Genetic Variation in *CYP11B2* and *AT1R* Influences Heart Rate Variability Conditional on Sodium Excretion

Katarzyna Stolarz, Jan A. Staessen, Kalina Kawecka-Jaszcz, Eva Brand, Giuseppe Bianchi, Tatiana Kuznetsova, Valérie Tikhonoff, Lutgard Thijs, Thomas Reineke, Speranta Babeanu, Edoardo Casiglia, Robert Fagard, Jan Filipovský, Jan Peleška, Yuri Nikitin, Harry Struijker-Boudier, Tomasz Grodzicki, on behalf of the European Project On Genes in Hypertension (EPOGH) Investigators

Abstract—Sympathetic tone increases with stimulation of the renin-angiotensin system and is under the influence of salt intake. In the European Project On Genes in Hypertension (EPOGH), we investigated whether polymorphisms in the genes encoding aldosterone synthase (*CYP11B2* C–344T) and the type-1 angiotensin II receptor (*AT1R* A1166C) affect the autonomic modulation of heart rate at varying levels of salt intake. We measured the low frequency (LF) and high frequency (HF) components of heart rate variability and their ratio (LF:HF) in the supine and standing positions in 1797 participants (401 families and 320 unrelated subjects) randomly selected from 6 European populations, whose average urinary sodium excretion ranged from 163 to 245 mmol/d. In multivariate analyses with sodium excretion analyzed as a continuous variable, we explored the phenotype–genotype associations using generalized estimating equations and quantitative transmission disequilibrium tests. Across populations, there was no heterogeneity in the phenotype–genotype relations. The genotypic effects differed according to sodium excretion. In subjects with sodium excretion <190 mmol/d (median), supine heart rate, LF, and LF:HF increased and HF decreased with the number of *CYP11B2* –344T alleles, and the orthostatic changes in LF, HF, and LF:HF were blunted in carriers of the *AT1R* 1166C allele. In subjects with sodium excretion >190 mmol/d, these associations with the *CYP11B2* and *AT1R* polymorphisms were nonsignificant or in the opposite direction, respectively. Thus, *CYP11B2* C–344T and *AT1R* A1166C polymorphisms affect the autonomic modulation of heart rate, but these genetic effects depend on sodium excretion. **(*Hypertension*. 2004;44:156-162.)**

Key Words: aldosterone ■ receptors, angiotensin ■ genetics ■ heart rate ■ sodium

Measurement of heart rate variability (HRV) in the frequency domain provides information on the autonomic nervous modulation of the cardiovascular system.[1] The high frequency (HF) component of HRV depends on vagal activity, whereas the low frequency (LF) component predominantly reflects sympathetic modulation.[1]

Sympathetic tone increases with stimulation of the renin-angiotensin system and is under the influence of salt intake.[2] Angiotensin II, via presynaptic type-1 receptors (*AT1R*), potentiates the release of norepinephrine.[3] This peptide, together with aldosterone, which is generated in the adrenal zona glomerulosa by aldosterone synthase (*CYP11B2*), maintains the circulating plasma volume that, in turn, through stimulation of cardiopulmonary and arterial mechanoreceptors, may influence sympathetic tone and increase HRV.[4]

Taken together, these observations raise the possibility that genetic variability in the renin-angiotensin-aldosterone system might have an impact on autonomic nervous activity as reflected by HRV. In the European Project On Genes in Hypertension (EPOGH), we therefore investigated whether HRV in basal conditions and after orthostatic stimulation was associated with the *CYP11B2* C–344T and *AT1R* A1166C polymorphisms.

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From the Study Coordinating Centre (K.S., J.A.S., T.K., L.T., R.F.), Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, University of Leuven, Belgium; First Cardiac Department (K.S., K.K.-J.) and Department of Internal Medicine and Gerontology (T.G.), Jagiellonian University, Kraków, Poland; University Medical Center Charité, Department of Internal Medicine (E.B., T.R.), Division of Endocrinology and Nephrology, Campus Benjamin Franklin, Berlin, Germany; Cattedra e Scuola di Nefrologia (G.B.), Universitá Vita e Salute San Raffaele, Milano, Italy; Institute of Internal Medicine (T.K., Y.N.), Novosibirsk, Russian Federation; Department of Clinical and Experimental Medicine (V.T., E.C.), University of Padova, Italy; San Luca Hospital (S.B.), Bucharest, Romania; Department of Internal Medicine II (J.F.), Charles University Medical School, Pilsen, Czech Republic; General Faculty Hospital (J.P.), Prague, Czech Republic; Department of Pharmacology and Toxicology (H.S.-B.), Cardiovascular Research Institute, Maastricht University, The Netherlands.

