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ORIGINAL ARTICLE

Left ventricular structure in relation to the human *SAH* gene in the European Project on Genes in Hypertension

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Earlier studies showed association of the human *SAH* (Spontaneously hypertensive rat-clone A-Hypertension associated) gene with hypertension and obesity. Left ventricular mass index (LVMI) increases with blood pressure and body mass index. In a family-based population study (54.5% women; mean age, 43.1 years), we measured LVMI, mean wall thickness (MWT) and the left ventricular internal diameter (LVID) at end-diastole in 699 non-Slavic and 493 Slavic participants. In multivariable-adjusted analyses, we investigated phenotype–genotype associations (*SAH G−1606A* and *−962ins/del* polymorphisms), while accounting for confounders and relatedness. Non-Slavic *−1606GG* homozygotes had a slightly greater LVID than *−1606A* allele carriers (48.6 *vs.* 48.0 mm; *P*=0.08). However, the between-family component of the variance in LVID was significant (*P*=0.005), suggesting that population stratification might explain the latter finding. Non-Slavic *−962del* carriers had higher LVMI (91.1 *vs.* 88.5 g m^{-2}; *P*=0.03) and MWT (9.61 *vs.* 9.44 mm; *P*=0.03) than *−962ins* homozygotes. Transmission of the *−962del* to non-Slavic offspring was also associated with higher MWT (*P*=0.03). In Slavic participants, in the absence of population stratification (*P*⩾0.69), *−1606GG* homozygotes had lower LVMI (96.5 *vs.* 102.3 g m^{-2}; *P*=0.004) and lower MWT (10.1 *vs.* 10.5 mm; *P*=0.003) than *−1606A* carriers. Sensitivity analyses showed that the latter associations were confined to founders. Transmission of the *−962del* allele to Slavic offspring was associated with lower MWT (*P*=0.007). In conclusion, LVMI and MWT, two phenotypes that are jointly influenced by blood pressure and obesity, might be related to variation in the human *SAH* gene.
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INTRODUCTION

The expression of *SAH* (Spontaneously hypertensive rat-clone A-Hypertension-associated) gene is markedly higher in the kidneys of spontaneously hypertensive rats compared with normotensive Wistar–Kyoto control rats.[1] In rats, SAH is expressed mainly in the proximal renal tubules.[2] The human *SAH* gene is located on chromosome 16p13.11.[3] Several earlier studies suggested association of the human *SAH* gene with hypertension and related metabolic phenotypes, such as obesity,[4] hypercholesterolemia[5] and hypertriglyceridemia.[6] However, we recently analyzed blood pressure, measures of obesity, serum lipids and blood glucose as continuous phenotypes in 2603 relatives

from 560 families and 31 unrelated subjects randomly recruited from six populations in the framework of the European Project on Genes in Hypertension (EPOCH). We genotyped our study population for *SAH G−1606A* and *−962ins/del*. In spite of sufficient statistical power, none of the phenotype–genotype associations approached statistical significance.[7]

Left ventricular mass index (LVMI) increases with blood pressure and body mass index.[8] This phenotype might therefore more readily pick up association with variation in the *SAH* gene. In this study, we explored possible association between left ventricular structure and the *G−1606A* and *−962ins/del* polymorphisms in the human *SAH* gene.

METHODS

Study population

We conducted the European Project on Genes in Hypertension according to the principles outlined in the Helsinki declaration for investigations in human subjects.[9] The Ethics Committee of each institution approved the study. In seven European countries, investigators randomly enrolled nuclear and extended families of White Caucasian ethnicity. Four centers took part in the substudy on echocardiography[10,11] and together enrolled 1522 subjects in Kraków (Poland; $n=325$), Novosibirsk (Russian Federation; $n=301$), Mirano (Italy; $n=345$) and Hechtel-Eksel (Belgium; $n=551$). The overall response rate was 67.8%. All participants gave informed written consent. We excluded 330 subjects from analysis, (1) because ECGs were either unavailable ($n=96$) or of insufficient quality ($n=52$), (2) because participants had left ventricular dysfunction due to myocardial infarction or valvular disorders ($n=52$), (3) because of lack of measurements required for our analysis ($n=58$), (4) because DNA failed to amplify ($n=63$) (5) or because of inconsistency in Mendelian segregation ($n=9$). Thus, the number of subjects statistically analyzed totaled 1192.

