Heritability of Ambulatory Heart Rate Variability

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Background—Reduced heart rate variability (HRV) is a prognostic factor for cardiac disease and cardiac mortality. Understanding the sources of individual differences in HRV may increase its diagnostic use and provide new angles for preventive therapy. To date, the contribution of genetic and environmental factors to the variance in HRV has not been investigated during prolonged periods of ambulatory monitoring in a naturalistic setting.

Methods and Results—In 772 healthy twins and singleton siblings, ambulatory ECG was recorded during 24 hours. Two time domain measures of HRV were used: the standard deviations of all normal-to-normal intervals across 5-minute segments (SDNN index) and the root mean square of successive differences between adjacent normal RR intervals (RMSSD). Multivariate genetic analyses across 4 periods of day (morning, afternoon, evening, night) yielded significant estimates for genetic contribution to the mean ambulatory SDNN index (ranging from 35% to 47%) and the mean ambulatory RMSSD (ranging from 40% to 48%).

Conclusions—Ambulatory HRV measures are highly heritable traits that can be used to support genetic association and linkage studies in their search for genetic variation influencing cardiovascular disease risk. **(*Circulation*. 2004;110: 2792-2796.)**

Key Words: genetics ■ heart rate ■ nervous system, autonomic

Heart rate variability (HRV) is a clinically relevant cardiovascular phenotype. Reduced HRV is an independent predictor of cardiac disease and cardiac mortality.[1–6] The major explanation for this predictive effect is that reduced HRV reflects a shift in cardiac sympathovagal balance from parasympathetic to sympathetic control over the heart rhythm.[7,8] Understanding the sources of individual differences in HRV may increase its diagnostic use and, if these differences can be traced to genetic polymorphisms, may provide new angles for preventive therapy. The first step in the establishment of genetic contribution to a clinical phenotype is the estimation of its heritability in samples of genetically related subjects. In laboratory studies, a significant genetic contribution to HRV has been established by twin and family studies. Heritability estimates at rest range from 13% to 39%,[9–13] but during exposure to various stress tasks the genetic contribution increases up to 51%.[9] This suggests that genetic influences are more pronounced when the subject is challenged by mentally and emotionally taxing tasks. Accordingly, we hypothesize that heritability of HRV measures will be even higher when recorded over prolonged periods in a naturalistic setting. To date, the genetics of HRV in such recordings have not been investigated. The purpose of the present study was to estimate the contribution of genetic and environmental factors to the variance in ambulatory measured HRV with the use of a twin family design.

Methods

Subjects

Participants were registered with the Netherlands Twin Register. All families were selected for a genetic linkage study in search of genes influencing personality traits, as described in detail elsewhere.[14] Briefly, the families were selected to have 2 siblings (dizygotic [DZ] twin pair, or sib-twin pair, or sib-sib pair) discordant or concordant for anxiety, neuroticism, or depression. In addition to these siblings, however, all other family members were recruited for study, and the resulting distribution of anxiety, neuroticism, and depression scores was near normal with only mild kurtosis.

Of the 1332 offspring who returned a DNA sample (buccal swabs) for the linkage study, 1008 were successfully contacted for a cardiovascular ambulatory monitoring study, of which 192 refused or were excluded. Reasons for exclusion were pregnancy, heart transplantation, pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. In 14 of the remaining 816 subjects, no data were available because of equipment failures. Ten subjects showed a very noisy ECG signal and were excluded from the analyses. Subjects (20 in total) using rhythm-altering medication (n=2) and HRV-reducing antidepressants (tricyclic antidepressants [n=2] and benzodiazepines [n=10]) and antihypertensive medication (β-blockers [n=13]) or a combination of these were excluded from the analysis. The final sample consisted of 218 monozygotic (MZ) twins (79 men), 301 DZ twins (107 men), and 253 singleton siblings (97 men) from 339 families. For the majority of the twin pairs, zygosity was determined by DNA typing; in a small part (8%), zygosity questionnaires were used. The mean age was 31.3 years (SD=10.6) for men and 30.8 years (SD=10.9) for women. The Ethics Committee of Vrije Universiteit approved the

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study protocol, and all subjects gave written consent before entering the study. No payment was made for participation.

Study Design

Subjects were visited at home on a weekday, before starting their normal daily activities. They were subjected to an interview on health status and current medication use. The Vrije Universiteit Ambulatory Monitoring System 46 (VU-AMS device[15,16]) was attached, and its operation was explained. Subjects wore the VU-AMS device the entire day and night until awakening the next morning. Every 30 (±10) minutes, the ambulatory device produced an audible alarm beep to prompt them to complete a detailed diary. Subjects wrote a chronological account of activity, posture, location, presence of other persons, and amount of perceived stress during each past 30 minutes. On the following day, the research assistant collected the device at home.