Correspondence to Jan A. Staessen, MD PhD, Study Coordinating Centre, Laboratory of Hypertension, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, Campus Gasthuisberg, University of Leuven, Herestraat 49, B-3000 Leuven, Belgium. E-mail jan.staessen@med.kuleuven.ac.be

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TABLE 1. Characteristics of the Study Participants by Country

Variable	Belgium	Czechia	Italy	Poland	Romania	Russia
Number	829	157	203	262	127	219
Clinical characteristics						
Age, y	40.7±14.9	37.8±13.7	39.5±14.0†	33.8±13.4†§	36.8±15.4	38.7±14.2¶
Gender, % female	51.4	50.3	56.7	53.8	59.8	58.9†
Body mass index, kg/m^2	24.8±4.4	24.8±3.7	24.7±4.3	24.7±4.3	24.6±5.3	25.0±4.8
Systolic pressure, mm Hg*	122.4±14.9	120.1±14.7	123.4±14.7	124.5±16.6	121.5±20.8	124.0±19.5
Diastolic pressure, mm Hg*	75.6±10.7	75.8±11.3	78.9±9.4†	77.6±11.2	77.3±11.1	79.9±11.9†‡
Questionnaire data						
Antihypertensive	3.3	7.0†	10.3†	7.6†	11.8†	11.4†
Treatment, %	28.7	19.8†	21.7†	28.6‡	26.0	27.4
Smokers, %	24.9	40.8†	41.9†	21.8‡§	21.3‡§	42.9†¶‖
Regular alcohol intake, %						
Urinary output						
Volume, L	1.51±0.65	1.88±0.68†	1.46±0.55	1.43±0.52‡	1.44±0.51‡	1.33±0.48†
Sodium, mmol/d	193±65	219±90†	184±71‡	245±88†‡§	163±86†‡¶	217±106†§¶‖
Potassium, mmol/d	71±28	67±30	63±24†	64±24†	59±29†	58±20†‡
Aldosterone, mmol/d	23.4	15.7	14.3	10.6	12.0	18.4
	(22.0 to 24.5)	(14.4 to 17.2)	(13.1 to 15.5)†	(9.7 to 11.5)†§	(10.5 to 13.5)†	(16.9 to 19.9)†§¶‖

Values are arithmetic means±SD, geometric means (95% CI), or the percentage of subjects.

*Average of 5 readings obtained at 1 home visit.

P values for between-center differences were adjusted for multiple comparisons (Tukey test): †$P \leq 0.05$ vs Belgium; ‡$P \leq 0.05$ vs Czechia; §$P \leq 0.05$ vs Italy; ¶$P \leq 0.05$ vs Poland; ‖$P \leq 0.05$ vs Romania.

Methods

Participants

The EPOGH project was conducted using epidemiological methods described elsewhere.[5,6] The Institutional Review Board of each center approved the study. Participants gave informed written consent.

Investigators from 6 European countries randomly recruited nuclear families of white ethnicity.[6] Overall, the response rate was 63.7%. HRV was measured in 3013 participants who were recruited in Romania (n=281), Poland (n=326), Belgium (n=1398), Italy (n=329), the Russian Federation (n=312), and the Czech Republic (n=367).[6]

We excluded 339 subjects from analysis because they had a history of myocardial infarction (n=19) or diabetes mellitus (n=83), or because they were taking sympatholytic drugs (n=255). In 201 subjects, we could not determine HRV in the supine position (n=65) or in both the supine and standing positions (n=136). We excluded 370 subjects whose urinary volume or creatinine excretion were outside published limits.[5] One or more genotypes were not determined in 266 subjects. In addition, we detected 40 cases of inconsistency in Mendelian segregation. Thus, the number of subjects analyzed statistically totaled 1797 for resting HRV and 1738 for the orthostatic change in HRV.