Echocardiographic measurements

In each center, one highly experienced observer performed all ECGs, using a commercially available device equipped with a 3.5-MHz transducer.[10,11] With subjects in left decubitus and breathing normally, the observers obtained M-mode echocardiograms of the left ventricle from the parasternal long-axis view under control of the two-dimensional image. They positioned the ultrasound beam just below the mitral valve at the level of the posterior chordae tendineae. The echocardiographic examinations were recorded on videotape and analyzed offline. The observers measured left ventricular internal diameter (LVID) and interventricular septal and posterior wall thickness at end-diastole, according to the recommendations of the American Society of Echocardiography.[12] Mean wall thickness (MWT) was half of the sum of the interventricular septal and posterior wall thickness at end-diastole. Left ventricular end-diastolic dimensions were used to compute left ventricular mass by an anatomically validated formula.[12] LVMI was left ventricular mass divided by body surface area, calculated as body weight 0.425 (in kg)×body height 0.725 (in cm)×0.007184. For statistical analysis, the measurements of three cardiac cycles were averaged.

Other measurements

With the subjects seated for at least 5 min, trained nurses obtained five consecutive blood pressure readings at the examination center. The blood pressure phenotype was the average of these five readings. Hypertension was a blood pressure of $\geqslant140$ mm Hg systolic or $\geqslant90$ mm Hg diastolic or the use of blood pressure lowering medications. Body mass index was weight in kilograms divided by the square of height in meters. We used a standardized questionnaire to collect information on each subject's medical history, smoking and drinking habits and intake of medications. The participants collected a 24-h urine sample in a wide-neck plastic container for the measurement of sodium, potassium and creatinine. If urinary volume or creatinine excretion were outside published limits,[13] the urinary measurements were considered to be missing and subjects were excluded from analysis.

Determination of genotypes

We extracted genomic DNA from WBCs, using the standard kits (Qiagen, Hilden, Germany). For genotyping, we used restriction fragment length polymorphism analysis of the respective PCR products at the frequent polymorphic loci $G-1606A$ (restriction enzyme *Ksp*AI) and $-962ins/del$ (*Xap*I). Because the $C-1808T$ was completely associated with $G-1606A$,[14] we did not genotype for this variant.

Using the published sequence of human *SAH* gene (accession number AC004381.1), primers were designed to amplify both fragments covering the appropriate SAH polymorphic sites. The PCR amplification was carried out in a 20-µl volume, containing 5 ng genomic DNA, 10 and 25 pmol l^{-1} of each primer (for both fragments, respectively), 2.5 mmol l^{-1} MgCl$_2$, 200 mol l^{-1} of each dNTP, 5 mmol l^{-1} betaine and 1 U AmpliTaq polymerase (Rapidozym, Berlin, Germany). The PCR was performed initially for 3 min at 94 °C and

followed by 35 cycles, each with 45 s at 94 °C, 45 s at 54 and 56 °C (depending on primer sequence), 60 s at 72 °C and final elongation of 10 min at 72 °C.

In the presence of the $-1606A$ allele, the PCR product (221 bp) was digested by *Ksp*AI into two fragments of 191 and 30 bp in length ($-1606G$ undigested). In the presence of the $-962del$ allele, the PCR product (339 bp) was digested by *Xap*I into three fragments of 180, 93 and 66 bp in length. For $-962ins$, the PCR product (344 bp) was digested into four fragments of 180, 82, 66 and 16 bp in length, visualized on ethidium bromide-stained 3% agarose gels (primer sequences and digestion conditions are available upon request).

Statistical analysis

For database management and statistical analysis, we used SAS software, version 9.1.3 (SAS Institute, Cary, NC, USA). We compared means and proportions, using the large-sample z-test and the χ^2-statistic, respectively. Our statistical methods also included single and multiple linear regressions. We included in our models covariables with known physiological relevance to the left ventricular phenotypes under study, using stepwise multiple regressions with the significance level for independent variables to enter and stay in the model set at 0.15.

We performed both population-based and family-based analyses. As in earlier analyses,[7] we combined the least frequent homozygous group with the heterozygous subjects. We tested linkage disequilibrium, using the SAS procedure PROC ALLELE. We applied a generalization of the standard linear model, as implemented in the PROC MIXED procedure of the SAS package, to test the associations between phenotypes and single-nucleotide polymorphisms, while accounting for relatedness and adjusting for covariables. We tested for heterogeneity, using appropriate interaction terms with the genotypes.

