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 Filipovsky, 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 C–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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The EPOGH project was conducted using epidemiological methods described elsewhere. The Institutional Review Board of each center approved the study. Participants gave informed consent.

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Methods
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Measurement of HRV
The method of analysis of HRV has been previously described in detail. 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. These powers were expressed in normalized or relative units (%) and used to calculate the low-to-high frequency power content ratio (LF:HF). 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.

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. 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 withingroup 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). 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. 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.

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 1. Characteristics of the Study Participants by Country

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

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<0.05 vs Belgium; ‡P<0.05 vs Czechia; §P<0.05 vs Italy; ¶P<0.05 vs Poland; ||P<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, 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 current smoking, alcohol consumption in excess of 5 g/d, family history of hypertension, and from 3 to 8 in 64 pairs of parents.

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).

The within-country frequencies of the genotypes (Table 3) complied with Hardy–Weinberg equilibrium (0.08<P<0.99). Both before and after adjustment for urinary sodium and potassium, 24-hour urinary aldosterone was not associated with the CYP11B2 C–344T polymorphism (P=0.47).

**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<0.99). For none of the phenotype–genotype relationships did we find significant interactions with gender (0.07<P<0.98), age (0.24<P<0.89), or generation (parents versus offspring; 0.25<P<0.90).

The phenotype–genotype relations were not significant (0.11<P<0.92) 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<0.04), as well as for the ATIR polymorphism in relation to the orthostatic changes in LF, HF, and LF:HF (0.02<P<0.03). Figure 2 illustrates these
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 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).

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. The theoretical basis for advocating routine adjustment for multiple comparisons is that chance serves as the first order explanation for observed phenomena. 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. Under such circumstances, adjustment for multiple comparisons is inappropriate.

AT1R receptors contribute to the renal and adrenal effects of angiotensin II. This octapeptide, 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 suggest that the circulating plasma volume might be an important determinant of autonomic nervous tone. Indeed, Veglio et al 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 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. In contrast, in patients with cardiomyopathy the para-

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP11B2</td>
<td>T</td>
<td>C</td>
</tr>
<tr>
<td>Belgium</td>
<td>963 (58.1)</td>
<td>695 (41.9)</td>
</tr>
<tr>
<td>Czechia</td>
<td>181 (57.6)</td>
<td>133 (42.4)</td>
</tr>
<tr>
<td>Romania</td>
<td>143 (56.3)</td>
<td>111 (43.7)</td>
</tr>
<tr>
<td>Italy</td>
<td>207 (61.0)</td>
<td>199 (49.0)</td>
</tr>
<tr>
<td>Poland</td>
<td>264 (50.4)</td>
<td>260 (49.6)</td>
</tr>
<tr>
<td>Russia</td>
<td>221 (49.5)</td>
<td>217 (50.5)</td>
</tr>
<tr>
<td>AT1R</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>Poland</td>
<td>410 (78.2)</td>
<td>114 (21.8)</td>
</tr>
<tr>
<td>Czechia</td>
<td>243 (77.4)</td>
<td>71 (22.6)</td>
</tr>
<tr>
<td>Russia</td>
<td>336 (76.7)</td>
<td>102 (23.3)</td>
</tr>
<tr>
<td>Italy</td>
<td>295 (72.7)</td>
<td>111 (27.3)</td>
</tr>
<tr>
<td>Romania</td>
<td>183 (72.0)</td>
<td>71 (28.0)</td>
</tr>
<tr>
<td>Belgium</td>
<td>1142 (68.9)</td>
<td>516 (31.1)</td>
</tr>
</tbody>
</table>

Values indicate number of alleles or subjects (%).

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

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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≤0.99). In off-spring, 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
sympathetic withdrawal was already detectable in baseline conditions and further increased with volume expansion.4

Connell et al18 hypothesized that CYP11B2 C-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 adrenocorticotropic. This mechanism might reset the aldosterone response to angiotensin II, giving rise to a phenotype characterized by an expanded extracellular volume19 and ultimately high blood pressure.19 We found that the sympathetic modulation of HRV increased in CYP11B2 C-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 increase in 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 Fur-
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 non-significant 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-
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.\(^5,7\)

Appendix

Coordination and Committees


EPOGH Centers

A complete list of the EPOGH investigators has been previously published.\(^6\)

Acknowledgments

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

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GENETIC VARIATION IN CYP11B2 AND AT1R INFLUENCES HEART RATE VARIABILITY CONDITIONAL ON SODIUM EXCRETION

Extended Methods Section

Participants

The EPOGH project was conducted according to the principles outlined in the Helsinki declaration for investigation of human subjects. 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 Caucasian extraction, including offspring with minimum age of 10 years in Belgium and 18 years in the other countries. Overall, the response rate was 63.7%. Heart rate variability was measured in 3013 participants, who were recruited in Bucharest (Romania, n=281), Cracow (Poland, n=326), Hechtel-Eksel (Belgium, n=1398), Mirano (Italy, n=329), Novosibirsk (Russian Federation, n=312), and Pilsen and Prague (Czech Republic, n=183 and 184, respectively).

