Sex Differences and Heritability of Two Indices of Heart Rate Dynamics: A Twin Study

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We investigated whether women show larger heart rate variability (HRV) than men after controlling for a large number of health-related covariates, using two indices of HRV, namely respiratory sinus arrhythmia (RSA) and approximate entropy (ApEn). In a twin design, the heritability of both indices was examined. The covariation between RSA and ApEn, a measure of heart rate dynamics derived from nonlinear dynamical systems theory, was decomposed into genetic and environmental components. Subjects were 196 male and 210 female middle-aged twins. Females showed larger HRV than men before (ApEn: $p < .001$; RSA: $p = .052$) and after adjustment for covariates (ApEn: $p < .001$; RSA: $p = .015$). This sex difference was confirmed by significant intrapair differences in the opposite-sex twin pairs for both ApEn ($p < .001$) and RSA ($p = .03$). In addition to sex, only heart period and age (both $p < .001$) were found to be independent predictors of ApEn, whereas RSA was also influenced by respiration rate and smoking (both $p < .001$). Age explained 16% and 6% of the variance in RSA and ApEn, respectively. Oral contraceptive use and menopausal status had no effect on HRV. Genetic model fitting yielded moderate heritability estimates for RSA (30%) and ApEn (40%) for both males and females. The correlation between RSA and ApEn ($r = .60$) could be attributed to genetic factors (48%), environmental factors (36%) and age (16%). The present study found support for a gender difference in HRV with women having greater HRV than men even after controlling for a large number of potential confounders. Indices of heart rate dynamics derived from nonlinear dynamical systems theory are moderately heritable and may be more sensitive than traditional indices of HRV to reveal subtle sex differences with important implications for health and disease.

Reduced heart rate variability (HRV) is considered to be an indication of diminished health. After acute myocardial infarction decreased HRV is a predictor of all-cause mortality, arrhythmic events and sudden death (Bigger et al., 1993; Kleiger et al., 1987; Reinhardt et al., 1996). Decreases in components of HRV have further been associated with onset of hypertension (Singh et al., 1998), congestive heart failure (Yoshikawa et al., 1999), severity of atherosclerosis (Hayano et al., 1991), advanced age (Parati et al., 1997), obesity (Karason et al., 1999), diabetic neuropathy (Freeman et al., 1991) and a range of psychopathologies including depression, anxiety, posttraumatic stress disorder, and schizophrenia (Friedman & Thayer, 1998).

A variety of measures have been used to operationalize HRV. Long-term measures like the standard deviation of all interbeat intervals in 24 hours, short-term measures like the standard deviation of 5-minute intervals and beat-to-beat measures like the root mean square of successive differences (RMSSD) have all been used. Respiratory sinus arrhythmia (RSA) is another measure and is defined as the change in heart period corresponding with the inspiratory and expiratory phases of the respiratory cycle. In addition, power spectral analysis of interbeat interval time series is frequently used to quantify HRV. The power spectrum of those time series contains two major components, a high (0.15–0.40 Hz) and low (0.01–0.15 Hz) frequency component reflecting cardiac vagal tone and a mixture of vagal and sympathetic influences, respectively. RSA, RMSSD and the high frequency component of the power spectrum are closely related, and all reflect vagal cardiac influence (Task Force, 1996). More recently, measures derived from nonlinear dynamics have been used to describe aspects of HRV. One such measure is approximate entropy (ApEn; Pincus, 2001; Pincus et al.,

Received 17 October, 2006; accepted 17 October, 2006.

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1991). It quantifies the complexity or irregularity of time series data. This and other 'chaos' derived parameters have proven valuable in the area of cardiovascular pathology, for example in discriminating cardiac patient groups with respect to the dynamics of their cardiovascular system. RSA and ApEn have been found to be significantly correlated (Newlin et al., 2000), but are by no means identical. For example, their mathematical foundation is quite different. It is, therefore, an important question which sources are responsible for similarities and differences among these two indices of heart rate dynamics.