In the family-based analysis, we performed transmission disequilibrium tests for quantitative traits (QTDT). We partitioned the phenotypic variance into between and within family components, using the orthogonal model[15] as implemented in Abecasis's QTDT software (version 2.6.0, available at http://www.sph.umich.edu/csg/abecasis/QTDT). The within-family component of phenotypic variance reflects the genetic effect and is robust to population stratification.

RESULTS

Characteristics of the participants

The study population consisted of 1014 relatives from 252 families and 178 unrelated subjects. There was significant heterogeneity between the non-Slavic (Mirano and Hechtel-Eksel) and Slavic (Kraków and Novosibirsk) participants in the association of MWT ($P=0.065$) and LVMI ($P=0.012$) with the *SAH* $G-1606A$ polymorphism. Moreover, non-Slavic and Slavic participants had different ($P<0.0001$) age (47.6 *vs.* 36.7 years), waist-to-hip ratio (0.86 *vs.* 0.83), pulse rate (66.0 *vs.* 70.7 beats per minute), urinary excretion of sodium (175.3 *vs.* 230.0 mmol day^{-1}) and potassium (69.4 *vs.* 62.1 mmol day^{-1}), and LVMI (87.5 *vs.* 94.9 g m^{-2}). For these reasons, in keeping with earlier reports,[10] we stratified all analyses according to ethnicity. Tables 1 and 2 list the subjects' characteristics by *SAH* genotypes for non-Slavic and Slavic participants, respectively. In both non-Slavic and Slavic subjects, LVMI increased with age (Figure 1).

Genotype frequencies

Table 3 reports the genotype and allele frequencies of the two *SAH* polymorphisms. The $-1606A$ allele frequencies were 11.7 and 12.1% in non-Slavic and Slavic participants, respectively (P for difference between ethnicities, 0.80). The frequency of the $-962del$ allele was higher in non-Slavic than Slavic subjects (30.4 *vs.* 26.0%; $P=0.02$).

In all four centers, the frequencies of the $G-1606A$ and $-962ins/del$ genotypes were complied with Hardy–Weinberg proportions ($0.20<P<0.59$ and $0.14<P<0.98$, respectively), except for the $G-1606A$ genotypes in Kraków ($P=0.02$). The two *SAH* polymorphisms were in complete linkage disequilibrium. Lewontin's disequilibrium coefficient D' was 1.00 ($P<0.0001$).

Table 1 Characteristics of participants by the *G−1606A* genotypes

Characteristic	Non-slavic		Slavic	
G−1606A	AA+GA (n=158)	GG (n=541)	AA+GA (n=115)	GG (n=378)
Anthropometry				
Women, *n* (%)	86 (54.4)	282 (52.1)	53 (46.0)	229 (60.6)*
Age (years)	47.9 ± 15.6	47.5 ± 14.6	35.2 ± 13.5	37.1 ± 14.3
Body height (cm)	168.4 ± 9.1	168.2 ± 9.5	170.4 ± 8.6	168.1 ± 9.2
Body weight (kg)	73.3 ± 12.7	72.8 ± 13.5	72.7 ± 15.2	71.8 ± 14.2
Waist-to-hip ratio	0.85 ± 0.08	0.86 ± 0.08	0.84 ± 0.08	0.82 ± 0.09
Body mass index (kg m^{-2})	25.8 ± 3.8	25.7 ± 4.0	25.0 ± 4.8	25.5 ± 5.1
Pulse rate (beats min^{-1})	66.4 ± 9.5	65.8 ± 10.3	70.1 ± 9.2	70.9 ± 9.8
Systolic pressure (mm Hg)	126.3 ± 15.5	126.5 ± 17.1	122.9 ± 16.3	125.6 ± 21.0
Diastolic pressure (mm Hg)	78.7 ± 9.2	79.5 ± 10.0	80.1 ± 10.5	80.8 ± 12.9
Questionnaire data				
Current smokers, *n* (%)	30 (19.0)	130 (24.0)	33 (28.7)	90 (23.8)
Using ⩾5 g alcohol per day, *n* (%)	66 (41.8)	237 (43.8)	42 (36.5)	103 (27.3)
On antihypertension treatment, *n* (%)	36 (22.8)	100 (18.5)	15 (13.0)	69 (18.3)
Family history of hypertension, *n* (%)	71 (44.9)	219 (40.5)	61 (53.0)	172 (45.5)
24-h urinary measurements				
Volume (l day^{-1})	1.76 ± 0.70	1.67 ± 0.72	1.47 ± 0.50	1.37 ± 0.50
Creatinine (mmol day^{-1})	10.9 ± 3.9	11.4 ± 4.1	12.2 ± 3.9	11.4 ± 3.9
Sodium (mmol day^{-1})	182.4 ± 73.6	173.3 ± 69.3	260.6 ± 113.6	220.7 ± 88.7[†]
Potassium (mmol day^{-1})	71.0 ± 28.8	68.9 ± 26.0	67.2 ± 24.9	60.5 ± 23.1*

Data are mean ± s.d. or number of subjects (%). Blood pressure was the average of five readings.
Significance of the difference between genotypes: *P⩽0.01; [†]P⩽0.001.