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) due to (tachy-) arrhythmia, artefacts, or an insufficient respiratory signal. We discarded 370
subjects, whose urinary volume or creatinine excretion were outside published limits. 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 HRV in the supine position and 1738 for the orthostatic change in HRV.

**Measurement of HRV**

To ensure stationarity, in each center, heart rate was recorded under standardized laboratory conditions in a quiet examination room, after the subjects had rested for 20 minutes in the supine position. Participants refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for at least 2 hours prior to the recording. A standard 12-lead electrocardiogram and the respirogram (obtained by means of a nasal thermistor) were recorded electronically for 15 minutes in the supine and free standing positions, respectively. Signals were sampled at 300 Hz. For the study of HRV, we selected the lead with the highest R-to-T-wave voltage ratio, usually lead II.

RR interval and respiratory frequency were derived from the recorded signals. The resulting tachogram and respirogram of each recording in each position were displayed on a computer screen. By visual inspection of the tachogram, we chose a stationary section, free of ectopic beats and artefacts as close as possible to the end of the 15-minute recordings. Analyses were performed on segments of 512 consecutive beats, unless only shorter periods appeared suitable for analysis (i.e. 256 or 128 beats). If there was no period of at least 128 beats without artefacts or extrasystoles, outlying values on the tachogram exceeding the mean RR interval ± 3
standard deviations could be removed. When it was impossible, the recording was not considered for further analysis. An accurate respiratory signal was always required.

The mean RR interval (ms) and its total variance or power (ms$^2$) were calculated. Power spectral analysis (PSA) was then performed to estimate the powers in the LF and HF ranges. Spectral components were expressed in equivalent Hertz (henceforth called Hz), defined as cycles per beat divided by the mean length of the heart interval. PSA was performed by fast Fourier transform (FFT). For FFT, the signals were subjected to Hamming windowing and then to the Cooley and Tukey algorithm. The LF and HF components included the powers from 0.04 to 0.15 Hz and from 0.15 to 0.40 Hz, respectively. These powers were expressed in normalized or relative units (%). Normalized or relative LF or HF power is the absolute power divided by the partial power, defined as the power between 0.03 and 0.40 Hz. In addition, the low-to-high frequency (LF: HF) power content ratio was calculated. Orthostatic changes in HRV were expressed as standing-to-supine ratios.

**Other Measurements**

The blood pressure phenotype was the average of 5 consecutive readings obtained at one home visit. Information on each subject's personal and familial medical history, smoking and drinking habits, and use of medications was obtained via a standardized questionnaire. The participants collected a 24-h urine sample in a wide-neck plastic container for the measurement of sodium, potassium, creatinine and aldosterone. Genomic DNA from white blood cells was amplified and genotyped for the CYP11B2 C–344T and the AT1RA1166C polymorphisms, as previously described.
**Statistical Analysis**

Database management and most statistical analyses were performed with SAS software version 8.1 (SAS Institute Inc., Cary, NC). Population means and proportions were compared by Tukey’s test for multiple comparisons and the $\chi^2$ statistic, respectively. If Shapiro-Wilk's test showed a significant departure from normality, we analyzed logarithmically transformed variables.

We performed both population-based and family-based analyses. In the population-based approach, we tested associations of continuous traits with the genotypes of interest by use of generalized estimating equations (GEE). GEE allows adjustment for covariates as well as for the non-independence of observations within families.\textsuperscript{12} In the GEE approach, we also tested for heterogeneity across populations, using appropriate interaction terms with the genotypes. We used a likelihood ratio test to compare nested GEE models including or excluding the interaction term between genotype and the 24-h urinary sodium excretion.

In the family-based analyses, we performed transmission disequilibrium tests for quantitative traits (QTDT) 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. in the QTDT software, version 2.3 (\url{http://www/well.ox.ac.uk/asthma/QTDT}).\textsuperscript{13} Second, using the approach proposed by Allison, we regressed the quantitative phenotypes of the offspring on their genotypes, while controlling for parental genotypes.\textsuperscript{14} To allow for residual correlation among offspring, we implemented Allison's model using GEE. 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.\textsuperscript{15}
References