There are large individual differences in HRV. Considering the potential relevance of low-HRV as a risk indicator, more insight into the origins of the individual differences is desirable. Part of the between subjects variation seems to be due to genetic factors. Studies in twins (Boomsma et al., 1990; Busjahn et al., 1998; De Geus et al., 2003; Snieder et al., 1997) and families (Singh et al., 1999; Sinnreich et al., 1999) suggest that up to 65% of the variance in HRV can be attributed to genetic influences. A potential candidate locus is the angiotensin converting enzyme insertion/deletion polymorphism (ACE I/D), which was found to be associated with HRV in both Caucasians (Busjahn et al., 1998) and African Americans (Thayer et al., 2003).

Sex differences in HRV have also been reported. Several studies have found women to have greater vagally mediated HRV in spite of having heart rates equal to or higher than men (Evans et al., 2001; Rossy & Thayer, 1998; Ryan et al., 1994; Thayer et al., 2003). The existence of a gender difference suggests a possible role of estrogens on vagal activity, which is supported by evidence from rat studies. The heart rate lowering effect of vagal stimulation is attenuated by ovariectomy (Du et al., 1994) and the suppressed parasympathetic activity after ovariectomy is restored by oestrogen replacement (McCabe et al., 1981).A higher vagal tone in women may protect them against fatal arrhythmias and sudden cardiac death and potentially explains their lower risk for these conditions compared to men (Kannel et al., 1998).

The first aim of the present study was to investigate whether women showed larger HRV than men as indexed by RSA and ApEn in a large sample of middle-aged male and female twins. We tested whether such a difference could be explained by any differences in health-related covariates of HRV between the sexes. Next, we estimated heritability for both indices. We further examined the relation between the two different indices of HRV in a bivariate twin model in which we tested to what extent the correlation between RSA and ApEn could be explained by genetic and environmental factors that are common to both traits.

Methods

Subjects

Subjects were part of a study on familial clustering of cardiovascular risk factors in middle-aged twins (Snieder et al., 1997) for which 213 twin pairs (aged between 34 and 63) were measured between 1992 and 1994. Informed consent was obtained from all subjects. One monozygotic (MZ) triplet was included in the sample by discarding the data from the second-born subject. Data from 10 pairs were excluded from analysis, because RSA or ApEn measurements were either incomplete or considered erroneous in at least one twin of those pairs. In total, data were available for 196 male and 210 female twins. In all same-sex twin pairs, zygosity was determined by DNA polymorphisms. Grouped according to their zygosity and sex the sample consisted of 43 pairs of monozygotic males (MZM), 36 pairs of dizygotic males (DZM), 47 pairs of monozygotic females (MZF), 39 pairs of dizygotic females (DZF) and 38 dizygotic pairs of opposite-sex (DOS).

Measures

Body weight was measured to the nearest 0.1 kg with a balance scale and subjects wearing light clothes only. Height was measured to the nearest 0.5 cm. Body mass index (BMI) was used as a measure of general obesity and calculated as weight divided by height squared (kg/m^2). Central obesity was measured as waist circumference at the level midway between the lower rib margin and the iliac crest.

Twins filled out questionnaires with items on health (including medication use), alcohol and tobacco use, exercise participation, and personality in the laboratory when they visited for the assessment of RSA and ApEn. In all subjects the questionnaires were screened for completeness by the experimenter during the laboratory session, and missing or ambivalent items were added or clarified.

Experimental Procedure

Twins always came in pairs (arriving around 10.00 a.m.) and were tested around the same time of day. All participants were asked to refrain from smoking, drinking alcohol, coffee or tea after 11.00 pm the night before. Subjects underwent mental stress testing interspersed by periods of quiet rest (for details see Snieder et al., 1997). Briefly, electrodes were attached for electrocardiogram (EKG) and impedance cardiogram recording and a strain-gauge was strapped around the waist to measure respiration. Participants were comfortably seated in reclined position in a dimly lit and sound shielded cabin in which they performed a number of mentally taxing tasks interspersed with periods of resting recovery. This paper focuses on the last of the resting conditions, an 8.5 minute period in which the twin was asked to sit back and relax as much as possible. The last resting period was selected to prevent influence of anticipatory arousal. All analyses will be based on the average values for RSA and ApEn obtained during this last resting condition.