Measurement of HRV

The method of analysis of HRV has been previously described in detail.[6] A standard 12-lead ECG was recorded electronically for 15 minutes in the supine and free-standing positions, respectively. The mean heart interval (ms) and its total variance or power (ms^2) were calculated for each position. Power spectral analysis was then performed to estimate the powers in the LF (0.04 to 0.15 Hz) and HF (0.15 to 0.40 Hz) ranges of the frequency domain.[1,7,8] These powers were expressed in normalized or relative units (%) and used to calculate the low-to-high frequency power content ratio (LF:HF).[9] Orthostatic changes in HRV were expressed as standing-to-supine ratios.

Genotypes

Genomic DNA from white blood cells was amplified and genotyped for the *CYP11B2* C–344T and the *AT1R* A1166C polymorphisms as previously described.[10,11]

Statistical Analysis

Database management and most statistical analyses were performed with SAS software version 8.1 (SAS Institute).

In the population-based approach, we tested associations of continuous traits with the genotypes of interest by use of generalized estimating equations (GEEs). GEEs allow adjustment for covariates as well as for the nonindependence of observations within families.[12] In the GEE approach, we also tested for heterogeneity across populations, using appropriate interaction terms with the genotypes.

In the family-based analyses, we performed transmission disequilibrium tests for quantitative traits (QTDT) using 3 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/csg/abecasis/QTDT).[13] Second, using the approach proposed by D.B. Allison, we regressed the quantitative phenotypes of the offspring on their genotypes, while controlling for parental genotypes.[14] Finally, in the PROC LOGISTIC procedure of the SAS package, we modeled the probability of the transmission of the allele of interest from each heterozygous parent as a function of the quantitative phenotype.[15]

Results

Characteristics of the Participants

The general characteristics of the study participants are summarized by country in Table 1. The present study included 1477 relatives from 401 families and 320 unrelated Belgian subjects. In addition to the unrelated subjects and 388 nuclear families, the Belgian sample also included 13 extended pedigrees spanning more than 2 generations. Across

TABLE 2. Heart Rate Phenotypes by Country

Variable	Belgium	Czechia	Italy	Poland	Romania	Russia
Rest						
Number	829	157	203	262	127	219
Heart rate, bpm	63.3±9.3	67.3±9.8*	67.1±9.6*	66.0±9.5*	70.7±10.2*†‡§	63.6±9.5†‡¶
Total power, ms^2	2089	2180	1930	2356	1625	2143
	(1948 to 2241)	(1875 to 2533)	(1678 to 2221)	(2076 to 2674)	(1356 to 1947)§	(1878 to 2446)
Low-frequency relative power, %	43.7±17.0	49.8±17.4*	52.2±16.2*§	42.4±15.7†	46.2±17.3‡	43.8±15.9†‡
High-frequency relative power, %	44.5±18.4	38.5±17.7*	36.3±17.5*§	47.0±18.2†	42.3±16.9	43.9±18.7†‡
Low-to-high frequency ratio	1.00	1.35	1.56	0.92	1.11	1.02
	(0.94 to 1.06)	(1.17 to 1.55)*	(1.37 to 1.76)*§	(0.83 to 1.02)†	(0.95 to 1.29)‡	(0.90 to 1.15)†‡
Respiratory frequency, breaths/min	14.8±3.1	15.1±4.2	15.5±3.5	15.5±3.5	16.9±3.2*†‡§	15.4±3.2¶
Orthostatic change						
Number	799	147	193	260	125	214
Heart rate, %	+30±17	+26±14	+27±16	+28±16	+25±21	+28±16
Low-frequency relative power, %	+51	+47	+43	+60	+49	+59
	(+47 to +56)	(+38 to +57)	(+35 to +51)	(+52 to +69)	(+38 to +61)	(+48 to +70)
High-frequency relative power, %	–57	–60	–64	–57	–57	–65
	(–59 to –55)	(–64 to –55)	(–68 to –59)*	(–60 to –53)	(–62 to –52)	(–68 to –61)*§
Low-to-high frequency ratio, %	+249	+265	+293	+269	+248	+347
	(+225 to +275)	(+211 to +329)	(+238 to +357)	(+228 to +315)	(+190 to +317)	(+285 to +418)*

Values are arithmetic means±SD or geometric means (95% CI).