Heart Rate Variability

The VU-AMS device continuously recorded the ECG from a 6-electrode configuration. Two HRV measures were extracted from the interbeat interval time series: the standard deviations of all normal-to-normal intervals (SDNN) and the root mean squares of the successive differences between adjacent normal-to-normal intervals (RMSSD). In addition to cardiac measures, the device also recorded vertical acceleration as a proxy for gross body movement. The vertical accelerometer information was combined with the diary information to divide the entire recording into smaller fragments that were stationary with regard to physical activity and posture, eg, within each fragment no shifts in activity/posture occurred. The fragments were never <5 minutes or >1 hour. They were coded for posture (lying, sitting, standing, walking, and bicycling), activity (eg, desk work, housekeeping, watching television), and location (eg, at home, at work, at a public place). SDNN was computed across all 5-minute periods that fitted in the coded fragment, effectively yielding the SDNN index. SDNN index and RMSSD were averaged over the entire fragment. On the basis of the reported times of dinner and lunch, awakening, and bedtime, mean RMSSD and SDNN index were computed across all fragments in the morning, afternoon, evening, and nighttime sleep periods. In 8% of the subjects, the exact time of dinner, lunch, awakening, or bedtime could not be extracted from either diary or body movement. For these subjects, the missing time was imputed with the use of the mean times of these events in the rest of the sample.

Statistical Analysis

Confounding

The individual differences in ambulatory HRV were expected to be sensitive to 3 main confounders: differences in sex and age[17] and differences in physical activity patterns on the measurement day.[18] All analyses below were adjusted for age and sex to control for the first 2 of these confounders. Because ambulatory recorded subjects may differ in their activity patterns, the potential influence of physical activity and postural changes on interindividual variance in HRV measures must be taken into account. This was done by calculating the twin correlations twice: once including the entire recording, containing data during all postures, and once including those fragments of the recording during which a subject was either sitting or lying.

Twin Correlations

MZ twins share all their genetic material, whereas DZ twins and siblings share on average 50% of their segregating genes. A larger resemblance of MZ than DZ twins or other first-degree relatives thus indicates that their larger genetic resemblance is associated with a larger phenotypic resemblance.[19] To determine the extent to which MZ twin pairs are more similar than DZ or sibling pairs, Pearson correlation coefficients were calculated per zygosity with the use of SPSS-11 (SPSS Inc). All possible MZ and DZ/sib pairs were used.

Structural Equation Modeling

To answer the question of to what extent genes and shared and unshared environment contribute to the variance of SDNN index and RMSSD, biometric genetic models were fitted to the data with the use of the structural equation program Mx.[20] First, nested univariate unconstrained models were fitted to test assumptions of the (extended) twin model. For each period of day, we tested the equality of means and variances for MZ twins, DZ twins, and singleton siblings. Likewise, we examined the presence of sex and age effects on the means and variances. In a final step, we tested for heterogeneity of correlations of men versus women and of DZ twins versus singletons.

The resulting most parsimonious unconstrained models were the ones against which the variance decomposition models were tested. The observed variance was decomposed into 3 sources: additive genetic influences (A), shared environment (C), and unshared environment (E), following Neale and Cardon.[21] For DZ twins and sibling pairs, similarity in shared environmental influences was fixed at 100%, and similarity of additive genetic influences was fixed at 50%. For MZ twins, similarities of additive genetic and shared environmental influences were fixed at 100%. Unshared environmental influences are uncorrelated in all twin and sibling pairs. After establishing the most parsimonious variance components model (ACE, AE, CE, or E) for each period of day, we used a full 4-variate Cholesky decomposition to test whether the same or different genetic and environmental factors influenced HRV at each of the 4 periods of the day. A priori, we expected a single genetic factor to underlie the variance across all 4 periods for both SDNN index and RMSSD. This was tested by contrasting a full Cholesky decomposition against a genetic factor model, which allows for a common genetic factor and specific additive genetic influences at each period. It was further tested whether unique environmental influences could also be better described by such a factor structure or whether a Cholesky decomposition should be preferred.

Nested models were compared by likelihood ratio test, with the use of twice the difference between the log-likelihoods of 2 models, which is asymptotically distributed as χ^2. A high χ^2 against a low gain in degrees of freedom will generate a significant probability value and denotes a worsening of the fit (related to the more parsimonious model).