Physiological Recording of RSA and ApEn

EKG and respiration signals were measured as described in detail by de Geus et al. (2003). The combined EKG and respiration signals were computer

scored to obtain RSA on a breath-to-breath basis. RSA is defined as the magnitude of change in heart period corresponding to the inspiratory and expiratory phases of the respiratory cycle. Heart period typically decreases during inspiration and increases during expiration. The stronger these respiratory-coupled variations in heart period, the larger the RSA and the stronger the vagal control of the heart. RSA was computed by the peak-to-valley method. The shortest interbeat interval was obtained during heart rate acceleration in the inspirational phase and the longest interbeat interval during deceleration in the expirational phase. The difference between the longest and shortest interval is used as an index of RSA. When no phase-related acceleration or deceleration was found, the breath was assigned a RSA score of zero. Mean RSA in milliseconds was computed for rest and task conditions by averaging the RSA values of all breaths falling within those conditions (including breaths with zero RSA). Automatic scoring of respiratory variables was checked by visual inspection of all respiratory signals in all conditions. Breathing cycles that showed irregularities like gasps, breath holding, coughing, and so forth, were not considered valid and were rejected and removed from further processing.

ApEn was calculated using the algorithm described by Pincus et al. (1991). Briefly, ApEn measures the regularity in the fluctuations of a signal such as heart period and reflects the logarithmic likelihood that a series of data points that are a certain predetermined distance apart (r) for a given number (m) of observations will remain so on subsequent incremental comparisons. Thus ApEn is like a template-matching procedure with fewer matches indicating greater complexity. As such, greater irregularity is associated with higher values of ApEn. To compute ApEn on a time series of a given fixed length two parameters must be specified and fixed for the data on which comparisons are to be made. The value m is the number of successive observations in the sequence to be used in the template as it were. Previous research suggests that m = 1 or 2 provides reasonable estimates for cardiac time series. The value r acts like a noise filter and is a proportion of the standard deviation of the time series. This value is usually set between 0.05 and 0.25 standard deviations units. We examined all the combinations of m = 1–3 and r = 0.05–0.25 in 0.05 increments. For the present analyses we chose the set of values that yielded the highest correlation with RSA which were m = 1 and r = 0.25.

Analytical Approach

Multiple Regression

To address the first aim of our study we tested for sex differences in mean values of RSA and ApEn and investigated whether such differences could be explained by differences between men and women in health-related covariates of RSA and ApEn using generalized estimating equations (GEE). GEE is a multiple regression technique that allows for nonindependence of twin or family data yielding unbiased standard errors and p values (Trégouët et al., 1997). In addition to sex, age, heart period and respiration rate, the following health-related HRV covariates were included as independent variables in the multiple regression model: current smoking (yes/no), physical activity (yes/no), antihypertensive medication use (yes/no), BMI and waist circumference. In addition to those, the effect of oral contraceptive use and menopausal status on RSA and ApEn were tested in women.

The 38 dizygotic (DZ) pairs of opposite sex in the study offered another way of examining sex differences. These pairs are naturally matched for age, part of their genetic background and a range of environmental confounders. Their intrapair difference in HRV (i.e., a paired t test) should therefore provide an accurate test for any existing gender difference. Sex differences were tested with two-sided tests, that is, p values for these differences are conservative.

Quantitative Genetic Model Fitting

We used multivariate structural equation modeling (SEM) to examine the genetic architecture of RSA and ApEn. We estimated the relative influence of genetic and environmental factors on RSA and ApEn and examined the extent to which the covariance between RSA and ApEn is determined by common genetic and/or environmental factors.

