

Angiotensin-Converting Enzyme and Angiotensinogen Gene Polymorphisms and Heart Rate Variability in Twins

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Decreased heart rate variability (HRV) is associated with congestive heart failure, post-myocardial infarction, ventricular arrhythmias, sudden cardiac death, and advancing age. A deletion/insertion polymorphism in the angiotensin-converting enzyme (ACE) gene and a substitution (M235T) in the angiotensinogen gene have been associated with risk for heart disease. The aim of this study was to determine the heritability of HRV and related parameters in monozygotic and dizygotic twins and to assess the influence of ACE and angiotensinogen polymorphisms. We studied 95 MZ pairs and 46 DZ pairs. We measured HRV and related parameters, ACE and angiotensinogen levels, plasma norepinephrine, ACE, and angiotensinogen genotypes. We found that

HRV and related parameters were significantly influenced by genetic variability, although nonshared genetic effects were also important. Angiotensinogen and plasma norepinephrine were generally correlated with decreased HRV, whereas ACE was correlated with perturbances of normal rhythmic HRV. Nevertheless, the DD ACE genotype was associated with increased HRV ($p < 0.05$), whereas angiotensinogen polymorphisms had no effect. We conclude that HRV and related parameters are in part heritable. Interestingly, the DD ACE genotype is associated with increased HRV. ©1998 by Excerpta Medica, Inc.

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Hear rate variability (HRV), defined as spontaneous fluctuations in sinus rate due to internal and external body processes, is an indicator of risk for death after a cardiac event.¹ HRV decreases with age and with certain diseases such as congestive heart failure, diabetic neuropathy, post-myocardial infarction, and in some forms of inducible ventricular tachycardia or ventricular fibrillation.^{1,2} HRV parameters are determined to a great extent by baroreceptor reflex-related mechanisms, and sympathetic and parasympathetic tone, all of which deteriorate with age.¹⁻³ Furthermore, HRV parameters show gender-related, as well as age-related differences, suggesting additional endocrine-mediated effects.⁴ Genetic variability exerts considerable influence on resting and stress-induced autonomic tone.⁵ The effect of genetic influences on HRV-related parameters has not been examined. We recruited monozygotic and dizygotic normal twin subjects to study genetic effects on HRV. Because an insertion/deletion (I/D) polymorphism in intron 16 of the angiotensin-converting enzyme (ACE) gene has been associated with myocardial infarction and left ventricular hypertrophy,^{6,7} and because a polymorphism in the angiotensinogen gene (M235T

substitution) is associated with increased blood pressure,⁸ we also determined if the ACE gene I/D alleles and the angiotensinogen M235T substitution were associated with HRV.

METHODS

We recruited 141 pairs of MZ (95 pairs) and DZ (46 pairs) twins by advertisement (print media) to participate in studies involving blood pressure and blood pressure reactivity to physical and mental stress.⁹ The subjects were all German Caucasians. They were recruited from various parts of Germany, which minimizes any regional effects. The protocol was approved by the University's committee on the protection of human subjects and written informed consent was obtained from all participants. Blood was obtained for the determination of zygosity. The zygosity was verified with the use of 5 highly polymorphic short tandem repeat polymerase chain reaction-amplified microsatellite markers, namely THO1, TPOX, FES/FPS, F13A1, and FGA.¹⁰ We used fluorescent-labeled primers in a multiplex automated genotyping system relying on a 373 DNA-sequencer and 672 GENESCAN and GENOTYPER software (all Applied Biosystems, Foster City, California). Plasma norepinephrine was measured with high-performance liquid chromatography with electrochemical detection,¹¹ ACE activity in plasma was determined by means of a synthetic substrate (FAPGG) as outlined elsewhere,¹² and plasma angiotensinogen was determined by radioimmunoassay.¹³

Blood pressure and heart rate were measured in the nondominant arm by an automated oscillometric

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TABLE I Demographic, Clinical, Echocardiographic, and Angiotensin-Converting Enzyme (ACE) and Angiotensinogen Plasma Level Data in Groups With Different ACE Genotypes

Variables	II (n = 31)	ID (n = 77)	DD (n = 33)
Age (yrs)	34 ± 14	33 ± 14	34 ± 14
Gender (M/F)	6/21	18/61	10/23
Height (cm)	169 ± 8	168 ± 8	170 ± 9
Weight (kg)	66 ± 9	66 ± 13	69 ± 14
BMI (kg/m ²)	23 ± 4	23 ± 4	23 ± 5
Systolic BP (mm Hg)	123 ± 17	123 ± 15	123 ± 14
Diastolic BP (mm Hg)	75 ± 10	73 ± 11	70 ± 10
HR (beat/min)	70 ± 9	73 ± 13	73 ± 9
ACE level (U/L) [‡]	25 ± 11*	44 ± 18*	57 ± 25*
Angiotensinogen (μg Ang I/ml) [§]	1.33 ± 0.35 [†]	1.31 ± 0.33 [†]	1.18 ± 28 [†]