Results

The valid ambulatory recording time was on average 22 hours and 13 minutes (SD=3 hours and 21 minutes), of which 51% was spent in a sitting or lying posture. Although the sample was previously selected on the basis of the presence of at least 2 family members with extreme scores on personality questionnaires, their scores did not correlate significantly with either SDNN index or RMSSD, which shows that the results were not biased by this selection criterion. At all 4 periods, SDNN index was significantly correlated with RMSSD (morning r=0.83, afternoon r=0.86, evening r=0.87, and night r=0.87). Because the RMSSD distribution was skewed at all time periods, its natural logarithm was used in all further analyses. Table 1 presents the untransformed means and SDs for SDNN index and RMSSD separately for each subject group, using HRV from all postures (top) or from fragments of sitting/lying only (bottom).

Twin and Sibling Correlations

Table 2 shows the resemblance between MZ and DZ/sibling pairs for SDNN index and RMSSD. All correlations were calculated twice: once on all available data and once with the use of fragments in which subjects were either sitting or lying. Despite the potentially large effects of differences in posture and physical activity on HRV similarity in twins and

TABLE 1. Means and SDs of SDNN Index and RMSSD for All Daily Periods

All Postures	MZM (70<n<78)	DZM (50<n<54)	MZF (131<n<138)	DZF (118<n<127)	DOS Men (47<n<54)	DOS Women (57<n<65)	Male Siblings (88<n<97)	Female Siblings (142<n<154)
SDNN index, ms								
Morning	82.3 (30.9)	78.6 (19.5)	67.8 (18.1)	67.3 (18.9)	81.4 (20.9)	72.7 (18.6)	73.9 (21.7)	69.7 (19.4)
Afternoon	78.5 (28.5)	77.1 (19.9)	64.7 (18.6)	62.5 (16.2)	77.6 (24.3)	68.8 (19.1)	69.0 (20.8)	64.9 (19.9)
Evening	78.7 (26.4)	83.6 (22.8)	69.7 (23.1)	63.5 (18.0)	82.1 (22.8)	68.4 (20.1)	73.5 (24.4)	66.4 (20.4)
Night	89.0 (31.7)	98.8 (26.8)	70.8 (28.9)	70.8 (22.7)	98.2 (27.4)	80.7 (27.2)	88.1 (29.7)	73.7 (24.0)
RMSSD, ms								
Morning	41.1 (26.6)	41.5 (30.1)	31.8 (14.3)	31.8 (16.0)	41.1 (18.7)	36.7 (22.4)	36.1 (17.7)	34.2 (19.1)
Afternoon	38.7 (22.7)	40.7 (24.5)	32.5 (15.8)	30.5 (12.6)	40.7 (19.5)	36.5 (23.9)	32.9 (14.8)	32.1 (17.9)
Evening	39.7 (22.4)	46.5 (21.8)	40.5 (21.3)	34.0 (15.3)	46.2 (22.1)	42.2 (39.0)	40.4 (23.2)	36.7 (19.5)
Night	57.4 (35.5)	70.8 (35.2)	58.7 (33.9)	49.1 (30.5)	72.0 (37.5)	60.9 (37.1)	59.1 (33.8)	50.7 (25.4)
Sitting and lying postures only								
SDNN index, ms								
Morning	73.5 (25.1)	73.8 (17.0)	64.4 (18.4)	63.3 (20.3)	79.4 (20.4)	67.3 (17.9)	70.7 (23.5)	65.9 (21.4)
Afternoon	73.3 (26.0)	72.8 (18.4)	61.7 (19.0)	59.0 (16.8)	74.8 (21.7)	65.1 (20.0)	65.7 (20.4)	61.5 (20.5)
Evening	72.6 (25.0)	77.0 (20.1)	67.0 (24.3)	60.6 (19.8)	80.1 (23.4)	62.6 (17.7)	69.0 (22.9)	63.8 (22.1)
Night	85.4 (30.4)	97.1 (25.4)	77.5 (26.2)	69.8 (21.2)	94.7 (23.9)	78.4 (23.0)	85.0 (26.1)	72.4 (21.8)
RMSSD, ms								
Morning	43.7 (28.8)	46.0 (40.1)	35.4 (16.9)	34.6 (19.7)	43.5 (25.7)	43.5 (25.7)	38.7 (21.6)	38.4 (22.8)
Afternoon	41.0 (26.2)	43.2 (28.8)	36.4 (19.1)	33.4 (14.3)	42.3 (25.2)	42.3 (25.2)	35.5 (17.3)	36.0 (21.6)
Evening	40.5 (23.6)	47.1 (22.7)	45.2 (27.2)	36.5 (18.1)	46.4 (32.6)	46.4 (32.6)	41.9 (23.9)	40.2 (22.6)
Night	58.1 (36.3)	71.4 (35.8)	60.2 (35.1)	50.1 (31.0)	65.5 (35.7)	65.5 (35.7)	59.6 (33.8)	51.2 (25.7)