Details of model fitting to twin data have been described elsewhere (Neale & Cardon, 1992; Snieder & MacGregor, 2005). The comparison of the variance-covariance matrices in MZ and DZ twin pairs allows separation of the observed phenotypic variance into additive (A) or nonadditive (D) genetic components and shared (C) and unique (E) environmental components. Age can spuriously introduce a common environmental effect if there is a significant correlation between the phenotype and age, because twins are always of the same age. Both RSA and ApEn decrease with age (Ryan et al., 1994; Snieder et al., 1997). By incorporating age into the model, the influence of age on the phenotypes can be quantified and controlled for (Snieder, 2000).

Data for the model-fitting analyses (age, RSA and ApEn measured in twin and co-twin) were summarized into 5 × 5 variance-covariance matrices for each of the zygosity groups and a triangular decomposition (Neale & Cardon, 1992) was used in the multivariate model fitting. The Cholesky decomposition represents the most general way in which the variance-covariance structure of the data can be decomposed into its genetic and environmental parts. It allows evaluation of (the significance of) the influence of genetic and environmental factors on RSA and ApEn and on their interrelation. The genetic correlation (r_g) between two traits gives an indication of the amount of overlap between (sets of) genes influencing those traits. r_g is calculated as the (additive) genetic covariance (COV_A)

Table 1

General Characteristics of Males and Females in the Study Sample

	Males	Females	*p*
n	196	210	
Age (y)	43.7 (6.4)	44.5 (6.6)	*ns*
BMI (kg/m^2)	25.0 (2.8)	24.1 (3.9)	< .05
Waist (cm)	92.3 (8.5)	80.6 (10.2)	< .001
Heart Period (ms)	951.8 (144.2)	903.5 (129.8)	< .01
Respiration rate (cycles/min)	15.6 (2.7)	16.0 (2.6)	*ns*
RSA (ms)	57.0 (28.6)	62.9 (36.0)	.052
ApEn	0.778 (0.210)	0.874 (0.223)	< .001
Current smoker (*n*[%])	73 (37.2)	65 (31.0)	*ns*
Physical inactivity (*n*[%])	82 (41.8)	90 (42.8)	*ns*
Antihypertensive use (*n*[%])	16 (8.2)	16 (7.6)	*ns*
Contraceptive use (*n*[%])	26 (12.4)		
Postmenopausal (*n*[%])[a]	62 (29.5)		

Note: [a]Includes all women with hysterectomy ($n = 17$).
Mean values (*SD*) are shown unless indicated otherwise. *n*, number of individuals; *ns*, nonsignificant.

between two traits divided by the square root of the product of the total genetic variance components (V_A) of each of the traits. Shared and unique environmental correlations are calculated in a similar fashion.

Model Fitting Procedure

A series of submodels nested within the full parameter ACE or ADE Cholesky model were fitted to the multivariate variance-covariance matrices. The significance of variance components A, C, D and age was assessed by testing the deterioration in model fit after each component was dropped from the full ACE or ADE model, leading to the most parsimonious model. Sex differences in variance components were examined by comparing the full model in which parameter estimates are allowed to differ in magnitude between males and females, with a reduced model in which parameter estimates are constrained to be equal across the sexes. The difference in χ^2 values between a submodel and full model is itself approximately distributed as χ^2, with degrees of freedom (*df*) equal to the difference in *df* of submodel and full model. Model selection was also guided by Akaike's Information Criterion (AIC = χ^2–2*df*).