P values for between-centers differences were adjusted for multiple comparisons (Tukey test): *$P \leq 0.05$ vs Belgium; †$P \leq 0.05$ vs Czechia; ‡$P \leq 0.05$ vs Italy; §$P \leq 0.05$ vs Poland; ¶$P \leq 0.05$ vs Romania.

all countries, mean age (±SD) was 51.0±8.4 years in 699 founders and 31.0±12.2 years in 1098 offspring. The number of sibs amounted to 1 in 304 pairs of parents, 2 in 284 pairs of parents, and from 3 to 8 in 64 pairs of parents.

Table 2 gives the heart rate phenotypes by country. Figure 1 shows the sex- and age-dependence of the HRV phenotypes. In previously published analyses,[6] we identified the determinants of HRV using stepwise multiple regression. We adjusted our genetic analyses for country, sex, age (linear and squared terms), body mass index, systolic pressure, use of antihypertensive drugs (other than sympatholytic agents), current smoking, alcohol consumption in excess of 5 g/d, sodium excretion, and respiratory frequency.

In all countries, aldosterone excretion adjusted for sex and age was correlated with urinary sodium and potassium and their ratio. With additional adjustment for country, the overall partial correlation coefficients were −0.14 for sodium, 0.25 for potassium, and −0.35 for the sodium-to-potassium ratio ($P<0.0001$, for all).

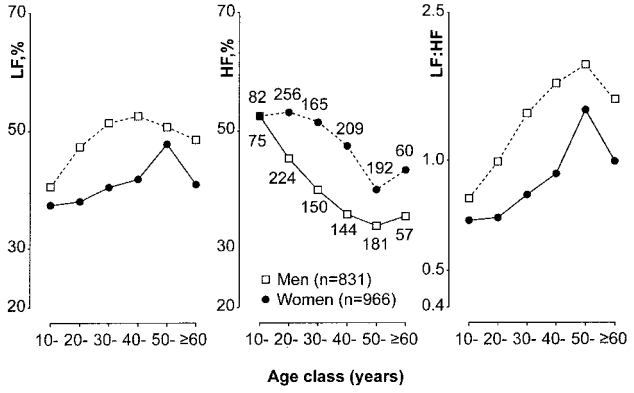

Figure 1. LF and HF relative power and LF:HF by sex and age class. Values are unadjusted means. The number of subjects contributing to each mean is presented in the middle section.

The within-country frequencies of the genotypes (Table 3) complied with Hardy–Weinberg equilibrium ($0.08<P\leq0.99$). Both before and after adjustment for urinary sodium and potassium, 24-hour urinary aldosterone was not associated with the *CYP11B2* C–344T polymorphism ($P\geq0.47$).

Population-Based Association Study

Because there is no agreed algorithm to construct the variance–covariance matrix for correlated data within extended pedigrees using GEE, we selected from the 13 Belgian families with such a structure the most informative nuclear unit with the largest number of phenotypes and genotypes.

In the population-based association study, we combined all countries because there was no heterogeneity in the phenotype–genotype relationships ($0.07<P\leq0.99$). For none of the phenotype–genotype relationships did we find significant interactions with gender ($0.07<P\leq0.98$), age ($0.24<P\leq0.89$), or generation (parents versus offspring; $0.25<P\leq0.90$).

The phenotype–genotype relations were not significant ($0.11<P\leq0.92$) in analyses, which did not account for the genotype-by-urinary sodium interaction (Table 4). We observed significant interactions between genotype and sodium excretion analyzed as a continuous variable (Table 4) for the *CYP11B2* polymorphism in relation to all supine heart rate–related phenotypes ($0.01<P\leq0.04$), as well as for the *AT1R* polymorphism in relation to the orthostatic changes in LF, HF, and LF:HF ($0.02<P\leq0.03$). Figure 2 illustrates these

TABLE 3. Genotype and Allele Frequencies by Country Ordered According to the Prevalence of the Major Allele