Table 2 Characteristics of participants by the *−962ins/del* genotypes

Characteristic	Non-Slavic		Slavic	
−962ins/del	DD+ID (n=367)	II (n=332)	DD+ID (n=221)	II (n=272)
Anthropometry				
Women, *n* (%)	183 (49.9)	185 (55.7)	138 (62.4)	144 (52.9)*
Age (years)	48.1 ± 14.8	47.0 ± 14.9	37.9 ± 14.4	35.7 ± 13.9
Body height (cm)	168.5 ± 9.3	168.0 ± 9.5	167.5 ± 9.3	169.6 ± 8.9
Body weight (kg)	73.5 ± 13.8	72.2 ± 12.8	72.1 ± 13.8	71.9 ± 14.9
Waist-to-hip ratio	0.86 ± 0.09	0.85 ± 0.08	0.83 ± 0.08	0.83 ± 0.08
Body mass index (kg m^{-2})	25.8 ± 4.0	25.6 ± 3.8	25.8 ± 5.2	25.0 ± 4.9
Pulse rate (beats min^{-1})	65.9 ± 10.7	66.0 ± 9.4	70.8 ± 10.2	70.7 ± 9.2
Systolic pressure (mm Hg)	127.0 ± 16.5	125.8 ± 16.9	126.4 ± 22.2	123.8 ± 18.0
Diastolic pressure (mm Hg)	78.0 ± 9.4	78.6 ± 10.2	81.7 ± 12.7	79.7 ± 12.1
Questionnaire data				
Current smokers, *n* (%)	87 (23.7)	73 (22.0)	56 (25.3)	67 (24.6)
Using ⩾5 g alcohol per day, *n* (%)	170 (46.3)	133 (40.0)	64 (29.0)	81 (29.8)
On antihypertension treatment, *n* (%)	73 (19.9)	63 (19.0)	38 (17.2)	46 (16.9)
Family history of hypertension, *n* (%)	154 (42.0)	136 (41.0)	91 (41.2)	142 (52.2)*
24-hour urinary measurements				
Volume (l day^{-1})	1.71 ± 0.71	1.66 ± 0.72	1.40 ± 0.46	1.39 ± 0.53
Creatinine (mmol day^{-1})	11.3 ± 4.0	11.3 ± 4.2	11.1 ± 3.8	12.0 ± 4.0*
Sodium (mmol day^{-1})	177.6 ± 70.3	172.8 ± 70.5	223.9 ± 92.5	234.9 ± 99.4
Potassium (mmol day^{-1})	70.5 ± 27.9	68.2 ± 25.1	61.8 ± 23.1	62.3 ± 24.1

Data are mean ± s.d. or number of subjects (%). Blood pressure was the average of five readings.
Significance of the difference between genotypes: *P⩽0.05.

Population-based association study

As in earlier analyses,[10,11] we adjusted the left ventricular phenotypes for center, sex, age, systolic blood pressure, body weight and height, waist-to-hip ratio, pulse rate, use of antihypertensive drugs and lifestyle factors, including smoking and alcohol consumption in excess of 5 g per day. Models with LVMI as the dependent variable were not adjusted for body weight and body height. With these adjustments applied, in both non-Slavic and Slavic participants, we did not detect any heterogeneity between single centers in the relations between the left ventricular phenotypes and the *G−1606A* and *−962ins/de*l polymorphisms.