*p <0.01; [†]p <0.05.
[‡]Group differences: II-ID, II-DD, ID-DD.
[§]Group differences: II-DD, ID-DD.
Ang I = angiotensin I; BP = blood pressure; BMI = body mass index; HR = heart rate.

method (Dinamap, Tampa, Florida). A detailed description of our HRV methodology and the mathematics involved in its calculation has been published.¹⁴ Analyses follow the guidelines published recently.¹⁵ For analysis of HRV, each subject was monitored for 4 hours with an ambulatory electrocardiographic Holter recorder (Avionics Stratascan, Del Mar Avionics, Irvine, California). All electrocardiograms were then checked and edited by a technician to remove all artifacts. The 30-minute period with the least number of artifacts was analyzed. Mean heart period refers to the average NN interval over 30 minutes. NN (successive normal beat-to-beat) intervals are the normal RR intervals in the tachogram after filtering the RR time series. We determined indexes of the time and frequency domain, where SDNN is the standard deviation of successive NN intervals, and RMSSD is the square root of the mean squared differences of successive NN intervals. Power spectral density estimates describe variance as a function of frequency. P is the total power of the frequency band 0.0 to 0.4 Hz. HF is the high-frequency power in the frequency band from 0.15 Hz up to 0.4 Hz and (ULF+VLF+LF)/P the normalized lower frequency power in the frequency band from 0.0 Hz up to 0.15 Hz. The spectra were estimated by use of the fast-Fourier transformation. These measures characterize the rhythmic variability of the heart rate. We also included measures of perturbed rhythmic HRV. PNNL10 and PNNL20 are the percentages of NN interval differences <10 and 20 ms, respectively, whereas PNN100 and PNN200 are the percentages of NN intervals >100 and 200 ms, respectively. High percentages indicate a perturbation of rhythmic HRV in either direction, i.e., fixed heart rate or large beat-to-beat (erratic) changes in heart rate. All data are normalized.

The I/D polymorphism of the ACE gene was identified with the polymerase chain reaction using a set of oligonucleotide primers flanking the polymorphic site according to the method described by Rigat et al.¹⁶ Allele frequencies and standard binomial errors were determined by the gene counting method. Allele-specific oligonucleotide hybridiza-

tion was used for the genotyping of angiotensinogen codons 174 and 235. Genomic DNA was subjected to 30 rounds of amplification using primers described elsewhere.¹⁷ The resulting 354-base pair fragment was denatured, dot-blotted in duplicate onto nitrocellulose, and then neutralized. The filters were subsequently hybridized to the appropriate ³²P-labeled oligonucleotides. Only 1 member of each twin pair was included for the association testing. Selecting either twin 1 or twin 2 did not influence the results. Genotype distributions of all groups were checked for Hardy-Weinberg equilibrium and were compared with each other by chi-square and likeli-

hood ratio methods. Relative risk figures (odds ratio statistics) and their 95% confidence intervals (CI) were calculated by using the linkage package 5.1 as outlined by Terwilliger and Ott.¹⁸

Statistical analysis was conducted using the SPSS program. To test for differences in the mean level of the cardiovascular measures, *t* tests for independent groups were performed. In addition to univariate methods, analysis of variance was employed as a true multivariate method for the simultaneous comparison of the 3 groups of genotypes. Post hoc comparisons between groups were done with the Scheffé test. Parameters of the quantitative genetic models were estimated by path analysis techniques using the LISREL 8 program developed by Jöreskog and Sörbom.¹⁹ Analogous to a regression analysis, the variability of any given phenotype (P) within a population can be decomposed in additive genetic influences (A), non-additive genetic influences (D), environmental influences shared (common) by the twins within a family (C) and random environment (E): $P = aA + dD + cC + eE$, with *a*, *c*, *e* as the estimated relative influence. For MZ and DZ the covariance of their phenotype is given by: $r_{MZ} = a^2 + d^2 + c^2 + e^2$ and $r_{DZ} = 0.5a^2 + 0.25d^2 + c^2 + e^2$. Path analysis in twin studies can estimate additive and nonadditive (dominance) components of genetic variability (estimated as *a*² and *d*²) as well as 2 environmental influences, shared (*c*²) and nonshared environmental influences (*e*²).²⁰ These values estimate the relative amount of the variable's influence on interindividual differences up to a sum of 1. Genetic as well as environmental effects were estimated by the best fitting model as selected by the chi-square value. The LISREL 8 output also gives estimates of the goodness-of-fit index, the adjusted goodness-of-fit index, and the Akaike information criterion. These estimates concurred with the results of the chi-square analysis, so we elected not to present these estimates.