MZM indicates monozygotic male twins; DZM, dizygotic male twins; MZF, monozygotic female twins; DZF, dizygotic female twins; DOS, dizygotic twins of opposite sex; and n, No. of subjects (varies slightly per daily period). Male and female siblings are singletons.

siblings, the correlations based on sitting/lying-only fragments differed only marginally from those that included the entire recording. Although the potential confounding by mixing data across different postures had little actual impact, we decided to restrict further model fitting to the most "pure" data, ie, fragments in which subjects had been sitting or lying (sleep).

Structural Equation Modeling

First, we fitted a series of univariate unconstrained models. The means and variances of both SDNN index and RMSSD were equal for MZ and DZ twins and singleton siblings. Importantly, equating male or female correlations or DZ correlations to correlations across any of the other sib/sib pairings (MZ twin/singleton sibling, DZ twin/singleton sibling, singleton sibling/singleton sibling) yielded no significant worsening in the fit of the model. This allowed us to reduce the number of parameters to be estimated but also implies that the results obtained in twins can be generalized to singletons. Both RMSSD and SDNN index decreased with age, in accordance with previous findings,[17] and were higher in men at all periods of day. The sex difference for SDNN index and RMSSD repeats previous findings,[22] although the opposite has been found for RMSSD.[17] In view of their effects, sex and age were retained as covariates in the final variance components analyses.

The resulting most parsimonious unconstrained models were contrasted against different multivariate variance components models (ACE, AE, CE, E). Only additive genetic (A) and unshared environmental (E) sources contributed significantly to individual variation in SDNN index and RMSSD. For the additive genetic variance, the genetic factor model showed the best fit. Unique environmental variance had to be left in full Cholesky decomposition. In this final model, the common genetic factor explained between 28% and 45% of the variance in SDNN index and between 32% and 48% of the variance in RMSSD (Table 3). Specific genetic influences on SDNN index were always present except for the afternoon and added between 2% and 12% to total heritability. Specific genetic influences on RMSSD were present only in the afternoon and during nighttime sleep and added 2% and 8%, respectively, to total heritability.

Discussion

Individuals characterized by low HRV are at increased risk for cardiac events,[1–3,5] sudden cardiac death,[6] and overall mortality.[5,23] On the basis of prolonged measurements of HRV in naturalistic settings obtained in a sample of 772 twins and singleton siblings, the present study showed that individual differences in 2 often-used HRV measures, SDNN index[24,25] and RMSSD,[25] are determined to a large extent by additive genetic factors. For ambulatory SDNN index, heritability estimates from genetic model fitting ranged from 35% to 47%. For ambulatory RMSSD, heritability estimates ranged from 40% to 48%.

TABLE 2. Age-Adjusted Twin and Sibling Correlations for SDNN Index and RMSSD

	SDNN Index		RMSSD*	
	r MZ	*r* DZ+Sib	*r* MZ	*r* DZ+Sib
Morning				
Men	**0.64/0.58**	**0.41/0.37**	**0.54/0.50**	**0.32/0.27**
Women	**0.58/0.45**	**0.22/0.18**	**0.51/0.45**	**0.24/0.23**
Opposite sex	...	**0.27**/0.04	...	0.11/0.08
Afternoon				
Men	**0.66/0.65**	**0.36/0.26**	**0.53/0.59**	**0.27/0.28**
Women	**0.57/0.51**	**0.21/0.21**	**0.49/0.58**	**0.17/0.23**
Opposite sex	...	**0.27**/0.07	...	**0.26**/0.13
Evening				
Men	**0.45/0.41**	**0.24**/0.13	**0.57/0.56**	**0.30/0.25**
Women	**0.44/0.48**	**0.26/0.28**	**0.49/0.56**	**0.18/0.28**
Opposite sex	...	**0.17**/0.13	...	0.12/0.09
Night				
Men	**0.71/0.69**	**0.25/0.25**	**0.63/0.57**	**0.36/0.31**
Women	**0.55/0.55**	**0.17/0.18**	**0.47/0.46**	**0.13/0.15**
Opposite sex	...	**0.15**/0.12	...	**0.18**/0.14

Shown are (1) twin correlations based on the entire recording including all postures and (2) twin correlations based exclusively on the fragments of the recording in which subjects were either sitting or lying. Correlations that were significant ($P<0.05$) are printed boldfaced.