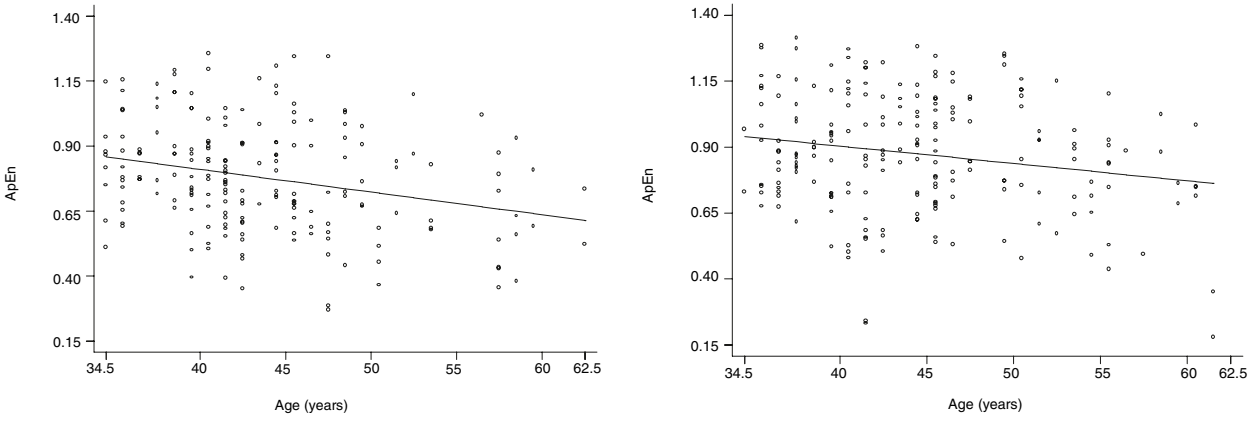
Prior to analysis, RSA was log transformed to obtain a better approximation of the normal distribution. The significance of phenotypic correlations and sex differences in mean values were tested within the SEM framework or with GEE. Both methods take account of nonindependency of twin data and yield unbiased *p* values. Data handling and preliminary analyses were done with STATA. All quantitative genetic modeling was performed with Mx (Neale et al., 1999).

Results

Characteristics of the sample for males and females are shown in Table 1. Males had a significantly higher BMI, a larger waist and a longer heart period. Females showed a significantly larger value for ApEn ($p < .001$) and a tendency for RSA ($p = .052$) in the same direction. Age, smoking, physical activity and antihypertensive use did not differ between the sexes. Zygosity did not have an effect on ApEn or RSA (results not shown).

ApEn values decreased significantly with age in males ($r = -.27$) and females ($r = -.19$; see Figure 1) and these correlations were not significantly different ($p = .71$). Figure 1 clearly shows the higher overall level of ApEn values in females compared to males.

Table 2 displays the phenotypic correlations between age, RSA, ApEn, heart period, respiration



a) males b) females

Figure 1

Scatterplot of the relation between ApEn and age in: (a) males ($r = -.27$) and (b) females ($r = -.19$).

Table 2

Correlations Between Age, ln(RSA), ApEn, Heart Period, Respiration Rate, Waist and Body Mass Index for Males (Lower Diagonal; $n = 196$) and Females (Upper Diagonal; $n = 210$)

	Age	ln(RSA)	ApEn	HP	RR	Waist	BMI
Age	*	**–.35**	**–.19**	–.03	.01	.09	.08
ln(RSA)	**–.44**	*	**.61**	**.47**	**–.35**	–.09	–.10
ApEn	**–.27**	**.60**	*	**.47**	.04	–.10	–.08
HP	–.04	**.42**	**.56**	*	–.09	–.10	–.10
RR	.03	**–.42**	–.11	–.13	*	.05	.06
Waist	**.18**	**–.32**	**–.24**	**–.29**	**.32**	*	**.90**
BMI	.12	**–.23**	–.15	**–.18**	**.28**	**.87**	*

Note: Significant ($p < .05$) correlations are in **bold**.
HP, heart period; RR, respiration rate; BMI, body mass index.

rate, waist circumference, and BMI for males and females. Correlations between age, RSA, ApEn, heart period and respiration rate were very similar for males and females. Interestingly, in both men and women respiration rate showed a strong inverse correlation with RSA, but not with ApEn. In men, waist (and BMI to a lesser extent) was inversely correlated with RSA, ApEn and heart period and positively with respiration rate, whereas these associations were absent in women.