RESULTS

Table I lists demographic data on all subjects divided into ACE genotypes. The ACE genotype

TABLE II Correlation Coefficients Between HRV Parameters and Blood Pressure, Heart Rate, Plasma Norepinephrine, Plasma Angiotensin-converting Enzyme Activity (ACE), and Plasma Angiotensinogen*

	Systolic Blood Pressure	Diastolic Blood Pressure	Heart Rate	Plasma Norepinephrine	ACE	Angiotensinogen
Mean heart period	-0.16	-0.39	—	-0.32	—	-0.35
SDNN	-0.25	-0.30	-0.48	-0.22	—	-0.33
RMSSD	-0.24	-0.28	-0.49	-0.27	—	-0.32
HF	-0.14	-0.14	-0.28	-0.19	—	—
Total power	-0.19	-0.24	-0.39	-0.20	—	-0.30
ULF + VLF + LF	0.20	—	0.15	0.14	—	—
PNNL10	0.44	0.37	0.55	0.32	0.18	0.23
PNNL20	0.43	0.39	0.56	0.34	0.15	0.28
PNN100	-0.15	-0.24	-0.45	-0.22	0.14	-0.31
PNN200	—	—	-0.24	-0.14	0.11	-0.23

*Only significant ($p < 0.05$) correlations are given.

HF = normalized power in the frequency band from 0.15 to 0.4 Hz; Mean heart period = average NN interval/30 minutes; PNNL10 and 20 = percentages of NN interval differences <10 and 20 ms, respectively; PNN100 and 200 = percentages of NN interval differences >100 and 200 ms, respectively; RMSSD = root-mean-square of NN interval successive differences; SDNN = standard deviation of filtered NN intervals; Total power = total power in the spectrum; ULF, VLF, and LF = power in the frequency band from 0 to 0.0033, 0.0044 to 0.04, and 0.04 to 0.15 Hz, respectively.

TABLE III Results for Twin Analysis (MZ = 95 pairs, DZ = 46 pairs)*

	a^2	d^2	c^2	e^2	r_{mz}/r_{DZ}	c^2/df
Mean heart period	0.17	—	0.37	0.46	0.58/0.38	1.77/3
SDNN	—	0.60	—	0.40	0.64/0.22	4.20/4
RMSSD	—	0.65	—	0.35	0.68/0.13	3.17/4
Total power	0.56	—	—	0.44	0.60/0.24	0.74/4
ULF + VLF + LF	—	0.47	—	0.53	0.50/0.05	0.33/4
HF	0.39	—	—	0.61	0.40/0.21	1.69/4
PNNL10	—	0.56	—	0.44	0.63/0.06	10.75/4
PNNL20	—	0.59	—	0.41	0.63/0.06	10.75/4
PNN100	—	0.58	—	0.42	0.58/0.16	2.4/4

*Additive genetic factors (a^2), nonadditive genetic factors (d^2), shared environmental (c^2), and nonshared environmental (e^2) factors are given.

Only significant ($p < 0.05$) data are presented.

Abbreviations as in Table II.

had a significant effect on plasma ACE levels; the D allele conferred a higher ACE level. The ACE genotype also had an effect on angiotensinogen levels. Persons with the DD ACE genotype had lower angiotensinogen levels. Table II lists the correlation coefficients between HRV parameters and blood pressure, heart rate, plasma norepinephrine, ACE activity, and angiotensinogen in the entire cohort. Only significant ($p < 0.05$) correlations are given. Numerous correlations were observed between HRV parameters and systolic blood pressure, diastolic blood pressure, and heart rate. Plasma norepinephrine was generally inversely correlated with rhythmic HRV, PNN100, and PNN200, and directly correlated with PNNL10, PNNL20, and ULF + VLF + LF. ACE activity and angiotensinogen concentrations were directly correlated with PNNL10 and PNNL20, as well as PNN100 and PNN200. Angiotensinogen was inversely correlated with SDNN, RMSSD, and total power. The correlations are consistent with the interpretation that plasma norepinephrine, and angiotensinogen concentrations are inversely related to HRV. The ACE inhib-

itor, however, is directly correlated with measures of arrhythmic HRV.

