated by mechanisms responsive to varying level of salt intake.

Intra-tissue formation of angiotensin II probably plays a critical role in cardiovascular remodelling [27]. According to current knowledge, systemic ACE activity does not change with salt intake [28]. In addition, in genetically modified mice expressing ACE in the liver, but not in the lungs and the endothelium, plasma ACE activity decreased by approximately 60%, but plasma angiotensin I and angiotensin II levels were significantly higher than in wild-type animals [29]. These experimental findings highlight the plasticity of the RAAS, and illustrated that angiotensin II can be generated via multiple pathways, including proteolysis by cathepsin G, chymostatin-sensitive angiotensin II-generating enzyme and chymase [30]. Nevertheless, Schmieder *et al.* [5] demonstrated that the plasma concentration of angiotensin II was positively correlated with plasma renin activity and decreased with higher sodium intake. Thus, impaired suppression of the RAAS may act as a stimulus increasing LVM [5]. ACE *II* homozygotes have lower plasma ACE activity than *D* allele carriers. For this reason, we speculate that the margin of adaptation of the RAAS in response to varying levels of sodium intake is smaller in *II* homozygotes than in *D* allele carriers. In line with this hypothesis, some investigators found that patients homozygous for the *I* allele experienced a significantly greater rise in blood pressure in response to a high salt intake, compared to *DD* homozygotes [31,32].

Aldosterone has both myocardial and renal effects that may have profound implications for left ventricular morphology [33]. Some investigators found that the presence of the *T* allele at the *–344C/T* locus in the promoter area of the aldosterone synthase gene was associated with higher plasma aldosterone levels [34]. However, we found no association between LVMI, urinary aldosterone excretion and the *–344C/T* polymorphism.

Our study has to be interpreted within the context of its limitations. We measured only one single nucleotide polymorphism per gene, and can therefore not exclude that full assessment of the haplotypes would have yielded even better relations with the phenotypes. We cannot exclude with certainty that our findings were due to random variability. Indeed, variance component models can be sensitive to the phenotypic distribution, especially in small or selected samples. However, empirical *P* values calculated from 1000 Monte-Carlo permutations confirmed the originally calculated estimate (0.023 versus 0.021) in the variance decomposition analysis, and therefore provides added confidence in our results. One 24-h urine collection may be insufficient to characterize an individual's habitual sodium intake, but it does reflect accurately the average salt consumption of groups of subjects [35]. On the other hand, in the two Slavic centres, there was consistency between the population- and family-based approaches.

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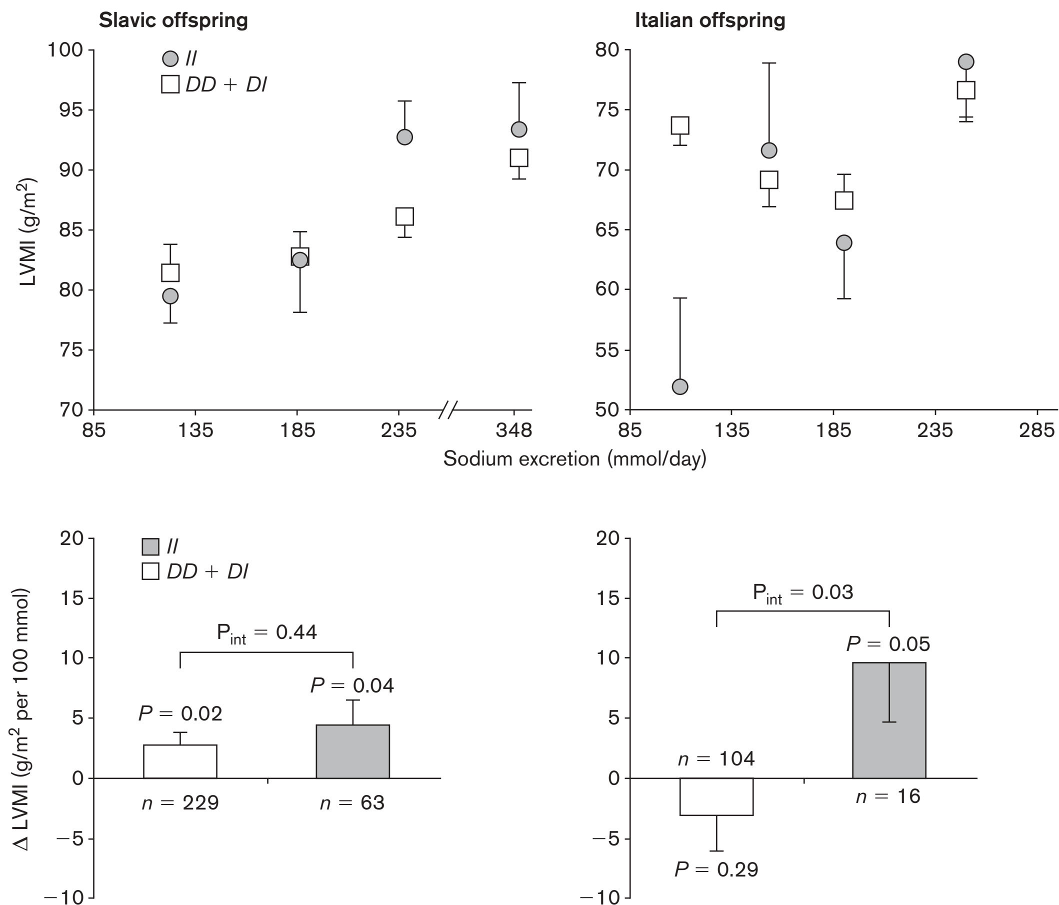
Table 5 Results of the transmission disequilibrium test (TDT) analyses for the angiotensin-converting enzyme (ACE) *D/I* and aldosterone synthase (CYP11B2) −344*C/T* polymorphisms in relation to left ventricular phenotypes