The sex effect on ApEn remained highly significant ($p < .001$) in a multiple regression model including antihypertensive use, smoking, physical activity, BMI, waist circumference, heart period, respiration rate and age. In addition to gender, only heart period ($p < .001$) and age ($p < .001$) were found to be independent predictors of ApEn. Contraceptive use and menopausal status had no effect on ApEn in females. Multiple regression models for RSA also revealed a significant gender effect ($p = .015$) with higher RSA levels in females. The only differences with the ApEn regression model were a significant effect for respiration rate ($p < .001$) and a significant positive effect of smoking, indicating a — somewhat unexpected — larger RSA in smokers ($p < .001$). However, amongst smokers no association was observed between RSA and number of cigarettes smoked per day ($r = .01$).

Intrapair differences in opposite-sex pairs confirmed significant sex differences in both ApEn (females: 0.928 vs. males: 0.772, $p < .001$) and RSA (females: 63.9 ms vs. males: 51.0 ms, $p = .03$).

Twin correlations for RSA and ApEn are shown in Table 3 for each sex by zygosity group. With the exception of the ApEn correlation in MZ males, twin correlations in MZ twin pairs were larger than those in DZ twin pairs, indicating genetic influences.

Model fitting results are shown in Table 4. Dominance genetic variation (D) did not contribute significantly and could be dropped from all ADE models (data not shown). Parameter estimates for males and females could be set equal without a significant loss in fit ($\Delta\chi^2 = 8.74$, $df = 11$, ns). Subsequent removal of the shared environmental component (C) from the ACE model without gender differences did not lead to a significant worsening of fit ($\Delta\chi^2 = 0.69$, $df = 3$, ns), implicating a model including additive genetic and unique environmental influences (AE model) without gender differences as the most parsimonious. Although this AE model showed the best fit — confirmed by the lowest AIC — an alternative model explaining familial resemblance through shared environment (CE model) could not be entirely dismissed, as dropping A from the ACE model failed to achieve significance ($\Delta\chi^2 = 4.16$, $df = 3$, ns). The influence of age could not be dropped from these models, which means that its influence was significant for both RSA and ApEn.

Table 5 shows parameter estimates and 95% confidence intervals (CIs) of the best fitting model. Moderate heritability estimates were found for RSA (30%) and ApEn (40%) with most of the phenotypic variance of both traits explained by environmental influences specific to the individual (54% for both RSA and ApEn). Age explained 16% and 6% of the variance in RSA and ApEn respectively. Figure 2 shows the large genetic (.83) and moderate environmental correlation (.40) between RSA and ApEn and factor loadings of the best fitting model. Squaring the factor loadings yields estimates of variance components explained by age, genetic and environmental factors as reported in Table 5. The factor loadings and

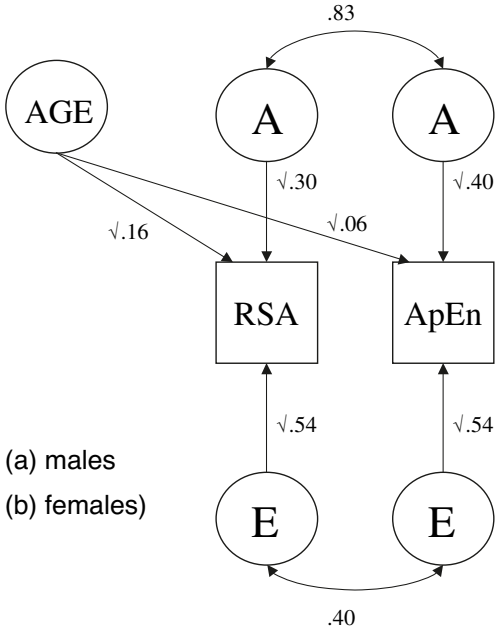


Figure 2

Genetic and environmental correlations and factor loadings of the best fitting model. Factor loadings (or path coefficients) are expressed as square roots to make clear that squaring those factor loadings yields estimates of variance components explained by age, genetic and environmental factors as shown in Table 5.