In non-Slavic participants (Table 4), the multivariable-adjusted mixed model showed a slightly greater (*P*=0.080) LVID in *−1606GG* homozygotes than in *−1606A* allele carriers. Furthermore, non-Slavic *−962del* carriers, compared with *−962ins* homozygotes, had higher LVMI (*P*=0.03) and MWT (*P*=0.03). In Slavic participants (Table 4), *−1606A* allele carriers had higher LVMI (*P*=0.004) and higher MWT (*P*=0.003) than *−1606GG* homozygotes.
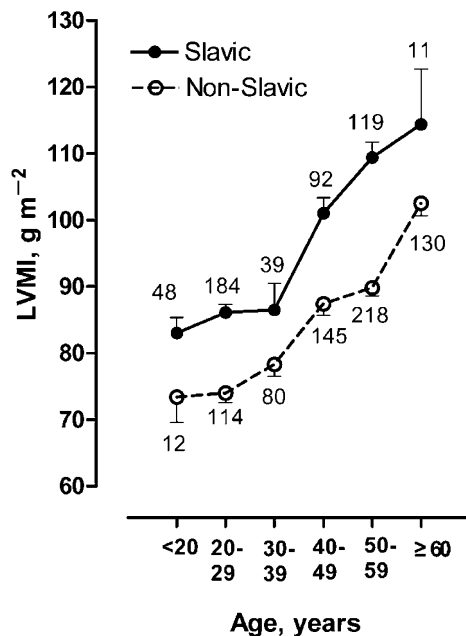

Figure 1 Left ventricular mass index (LVMI) by ethnicity and age. Values are means ± standard errors. Numbers are counts of subjects included in each plotted point.

Family-based association study

Our family-based analyses included 651 offspring and 541 parents (including unrelated subjects). We adjusted the QTDT analyses, as described above. Table 5 lists the between-family and within-family effect sizes associated with the *G−1606A* and *−962ins/del* polymorphisms. In non-Slavic participants, the between-family component of variance in LVID was significant for the *G−1606A* polymorphisms (*P*=0.005), and transmission of the *−962del* to informative offspring was associated with higher MWT (*P*=0.03). In Slavic subjects, the between-family component of variance in MWT was borderline significant (*P*=0.055) for the *−962ins/del* polymorphism. Transmis-

Table 4 Left ventricular phenotypes in relation with variation in the *SAH* gene

	Non-Slavic		
G−1606A	*AA+GA* (n=158)	*GG* (n=541)	P-*values*
LVMI (g m^{-2})	88.1 ± 1.5	90.4 ± 1.1	0.12
LVID (mm)	48.0 ± 0.4	48.6 ± 0.3	0.08
MWT (mm)	9.49 ± 0.11	9.54 ± 0.08	0.63
−962ins/del	*DD+ID* (n=367)	*II* (n=332)	P-*values*
LVMI (g m^{-2})	91.1 ± 1.2	88.5 ± 1.2	0.03
LVID (mm)	48.4 ± 0.3	48.5 ± 0.3	0.83
MWT (mm)	9.61 ± 0.08	9.44 ± 0.08	0.03
	Slavic		
G−1606A	*AA+GA* (n=115)	*GG* (n=378)	P-*values*
LVMI (g m^{-2})	102.3 ± 2.0	96.5 ± 1.4	0.004
LVID (mm)	48.6 ± 0.4	48.3 ± 0.3	0.45
MWT (mm)	10.5 ± 0.13	10.1 ± 0.09	0.003
−962ins/del	*DD+ID* (n=221)	*II* (n=272)	P-*values*
LVMI (g m^{-2})	97.6 ± 1.7	98.2 ± 1.5	0.70
LVID (mm)	48.5 ± 0.3	48.2 ± 0.3	0.49
MWT (mm)	10.1 ± 0.11	10.2 ± 0.10	0.20

Data are mean ± s.e. LVMI, LVID and MWT indicate left ventricular mass index, left ventricular end-diastolic internal diameter and mean wall thickness, respectively. *P*-values refer to the difference between genotypes.

Table 3 Frequencies of genotypes and alleles

	Genotypes			*Alleles*	
Variable	*GG*	*GA*	*AA*	*G*	*A*
G−1606A					
Non-Slavic (*n*=699)	541 (77.4%)	152 (21.8%)	6 (0.86%)	1234 (88.3%)	164 (11.7%)
Slavic (*n*=493)	378 (76.7%)	111 (22.5%)	4 (0.80%)	867 (87.9%)	119 (12.1%)
	DD	*DI*	*II*	*D*	*I*
−962ins/del					
Non-Slavic (*n*=699)	58 (8.3%)	309 (44.2%)	332 (47.5%)	425 (30.4%)	973 (69.6%)
Slavic (*n*=493)	35 (7.1%)	186 (37.7%)	272 (55.2%)	256 (26.0%)	730 (74.0%)

Values indicate number of subjects or alleles (%).