Gene	Number of informative probands all siblings	LVMI χ^2	LVMI *P*	LVID χ^2	LVID *P*	MWT χ^2	MWT *P*
ACE							
Orthogonal model							
Slavic	182/311	5.12	0.02	3.41	0.06	0.95	0.33
Mirano	85/125	1.45	0.23	0.28	0.60	0.38	0.54
Logistic model							
Slavic	181	4.72	0.03	2.49	0.11	0.65	0.42
Mirano	89	0.13	0.72	0.49	0.49	0.42	0.52
CYP11B2							
Orthogonal model							
All populations	284/436	0.49	0.48	1.45	0.23	0.01	0.93
Logistic model							
All populations	285	0.36	0.55	0.30	0.59	0.49	0.48

LVMI, left ventricular mass index; LVID, left ventricular internal diameter; MWT, mean wall thickness. The orthogonal model accounted for between- and within-family variability components. Analyses were adjusted for centre (Slavic subjects), sex, age, systolic blood pressure, antihypertensive treatment, smoking and alcohol intake. In addition, LVID and MWT were also adjusted for body weight and height.

Fig. 3



Association between left ventricular mass index (LVMI) and 24-h urinary sodium excretion by angiotensin-converting enzyme (ACE) genotype in 412 untreated offspring of Slavic or Italian origin. LVMI was adjusted for centre (Slavic offspring), sex, age, systolic blood pressure, smoking and alcohol intake. Upper panels show the association in quartiles of the distribution of sodium excretion. Lower panels show differences in LVMI associated, in multiple regression analysis, with a 100 mmol increase in the 24-h sodium excretion. P_{int} indicates the *P* values for interaction between the ACE genotype and 24-h urinary sodium excretion analysed as a continuous variable.

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We did not find significant population stratification in any of the three centres. The same laboratory performed all measurements of ACE activity and urinary aldosterone. LVM is a quantitative trait prone to measurement error, but in each centre only one experienced observer performed all ultrasound examinations and echocardiographical measurements, with high intra-observer inter-session reproducibility. We explored the relationship between LVMI and urinary sodium excretion only in offspring not taking antihypertensive drugs. In comparison with the founder generation, in offspring, the relationship between LVMI and salt intake is less likely to be confounded by chronic age-related disorders of the cardiovascular system, including hypertension, and by other lifestyle factors, such as heavy manual work or drinking alcohol.

In conclusion, the relationship between LVMI and the ACE *D/I* polymorphism differs across populations, possibly as a consequence of varying levels of salt intake. Our findings are hypothesis-generating and should be confirmed in experiments with measurement of the intermediate pathophysiological pathways.

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