Table 3

Twin Correlations for ln(RSA) and ApEn

	N	ln(RSA)	ApEn
MZM	43	.53	.29
DZM	36	.22	.49
MZF	47	.47	.55
DZF	39	.35	.14
DOS	38	.26	.32

Note: *N*, number of twin pairs; MZM, monozygotic male; DZM, dizygotic male; MZF, monozygotic female; DZF, dizygotic female; DOS, dizygotic opposite sex

correlations can be used to calculate the proportion of the phenotypic correlation between RSA and ApEn (r = .60) explained by genetic factors (48%), environmental factors (36%) and age (16%) as predicted by this best fitting model.

Discussion

The present study investigated whether women show larger HRV than men as indexed by RSA and ApEn and found this to be significant for ApEn and marginally significant for RSA. Adjusting for a range of health-related covariates of HRV could not explain this gender effect. On the contrary, the gender effect remained highly significant for ApEn and became significant for RSA. These results were further corroborated by significant gender differences in the 38 twin pairs of opposite sex for both ApEn and RSA. We further investigated the relation between the two different indices of HRV in a bivariate twin model and tested to what extent the correlation between RSA and ApEn could be explained by genetic and environmental factors that are common to both traits. Moderate heritabilities of 30% and 40% were found for RSA and ApEn respectively with a large genetic correlation between these traits of .83 indicating a considerable amount of overlap between (sets of) genes influencing these two indices of heart rate dynamics.

Numerous previous studies have reported greater HRV in women compared to men (Evans et al., 2001; Fagard et al., 1999; Rossy & Thayer, 1998; Ryan et al., 1994; Thayer et al., 2003; but see Liao et al., (1995) for a negative finding). However, the present study extends the previous findings in at least two important ways. First, this study examined a wider range of potential confounding covariates than had previously been investigated. The present findings showed that women had greater HRV as indexed by ApEn and RSA even after controlling for antihypertensive use, smoking, physical activity, BMI, waist circumference, respiration rate, resting heart period, and age. Moreover, contraceptive use and menopausal status had no effect on ApEn and RSA in women. Second, this is the largest study that examined gender differences to use ApEn as an index of HRV. ApEn is an index of the irregularity or complexity of a time series (Pincus, 2001). It has been suggested that disease states are associated with a loss of complexity in the cardiac time series and the gender differences observed in this study may be linked to important health disparities between men and women.

Although the AE model without gender differences was the most parsimonious, an alternative model explaining familial resemblance through shared environment (CE model) could not be entirely dismissed. One possible reason for the lack of discriminative power between these alternative explanations of the data can be found in the diverging pattern of MZ/DZ ApEn correlations between males and females (i.e., the unexpectedly low MZ male correlation).

Our best fitting model indicated that 40% of individual differences in ApEn could be explained by genetic factors in both men and women. However, this sample of around 200 twin pairs had only moderate power to detect a difference in heritability estimates between men and women. More specifically, we had 80% power (alpha = .05) to detect a heritability difference of about 20%. Small gender differences in heritabilities can therefore not be excluded.

Age is another source of individual differences in ApEn and we confirmed its negative relationship with

Table 4

Model Fitting Results of the Bivariate Cholesky Decomposition Model, With and Without Sex Differences

Model	χ^2	*df*	*p*	AIC	Vs.	$\Delta\chi^2$	Δdf	*p*
Gender differences								
(1) ACE	63.45	52	.133	–40.55				
(2) AE	68.27	58	.168	–47.73	1	4.82	6	.57
(3) CE	70.24	58	.130	–45.76	1	6.79	6	.34
No gender differences								
(4) ACE	72.19	63	.200	–53.82	1	8.74	11	.65
(5) AE	**72.88**	**66**	**.262**	**–59.12**	**4**	**0.69**	**3**	**.88**
(6) CE	76.35	66	.180	–55.65	4	4.16	3	.24

Note: χ^2, Chi-square goodness of fit statistic; *df*, degrees of freedom; *p*, *p* value; AIC, Akaike's Information Criterion; *ns*, nonsignificant; Vs., versus; and indicates with which model the submodel is compared. All models included age. Most parsimonious solution is printed in **bold**.