Gene	Allele		Genotype		
CYP11B2	T	C	TT	CT	CC
Belgium	963 (58.1)	695 (41.9)	282 (34.0)	399 (48.1)	148 (17.9)
Czechia	181 (57.6)	133 (42.4)	54 (34.4)	73 (46.5)	30 (19.1)
Romania	143 (56.3)	111 (43.7)	37 (29.1)	69 (54.3)	21 (16.5)
Italy	207 (51.0)	199 (49.0)*	57 (28.1)	93 (45.8)	53 (26.1)
Poland	264 (50.4)	260 (49.6)*	63 (24.0)	138 (52.7)	61 (23.3)
Russia	221 (49.5)	217 (50.5)*	57 (26.0)	103 (47.0)	59 (26.9)
AT1R	A	C	AA	AC	CC
Poland	410 (78.2)	114 (21.8)	160 (61.1)	90 (34.3)	12 (4.6)
Czechia	243 (77.4)	71 (22.6)	94 (59.9)	55 (35.0)	8 (5.1)
Russia	336 (76.7)	102 (23.3)	123 (56.2)	90 (41.1)	6 (2.7)
Italy	295 (72.7)	111 (27.3)†	108 (53.2)	79 (38.9)	16 (7.9)
Romania	183 (72.0)	71 (28.0)†	70 (55.1)	43 (33.9)	14 (11.0)
Belgium	1142 (68.9)	516 (31.1)†‡	404 (48.7)	334 (40.3)	91 (11.0)

Values indicate number of alleles or subjects (%).

P values for between-center differences: *$P \leq 0.05$ vs Belgium; †$P \leq 0.05$ vs Poland; ‡$P \leq 0.05$ vs Czechia.

interactions according to the country- and sex-specific median sodium excretion (approximately 190 mmol/d) for the *CYP11B2* and the *AT1R* polymorphisms in relation to the supine LF:HF ratio and the orthostatic change in the LF:HF ratio, respectively. *P* values for the joined effects of genotype and the genotype-by-sodium interaction are given in Table 4. We did not observe any interaction between the genotypes under study and body mass index ($0.20 < P \leq 0.99$) or systolic blood pressure ($0.07 < P \leq 0.83$) in relation to the heart rate–derived phenotypes. Moreover, additional adjustment for resting heart rate and the genotype-by-heart rate interaction, which was not significant ($0.27 < P \leq 0.97$), did not materially alter the results reported in Table 4. Analyses including only Belgian participants were consistent with the overall results in Table 4.

Empirical power calculations involving subsamples of the total study population, in which we randomly reduced the number of subjects kept in the analyses by 10% steps, demonstrated that significant genotype-by-sodium interaction term remained significant until the sample size was reduced by 30% to 60%.

Family-Based Association Study

For supine heart rate, LF, HF, and LF:HF, as well as for the orthostatic changes in these phenotypes in relation to the 2 genotypes, Abecasis' orthogonal model did not reveal population stratification in any country ($0.13 < P \leq 0.99$). In offspring, none of the QTDT approaches showed significant phenotype–genotype relations (Table 5).

Discussion

Our main finding was that in subjects with sodium excretion <190 mmol/d, the supine LF:HF ratio increased with the number of *CYP11B2* –344T alleles, whereas the orthostatic change in LF:HF was blunted in carriers of the *AT1R* 1166C allele. In subjects with sodium excretion >190 mmol/d, these associations with the *CYP11B2* and *AT1R* polymorphisms were nonsignificant or in the opposite direction, respectively.

We did not adjust for multiple testing. However, in view of the physiological consistency in the phenotype–genotype relations, it is unlikely that our findings arose just by chance. Adjustment for multiple comparisons is usually recommended to avoid rejecting null hypotheses too readily.[16] The theoretical basis for advocating routine adjustment for multiple comparisons is that chance serves as the first order explanation for observed phenomena.[16] This hypothesis undermines one of the basic premises of epidemiological research, which holds that human biology follows regular laws that may be studied through observation of populations. Moreover, if as in the present study phenotypes are correlated, then multiple testing is not indicated because each new test does not provide a completely independent opportunity for a type I error.[16] Under such circumstances, adjustment for multiple comparisons is inappropriate.