Table 5 Between- and within-family effect sizes associated with transmission of alleles

Variable	N	Effect size ± s.e.			
		Between-family		Within-family	
		Estimate	P-values	Estimate	P-values
Non-Slavic					
−*1606A transmitted*					
LVMI (g m⁻²)	217	+2.89 ± 2.57	0.26	−2.11 ± 1.94	0.28
LVID (mm)	217	−1.93 ± 0.68	0.005	−0.55 ± 0.50	0.27
MWT (mm)	217	+0.15 ± 0.18	0.42	−0.09 ± 0.15	0.53
−*962del transmitted*					
LVMI (g m⁻²)	279	+1.62 ± 1.67	0.33	+1.87 ± 1.44	0.20
LVID (mm)	279	+0.12 ± 0.45	0.79	−0.49 ± 0.38	0.19
MWT (mm)	279	+0.12 ± 0.12	0.33	+0.24 ± 0.11	0.03
Slavic					
−*1606A transmitted*					
LVMI (g m⁻²)	79	+0.48 ± 3.04	0.88	+2.63 ± 3.03	0.39
LVID (mm)	79	−0.03 ± 0.67	0.96	+0.09 ± 0.69	0.89
MWT (mm)	79	+0.08 ± 0.20	0.69	+0.21 ± 0.21	0.31
−*962del transmitted*					
LVMI (g m⁻²)	110	−1.77 ± 2.14	0.41	−3.33 ± 2.18	0.13
LVID (mm)	110	+0.40 ± 0.48	0.41	+0.36 ± 0.49	0.48
MWT (mm)	110	−0.27 ± 0.14	0.055	−0.39 ± 0.14	0.007

N indicates the number of informative offspring. LVMI, LVID and MWT indicate left ventricular mass index, left ventricular end-diastolic internal diameter and mean wall thickness, respectively.

sion of the −*962del* to Slavic offspring was associated with decreased MWT (*P*=0.007).

Sensitivity analyses

Among non-Slavic participants, the 24-h urinary sodium excretion was lower in Hechtel-Eksel than in Mirano (169.7 *vs.* 184.8 mmol day⁻¹; *P*=0.008). Adding a genotype-by-sodium interaction term to the multivariable-adjusted mixed model enhanced the significance of the association between LVID and the G−*1606A* polymorphism (effect size in carriers of the −*1606A* allele, −2.19 mm; *P*=0.02). The interaction term was borderline significant (*P*=0.07). It indicated that a 100 mmol day⁻¹ higher sodium excretion was associated with a 0.57 mm increase in LVID in −*1606A* allele carriers, but with a 0.30 mm decrease in −*1606GG* homozygotes.

Among 231 Slavic founders (Figure 2), −*1606A* allele carriers had higher LVMI (115.8 *vs.* 107.4 g m⁻²; *P*=0.009) and higher MWT (11.6 *vs.* 11.1 mm; *P*=0.005) than −*1606GG* homozygotes. Among 262 Slavic offspring, these phenotype–genotype associations were not significant. The multivariable-adjusted estimates (−*1606A* carriers *vs.* −*1606GG* homozygotes) were 87.8 *vs.* 85.8 g m⁻² (*P*=0.37) for LVMI and 9.3 *vs.* 9.1 mm (*P*=0.20) for MWT.

Among Slavic founders, the −*962ins/del* genotype did not have any significant association with the left ventricular phenotypes; the multivariable-adjusted estimates (−*962del* carriers *vs.* −*962ins* homozygotes) were 110.6 *vs.* 108.8 g m⁻² (*P*=0.51) for LVMI and 11.3 *vs.* 11.2 mm (*P*=0.41) for MWT. In contrast, in Slavic offspring, −*962del* carriers had lower LVMI (83.9 *vs.* 87.4 g m⁻²; *P*=0.07) and MWT (8.8 *vs.* 9.3; *P*=0.0003) than −*962ins* homozygotes (Figure 2).