Table 5

Standardized Estimates of Most Parsimonious Model (AE No Sex Diff.)

	h^2 (95% CI)	e^2 (95% CI)	age^2 (95% CI)
ln(RSA)	.30 (.16–.43)	.54 (.41–.68)	.16 (.09–.24)
ApEn	.40 (.26–.53)	.54 (.41–.69)	.06 (.02–.12)

Note: h^2 = heritability, e^2 = unique environmental variance component, age^2 = variance component due to age.

ApEn (Ryan et al., 1994). However, this relationship was similar in males and females and could not explain the sex difference. Whereas other researchers have suggested that estrogen may be associated with the finding of greater HRV in women the present results failed to find an effect of indices of hormonal status (contraceptive use and menopausal status) on ApEn and/or RSA in females. Another potential source for this difference may be the neural control of cardiac chronotropy. We have reported that the inhibitory effect exerted by the prefrontal cortex on sympathoexcitatory subcortical circuits as indexed by heart rate and HRV is greater in females than in males particularly for the right hemisphere (Ahern et al., 2001). Future research in animals and humans will be needed to explicate the basis for this gender difference.

Another important result of the present study concerns the similarity and differences among the two indices of heart rate dynamics. The mathematical foundations of RSA and ApEn are quite different. Whereas we and others (Newlin et al., 2000) have found RSA and ApEn to be significantly correlated, we also find that these indices are not identical. Importantly, in a number of settings ApEn has been found to be more sensitive than traditional indices of HRV (Nabors-Oberg et al., 2002; Newlin et al., 2000; Ryan et al., 1994). In the present study we found that the genetic bases of these indices were significantly related. Nonetheless we found that these indices were differentially sensitive to the gender difference with the ApEn index showing a much larger and more significant difference between men and women even after controlling for a large number of covariates. Thus indices derived from nonlinear dynamical systems theory may have utility in the investigation of subtle differences in heart rate dynamics.

RSA is a reflection of tonic vagal firing of vagal motoneurons as modulated with a respiratory related phasic signal by the output of the central respiratory generator, as outlined in the model of Berntson et al. (1997). Respiration parameters, however, and mainly respiration rate, influence RSA to some extent, without a corresponding influence on cardiac vagal tone. The influence of respiration rate on RSA was confirmed by the high correlation we observed in this study between these two variables. It is therefore advised to correct for respiration rate in between subjects/groups comparisons, as we did for our examination of gender effects. However this correction did not substantively alter the present findings. With regard to the quantitative genetic modeling, we clearly showed in a previous analysis of this twin cohort that taking respiration rate into account did not appreciably alter the estimates of heritability of RSA under either resting or stressful conditions (Snieder et al., 1997). ApEn was not correlated with respiration rate in our data. This implies that to the extent ApEn reflects cardiac vagal tone it reflects an estimate unbiased by respiratory influences. On the other hand several other mechanisms apart from respiratory modulation influence cardiac vagal tone (Houtveen et al., 2002) and nonvagal mechanisms may also contribute to individual differences in magnitude of ApEn, because RSA and ApEn only partially overlap.

In summary, the present study showed that indices of heart rate dynamics derived from nonlinear dynamical systems theory are moderately heritable and of the same order of magnitude as RSA. The sets of genes underlying these two indices of HRV showed significant genetic overlap, but were not identical. The present study found support for a sex difference in HRV with women having greater HRV than men even after controlling for a large number of potential confounders. These findings confirm and extend previous studies that have found women to have greater HRV with potentially important implications for health and disease.

Acknowledgments

This study was funded by the Netherlands Hearts Foundation (90–313).

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