ATR1 receptors contribute to the renal and adrenal effects of angiotensin II. This octopeptide, together with aldosterone, maintains or expands the circulating plasma volume under sodium-deplete or sodium-replete conditions, respectively. By and large, the present findings together with other evidence[4,17] suggest that the circulating plasma volume might be an important determinant of autonomic nervous tone. Indeed, Veglio et al[17] reported that the LF variability of systolic and diastolic blood pressure and the corresponding LF:HF ratios were significantly higher in patients with primary or idiopathic hyperaldosteronism than in normotensive controls. Spinelli et al[4] investigated the effect of acute isotonic volume expansion on HRV in 10 patients with dilated cardiomyopathy and in age- and sex-matched normal volunteers. In controls, HRV rose during volume expansion, possibly as a consequence of parasympathetic activation, mediated by stimulation of cardiopulmonary and arterial mechanoreceptors.[4] In contrast, in patients with cardiomyopathy the para-

TABLE 4. Heart Rate Phenotypes by Genotypes in Nuclear Families

		Parameter Estimates Not Accounting for the Genotype-By-Sodium Interaction*				*P* Values Accounting for the Genotype-By-Sodium Interaction	
Polymorphism	Phenotype	Means±SE or Geometric Means (95% CI)			P_g	$P_{g\|int}$	P_{int}
CYP11B2 C-344T		CC	CT	TT			
Rest	N	313	709	423			
	HR	64.8±0.6	65.7±0.4	65.7±0.5	0.41	0.01	0.01
	LF	44.4±1.0	45.4±0.6	45.9±0.8	0.49	0.05	0.04
	HF	43.8±1.0	42.5±0.6	42.3±0.8	0.47	0.008	0.02
	LF:HF	1.02 (0.93 to 1.13)	1.10 (1.04 to 1.18)	1.12 (1.03 to 1.21)	0.34	0.01	0.04
Standing	N	300	685	413			
	ΔHR	+27.2±0.8	+26.5±0.6	+25.8±0.7	0.43	0.15	0.12
	ΔLF	+53 (+45 to +61)	+47 (+43 to +52)	+51 (+45 to +58)	0.33	0.37	0.28
	ΔHF	–57 (–60 to –54)	–58 (–60 to –56)	–58 (–61 to –55)	0.92	0.57	0.37
	ΔLF:HF	+255 (+216 to +299)	+248 (+224 to +275)	+262 (+227 to +301)	0.83	0.51	0.35
AT1R A1166C		AA	AC	CC			
Rest	N	787	545	113			
	HR	65.7±0.4	65.2±0.4	65.2±1.0	0.61	0.64	0.84
	LF	45.4±0.6	44.7±0.7	48.0±1.5	0.11	0.13	0.23
	HF	42.6±0.6	43.2±0.7	40.8±1.5	0.29	0.11	0.16
	LF:HF	1.09 (1.02 to 1.16)	1.06 (0.99 to 1.14)	1.25 (1.07 to 1.45)	0.17	0.10	0.18
Standing	N	762	527	109			
	ΔHR	+26.5±0.5	+26.5±0.6	+25.7±1.2	0.84	0.48	0.50
	ΔLF	+49 (+44 to +53)	+53 (+47 to +59)	+42 (+32 to +53)	0.21	0.03	0.03
	ΔHF	–58 (–60 to –56)	–57 (–60 to –55)	–58 (–63 to –52)	0.87	0.01	0.02
	ΔLF:HF	+254 (+229 to +282)	+256 (+227 to +288)	+239 (+181 to +308)	0.90	0.01	0.02

HR, LF, HF, and LF:HF indicates heart rate (beats per minute), low frequency relative power (%), high frequency relative power (%), and low-to-high frequency ratio (LF:HF) in the supine position; ΔHR, ΔLF, ΔHF, ΔLF:HF indicates orthostatic changes in HR, LF, HF and LF:HF expressed as percentage of the values in the supine position.

P_g and $P_{g|int}$ are *P* values for genotypes in models excluding or including a genotype-by-sodium interaction term.

P_{int} is the probability of the genotype-by-sodium interaction.

*To account for the correlated data structure, parameter estimates were derived by GEE analysis (see Methods). Adjustments included country, sex, age (linear and squared terms), body mass index, systolic pressure, current smoking, alcohol intake, sodium excretion, and respiratory frequency.

sympathetic withdrawal was already detectable in baseline conditions and further increased with volume expansion.[4]