DISCUSSION

The key finding of our study was that in multivariable-adjusted analyses, which also accounted for relatedness, LVMI and MWT were associated with genetic variation in the human *SAH* gene. However, there was heterogeneity in the phenotype–genotype associations between non-Slavic and Slavic participants. Indeed, in non-Slavic participants, −*962del* carriers had higher LVMI and MWT than −*962ins* homozygotes. Transmission of the −*962del* to non-Slavic offspring was also associated with higher MWT. In Slavic participants, −*1606GG* homozygotes had lower LVMI and lower MWT than −*1606A* carriers, but the latter association was confined to founders. Transmission of the −*962del* allele to Slavic offspring was associated with lower MWT. These findings underscore that phenotype–genotype associations can only be reasonably investigated, while accounting for ecogenetic context.[16,17]

We stratified our analyses of the phenotype–genotype associations according to ethnicity, because of the differences between non-Slavic and Slavic subjects in LVMI, anthropometric characteristics, sodium excretion and the frequency of the −*962ins/del* genotypes. In earlier reports, we already noticed ethnic diversity between Italian and Slavic populations in the frequencies of the *AGTR2 G1675A*,[11] *ACE del/ins*[10] and *AGT C−532T*[18] polymorphisms. In the latter study,[18] Slavic compared with Italian subjects also had higher LVMI. Other investigators reported ethnic diversity between western European and Slavic populations in various genetic polymorphisms,[19–21] which is probably due to differing genetic evolution along the migration patterns when Europe started to be populated from the Middle East and North Africa.[22–24]

Research into the *SAH* gene found its roots in studies of genes, which are overexpressed in genetically hypertensive rats.[1,25] The Suita population survey[6,26] and subsequent case–control studies[4,5,14] reported significant associations of hypertension and related phenotypes, such as obesity, hypercholesterolemia and hypertriglyceridemia, with genetic variation in the *SAH* gene. Telgmann *et al.*[14] identified five polymorphisms in the *SAH* promoter region (*C−1808T*, *G−1606A*, −*962ins/del*, *G−451A*, *T−67C*), two polymorphisms in introns 5 and 7 (*C+9/In5T*, *A+20/In7T*) and one non-functional missense variant (*K359N*). In the PEGASE (Project d'Etude des Gènes de l'Hypertension Artérielle Sévère à Modérée Essentielle) case–control study for essential hypertension, Telgmann *et al.*[14] reported association between obesity-related hypertension and the *SAH G−1606A* and −*962ins/del* polymorphisms. However, we recently analyzed blood pressure, measures of obesity, serum lipids and blood glucose as continuous phenotypes in 2603 relatives from 560 families and 31 unrelated subjects randomly recruited from six populations in the framework of the EPOCH. We also genotyped the *G−1606A* and −*962ins/del* polymorphisms. In spite of sufficient statistical power, none of the phenotype–genotype associations approached statistical significance.[7]

Left ventricular mass index and MWT depend on a complex interaction between genetic and environmental factors, including lifestyle. They increase with both blood pressure and body mass index. This might explain why, in our current analyses, we were able to show the association of these left ventricular phenotypes with variation in the SAH gene, whereas our earlier study was negative.[7] The PEGASE researchers[14] reported that carriers of the −*1606A* allele had higher blood pressure and increased body mass index compared with −*1606GG* homozygotes. Blood pressure and body mass index increase with advancing age. This might explain, why in our Slavic participants the association of LVMI and MWT with the *G−1606A*
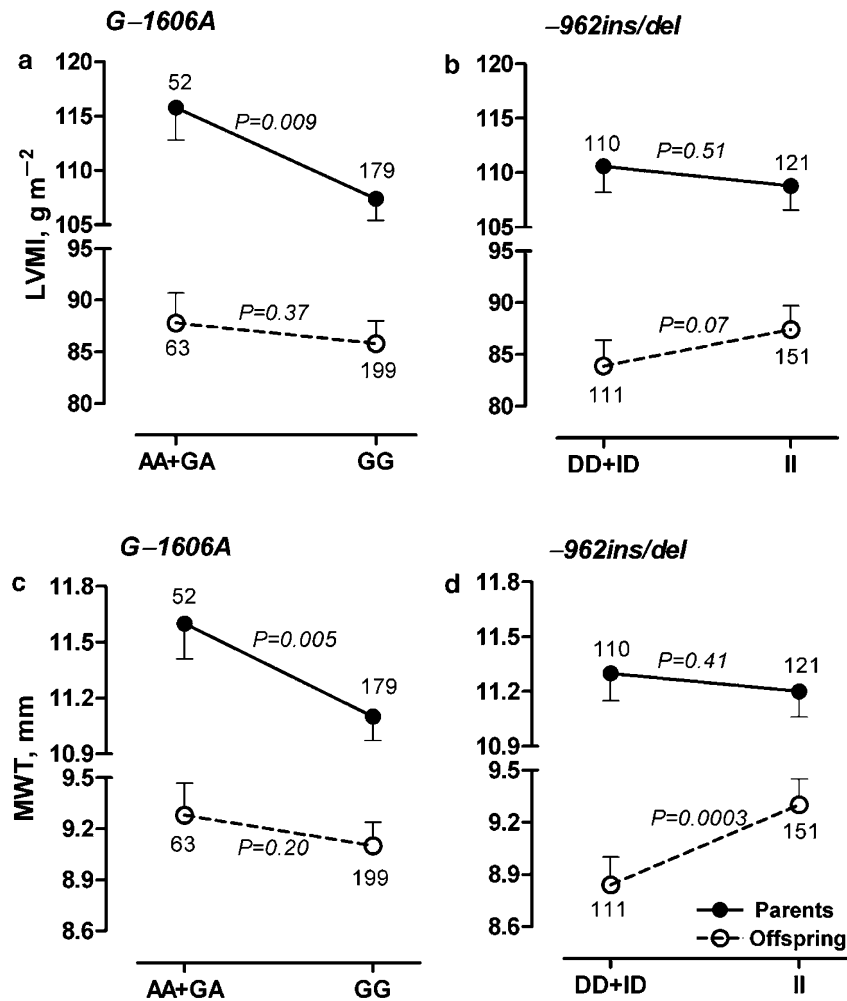
Figure 2 Left ventricular mass index (LVMI; (**a** and **b**)) and mean wall thickness (MWT; (**c** and **d**)) in relation to the G−1606A (**a** and **c**) and −962ins/del (**b** and **d**) polymorphisms in the human *SAH* gene in Slavic parents and offspring. Values are means ± standard error adjusted for relatedness, center, sex, age, systolic blood pressure, pulse rate, waist-to-hip ratio, use of antihypertensive drugs, smoking and alcohol intake in excess of 5 g day^{-1}. MWT was additionally adjusted for body weight and height.