Table III lists the significant heritability estimates. Additive genetic factors (a^2), nonadditive genetic factors (d^2), shared environmental (c^2), and nonshared environmental (e^2) factors are given. Only significant ($p < 0.05$) data are presented in the Table. Significant additive and non-additive genetic factors were commonly identified. Shared environmental factors were unusual. A significant effect was found only for the mean heart period. Nonshared environmental factors were significant in every instance.

The 282 twins were genotyped according to the ACE intron deletion polymorphism. The twins met the Hardy Weinberg equilibrium criteria. When we used twin 1 of any given twin pair, 33 had the DD, 77 had ID, and 31 had II genotype. Thus, 33 had DD, whereas 108 had ID or II genotypes. Table IV lists the differences between the DD and II/ID genotypes. The standard deviation of NN intervals, the root-mean-square of NN intervals, the power in the high-frequency band, and probability of unpredictable changes in heart rate (PNN100 and PNN200) were significantly different comparing subjects with DD genotype with the II or ID genotypes. Figure 1 shows the root-mean-square of successive differences of all normal-to-normal NN intervals as a function of ACE genotypes. The value was significantly greater for the DD genotype than for II or ID genotypes. In general, these data indicate that persons with the DD genotype had greater HRV than persons with the I allele. The angiotensinogen M235T substitution was not associated with any of the HRV-related parameters.

TABLE IV Angiotensin-Converting Enzyme Genotypes and Heart Rate Variability Parameters

	II + ID (n = 108)	DD (n = 33)
Age	31.5 ± 11.4	30.1 ± 10.2
SDNN	2.1 ± 0.8	2.8 ± 1.4*
RMSSD	1.3 ± 0.7	2.2 ± 1.6*
HF	0.5 ± 0.5	1.4 ± 2.0*
Power	1.0 ± 0.8	1.6 ± 1.5
PNN100	0.47 ± 0.8	1.34 ± 1.5*
PNN200	0.13 ± 0.3	0.94 ± 2.2*

*p <0.05, comparing II + ID with DD.
Values expressed as mean ± SD.
Abbreviations as in Tables II and III.

DISCUSSION

The important findings in this study are that HRV and related parameters show correlations with blood pressure, heart rate, plasma norepinephrine, angiotensinogen, and ACE levels. HRV and related parameters are heritable, and are more pronounced in persons carrying the ACE intron 16 DD genotype than in those possessing the I allele. We found no genetic association between HRV and the angiotensinogen locus. From the heritability estimates, we concluded that genetic influences on HRV were mostly dominant. Codominant effects were assessed by analysis of variance, which showed no difference between II and ID genotypes, whereas both were significantly different from the DD genotype. For this reason, we compared the DD genotype with the II and ID genotypes. To our knowledge, no earlier studies have examined genetic influences on HRV. The HRV parameters all reflect the influence of different physiologic systems (sympathetic tone, parasympathetic tone, and so forth) that are heritable to varying degrees. The range of genetic variability we observed for HRV lay between 39% to 65%; the rest could be attributed to environmental influences. Genetic influence on HRV was significantly greater than that on heart rate, which was only 17%. Common environmental effects were unusual except for heart rate. Thus, the family-related environment such as lifestyle, social economic status, etc., seemed not to have a major influence on HRV. On the other hand, the nonshared environmental effects, e.g., differences in how 1 twin slept the night before compared with the other twin, differences in illness spectrum, differences in alcohol intake, etc., had a major influence on HRV and related parameters. We did not measure these environmental effects precisely and are not able to speculate on which were important.

Several studies in humans and experimental animals demonstrate convincingly that impaired baroreceptor reflex sensitivity and reduced HRV increases the risk of cardiac mortality.²¹ Particularly, the low-frequency spectral component of HRV, rather than a reduced high-frequency component, are associated with an increased risk of life-threatening arrhythmia after myocardial infarc-

tion.^{22,23} Although HRV, assessed by both time and frequency domain parameters, is very stable and reproducible over shorter periods of time, HRV decreases with advancing age.²⁻⁴ Impaired cardiovascular autonomic regulation, as reflected by HRV, may be partly responsible for the greater mortality after myocardial infarction observed in the elderly. Plasma norepinephrine concentrations increase with advancing age.²⁴ Thus, we were interested in the fact that plasma norepinephrine was inversely correlated with HRV. The same was true for angiotensinogen. A long-term association between angiotensin II and sympathetic activation has been suggested.²⁵ These findings may be relevant to the previously described decrease in HRV with increasing age and the greater degree of mortality after myocardial infarction in the elderly.