Connell et al[18] hypothesized that *CYP11B2* –344T allele carriers might have a relative impairment of the adrenal 11β-hydroxylation, less conversion of deoxycortisol to cortisol, which might chronically increase the stimulation of the adrenal cortex by adrenocorticotropin. This mechanism might reset the aldosterone response to angiotensin II, giving rise to a phenotype characterized by an expanded extracellular volume[19] and ultimately high blood pressure.[19] We found that the sympathetic modulation of HRV increased in *CYP11B2* –344T allele carriers who had a lower than median sodium excretion, whereas at higher sodium output we did not observe an association between sympathetic modulation and the *CYP11B2* C–344T polymorphism. We speculate that an excessive salt intake might be associated with an expanded circulating plasma volume,[20] which in turn might mask the genetic influence of the *CYP11B2* C–344T polymorphism on heart rate variability.[4,17]

The expression of *AT1R* receptors depends on salt intake. Indeed, on a sodium-rich diet, *AT1R* receptors are upregulated in the brain and the adrenal gland.[21,22] Furthermore, Vuagnat et al[23] found that in sodium-deplete conditions, hypertensive siblings homozygous for the *AT1R* 1166C allele, compared with A allele carriers, showed less increase in their blood pressure in response to angiotensin II. In hypertensive patients monitored for 1 week on a high sodium diet, CC homozygotes compared with A-allele carriers displayed an exaggerated response of glomerular filtration, effective renal plasma flow, and vascular resistance to angiotensin II.[24] Thus, depending on salt intake, the *AT1R* 1166 CC genotype may or may not be associated with a tendency for volume expansion, which might explain the differential autonomic modulation of HRV in CC homozygotes as observed in the present study.

In a post-hoc analysis of the diabetic patients enrolled in the Losartan Intervention For Endpoint reduction trial (LIFE), the incidence of sudden death decreased by 49% in the losartan compared with the atenolol group.[25] The LIFE investigators speculated that *AT1R* blockade might have an antiarrhythmic effect because of the more pronounced regression of left ventricular mass on losartan.[25] However, the present findings suggest that *AT1R* antagonism might also be antiarrhythmic by blunting the facilitating effects of angio-

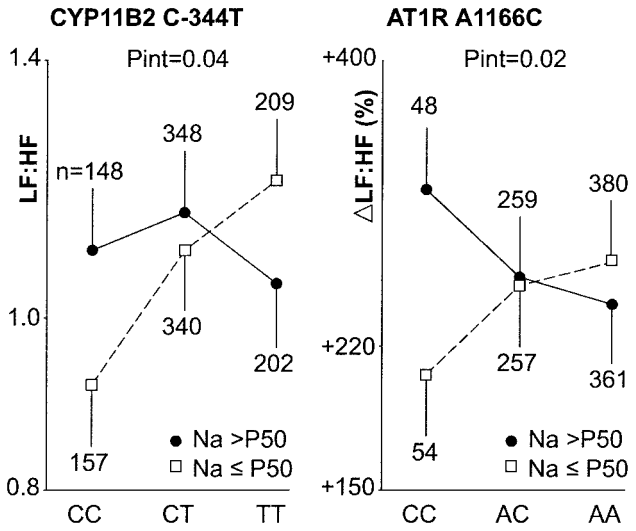

Figure 2. Low-to-high frequency ratio in the supine position (LF:HF) and orthostatic change in this ratio expressed as a percentage of its value in the supine position (ΔLF:HF) in relation to the *CYP11B2* and *AT1R* polymorphisms, respectively. Associations were plotted for 2 groups, based on country- and sex-specific medians of sodium excretion. The probability of the interaction (P_{int}) between genotype and sodium excretion analyzed as a continuous variable was derived by GEE and accounts for nonindependence within families and covariates (see Table 4).

tensin II on sympathetic tone, especially in *AT1R* 1166 CC homozygotes. However, the latter hypothesis remains to be proven.

The present study has to be interpreted within the context of its limitations and strengths; we only investigated the short-term sympathovagal modulation of heart rate. Further studies must clarify whether our findings can be extrapolated to the long-term autonomic regulation of the cardiovascular-renal system. The present findings also reflect our research strategy. Indeed, for practical reasons we first dealt with genetic variation in the renin-angiotensin-aldosterone system. We excluded association between HRV and the angiotensin-converting enzyme (*ACE*) I/D and the angiotensinogen G–6A polymorphisms (data not shown), while research addressing genetic variation in various subtypes of adrenoceptors is currently in the planning stage. Moreover, the insight that accounting for the genotype-by-sodium interaction was necessary originated from previous work on the *ACE* gene polymorphism in relation to left ventricular mass.[26] In general, there is a growing body of evidence showing that complex traits, such as heart rate variability, should be studied within their ecogenetic context. The publicly available QTDT software does not allow the direct investigation of interactions between environmentally determined factors, such as sodium excretion, and the probability of allele transmission within families in relation to continuous traits, such as HRV. Our QTDT results therefore reflect the nonsignificant genetic effects observed in the population-based approach, which did not account for the genotype-by-sodium interaction.