was confined to founders and why we did not pick it up in the family-based analysis of offspring.

In our analyses of non-Slavic subjects, we noticed that LVID was slightly greater in −1606GG than −1606A carriers. In the family-based analyses, the between-family component of phenotypic variation in LVID was significant, whereas the within-family component was not. The between-family component reflects population stratification or admixture, whereas the within-family component reflects the genetic effect and is robust against population substructure. The 24-h urinary sodium excretion was 15.1 mmol day^{-1} lower in Belgians than in Italians. Adding a genotype-by-sodium interaction term to the multi-variable-adjusted mixed model enhanced the significance of the association between LVID and the G−1606A polymorphism. This interaction term was borderline significant. Thus, we suggest that the association in the non-Slavic participants between LVID and the G−1606A polymorphism might reflect population stratification, possibly explained by differences in sodium intake.

We observed complete linkage disequilibrium between *SAH* G−1606A and −962ins/del polymorphisms. Using the PROC HAP-LOTYPE procedure as implemented in the SAS package, version 9.1.3., we inferred three haplotypes: the combinations of −1606G with

−962ins (59.6%), −1606G with −962del (28.6%) and −1606A with −962ins (11.9%). The combination of −1606A with −962del did not exist. Studying the associations of the left ventricular phenotypes with the haplotypes did not produce any additional information, over and beyond the reported associations with single-nucleotide poly-morphisms (data not shown).

Our study has to be interpreted within the context of its limitations. First, our sample size was relatively small and we only studied White Europeans of non-Slavic and Slavic origin. Our results might therefore not be generalizable to other ethnic groups. Second, LVM is a quantitative trait prone to measurement error. However, in each center only one experienced observer performed all ultrasound exam-inations. As reported elsewhere,[18] the intra-observer intersession reproducibility coefficient, computed according to Bland and Altman's method,[27] was less than 3% across centers. Third, the differences between genotypes in MWT and LVID were small. However, in the population-based analysis of the G−1606A polymorphism, the differ-ence in LVMI was 5.8 g m^{-2}. In the family-based analysis, the effect size associated with −962del transmission was 3.33 g m^{-2}. These estimates represent 41.4% and 23.8% of the first-year changes in LVMI on blood pressure-lowering treatment in the Losartan

Intervention For Endpoint Reduction in Hypertension (LIFE) trial.[28]

In conclusion, our population-based and family-based studies showed that LVMI and MWT, two phenotypes that are jointly influenced by blood pressure and obesity, might be related to the variation in the human *SAH* gene. However, pending confirmation in other cohorts, the mechanisms underlying such association still need to be clarified in future research.

DISCLOSURE/CONFLICT OF INTEREST

None of the authors has a conflict of interest.

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