We are not able to explain why the ACE DD genotype would be associated with higher HRV. Persons with the D allele had higher ACE levels. We were recently able to show that the ACE gene locus is responsible for almost all of the genetic variance in ACE levels.²⁶ We found that ACE levels were correlated with perturbed rhythmic HRV, namely PNNL10, PNNL20, PNN100, and PNN200. However, we found no correlation between all measures of rhythmic HRV and ACE levels. We did observe an inverse correlation between ACE levels and angiotensinogen concentrations. This finding suggests that higher ACE activity may result in a greater degree in angiotensinogen consumption, consistent with the notion that ACE is a rate-limiting step in angiotensin II generation.²⁷ Angiotensinogen itself was inversely correlated with rhythmic HRV. Taken together, these observations are consistent with a functional effect of the DD genotype on rhythmic HRV via renin-angiotensin-mediated influences.

Conceivably, 2 pathways may be operative: 1 may involve the renin-angiotensin system, while another could involve the kinin system. Bradykinin, which is degraded by ACE, reduces blood pressure and increases heart rate.²⁸ Finally, there is a substantial interaction between the renin-angiotensin system and the baroreceptor reflex, which is under sympathetic and parasympathetic nervous system control. We found a significant interaction between ACE activity and plasma norepinephrine. ACE levels were correlated not only with PNNL10 and PNNL20, which indicate periods of inflexible heart rate, but also with PNN100 and PNN200, which indicate larger unpredictable beat-to-beat variations in heart rate. The direction of these changes are dependent on plasma norepinephrine values, because plasma norepinephrine is directly correlated with PNNL10 and PNNL20, and inversely correlated with PNN100 and PNN200. These results are consistent with earlier observations in humans.²⁹

The ACE DD genotype, which has been associated with risk for myocardial infarction⁶ and left ventricular hypertrophy,⁷ is also associated with longevity. Schächter et al³⁰ studied 338 adults aged ≥100 years and compared these subjects with

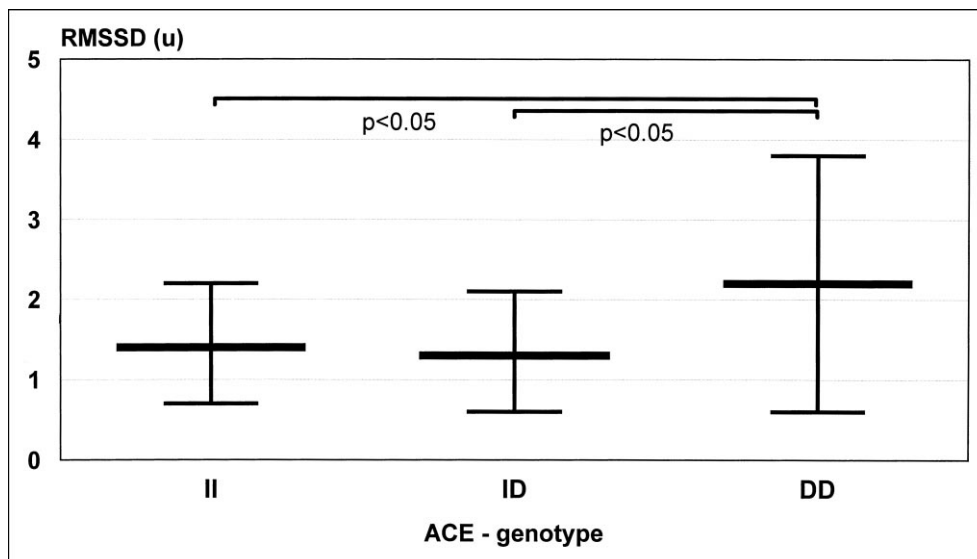


FIGURE 1. Relation between root-mean-square of the NN interval successive differences (RMSSD) (ms) and angiotensin-converting enzyme (ACE) I/D genotypes. The ACE I allele was associated with lower values, indicating a lower heart rate variability. The ACE DD genotype had significantly higher RMSSD values than the genotypes containing the I allele.

adults aged 20 to 70 years. They found that the ACE DD genotype was significantly increased among centenarians compared with the younger control cohort and with the expected genotype distribution. We were able to confirm these findings in a sample of 349 Berliners aged >80 years (unpublished observations). These results imply that persons with the ACE DD genotype, providing they avoid the pitfalls of possible early increased cardiovascular risk, may be more likely to be among the “old” elderly than those with the I allele. Our observation that persons with the DD genotype have greater HRV may explain the unexpected finding that an ACE variant, which may predispose to coronary heart disease, is unexpectedly frequent in French centenarians and German octogenarians. We suggest that the ACE gene may exert pleiotropic, age-dependent effects on longevity.

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