To the best of our knowledge, our population study is the largest family-based resource of HRV currently available. Family-based analyses neither revealed significant population stratification within countries nor demonstrated heterogeneity in the phenotype–genotype relations across countries. Analyses confined to the large group of Belgian participants or random subsamples representing 40% to 70% of the total study population were consistent with our overall results and the interpretation that our findings were not because of heterogeneity between countries. Finally, the observation that the correlations between urinary aldosterone, sodium, and potassium were consistent with physiological expectations and statistically significant within all countries provided an internal validation of our data set.

Perspectives

In subjects consuming <190 millimoles of sodium per day, sympathetic modulation of heart rate is significantly associ-

TABLE 5. QTDT Analyses of Heart Rate Phenotypes

Polymorphism	Condition	Model	N of sibs (informative/all)	χ^2 HR	χ^2 LF	χ^2 HF	χ^2 LF:HF
CYP11B2 C-344T	Basal	Orthogonal	825/1098	0.08	0.37	0.69	0.44
		Allison	356/1098	5.18*	0.16	0.43	0.55
		Logistic	725	5.34*	0.18	0.35	0.32
	Orthostatic change	Orthogonal	796/1063	3.12*	0.12	3.17*	3.55*
		Allison	344/1063	4.72*	0.06	0.37	3.42
		Logistic	697	2.75	0.20	0.38	1.90
AT1R A1166C	Basal	Orthogonal	651/1098	0.19	0.43	0.18	0.29
		Allison	267/1098	4.46	2.42	1.38	1.43
		Logistic	380	0.02	0.79	0.40	0.81
	Orthostatic change	Orthogonal	628/1063	0.03	0.23	0.42	0.04
		Allison	244/1063	4.10	1.24	0.27	0.08
		Logistic	366	0.21	0.11	0.28	0.21

HR indicates supine heart rate; LF indicates low frequency relative power; HF indicates high frequency relative power; LF:HF indicates low-to-high frequency ratio. Adjustments included sex, age, body mass index, systolic pressure, current smoking, alcohol intake, sodium excretion, and respiratory frequency.

The χ^2 statistics did not attain statistical significance; an asterisk marks those with $P<0.1$.

ated with the *CYP11B2* C–344T and *AT1R* A1166C polymorphisms in the supine and standing positions, respectively. Our findings, in keeping with other reports in the literature,[4,17] support the hypothesis that genetic polymorphisms or lifestyle factors leading to expansion of the circulating plasma volume might significantly affect the autonomic nervous regulation of the cardiovascular system. The present study also underscores the necessity to investigate genetic determinants of complex quantitative traits within their eco-genetic context. Moreover, if confirmed, our findings might open new perspectives for individualized cardiovascular prevention. Indeed, in *AT1R* 1166 CC homozygotes reducing salt intake, or angiotensin II type-1 receptor blockade, or both, might decrease the sympathetic predominance of HRV and the cardiovascular complications associated with this condition.[27]

Appendix

Coordination and Committees

Project Coordinator: J.A.S.; Scientific Coordinator: K.K.-J.; Steering Committee: S.B. (Romania), E.C. (Italy), J.F. (Czech Republic), K.K.-J. (Poland), C. Nachev (Bulgaria), Y.N. (Russian Federation), J.P. (Czech Republic), J.A.S. (Belgium); Data Management Committee: T.K., J.A.S., K.S., V.T., J.G. Wang; Publication Committee: E.C., K.K.-J., Y.N.; Advisory Committee on Molecular Biology: G.B.(Milan), E.B. (Berlin), S.M. Herrmann (Münster), H.A.S.-B. (Maastricht); EPOGH-EurNetGen Liaison: A.F. Dominiczak (Glasgow), J.A.S. (Leuven).

EPOGH Centers

A complete list of the EPOGH investigators has been previously published.[6]

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