*RMSSD was transformed by taking the natural logarithm to approach normality in the data.

Our heritability estimates correspond well with those in a previous study in which much shorter ambulatory recording periods (<4 hours) were used. Using segregation analysis, the Kibbutzim family study[12] found genetic influences to account for 45% of age- and sex-corrected RMSSD. However, our estimate for SDNN index is substantially larger than the estimate from a family study based on the Framingham Heart Study and the Framingham Offspring Study.[11] In this latter study, SDNN was obtained rather than SDNN index. SDNN was averaged over a 2-hour fragment obtained during a routine, scheduled examination at the Framingham Heart Study clinic. Genetic factors accounted for only 19% of the interindividual variation in SDNN. A major difference in the genetic study design might account for these diverging findings. The Framingham studies used spouse and sibling correlations to produce synthetic estimates of variance components. Because a significant spouse correlation was found, the resemblance between siblings was attributed in part to a shared household. Spouse correlation, however, may also reflect assortative mating for exercise behavior, a variable known to be associated with SDNN index.[26] It is of note that the age-corrected sibling correlations in the Framingham Study (0.23 to 0.26) correspond very closely to our age-corrected sibling correlations, suggesting that, when the studies can be compared directly, they are actually very consistent.

Our study made use of an extended twin design that strongly increases statistical power to distinguish between components A, C, and E compared with a design including only MZ and DZ twins.[27] Although there was sufficient power (at $\beta=0.80$, $\alpha=0.05$) to detect effects of $\geq 23\%$, no significant common environmental effect was found. The extended twin design further allowed us to test the possibility that results obtained on singleton sibling pairs were identical to those obtained in twin pairs. This is important because the much lower birth weight in twins might be considered to reflect an impaired fetal environment, which according to the "Barker hypothesis" may influence autonomic function.[28,29] MZ or DZ twins did not differ from singleton siblings in means, variances, and covariances on any of the measures. The absence of any twin-singleton difference repeats previous findings in other cardiovascular risk factors[30] and indicates that our results can be generalized safely to the population at large.

We separated the entire ambulatory recording into 4 periods of day to allow for the possibility that different genetic factors would affect heart rate regulation during awake and sleeping periods or during leisure (evening) and work (morning, afternoon) periods. Although evidence was found for separate genetic factors influencing HRV at different daily periods, their contribution to the total genetic variance was marginal in comparison to the common genetic factor that influenced HRV at all times of day. From a gene-finding point of view, the common genetic factor structure is advantageous on at least 2 accounts. First, using highly genetically correlated multivariate phenotypes can yield higher statistical power to find genes in linkage analysis.[31] Second, these genes, by virtue of having a pervasive influence on HRV across all situations, will also have the largest clinical relevance. This assumes that the genes causing low HRV in this relatively young population remain of

TABLE 3. Multivariate Heritability Estimates for SDNN Index and RMSSD

	SDNN Index			RMSSD*		
	Common h^2	Specific h^2	Total h^2	Common h^2	Specific h^2	Total h^2
Morning	0.28 (0.14 to 0.44)	0.07 (0.02 to 0.14)	0.35	0.41 (0.27 to 0.53)	0.00 (−0.04 to 0.04)	0.41
Afternoon	0.36 (0.20 to 0.50)	0.00 (−0.04 to 0.04)	0.36	0.46 (0.32 to 0.59)	0.02 (−0.06 to 0.06)	0.48
Evening	0.45 (0.31 to 0.58)	0.02 (−0.08 to 0.08)	0.47	0.48 (0.35 to 0.60)	0.00 (−0.03 to 0.03)	0.48
Night	0.31 (0.18 to 0.45)	0.12 (0.04 to 0.21)	0.43	0.32 (0.19 to 0.45)	0.08 (0.02 to 0.15)	0.40

Shown are the heritability estimates (h^2) for SDNN index and RMSSD. Total heritability has been categorized into heritability caused by shared genetic components and heritability caused by time-specific genetic components. Between parentheses are the lower and upper boundaries for the 95% CIs around the estimates.

*RMSSD was transformed by taking the natural logarithm to approach normality in the data.

importance in later life. Note that we cannot exclude expression of different HRV genes throughout the life span.

In conclusion, this study provides a strong confirmation that genes are important in the regulation of ambulatory HRV, a clinically relevant phenotype for a wide range of cardiovascular diseases.[3,5,6,24] The next step is to trace the actual genetic polymorphisms that influence ambulatory HRV to provide new angles for preventive therapy. The first whole-genome screens and candidate gene studies have been initiated already,[13,32] and these initiatives should be rapidly extended. Because the power to detect genes increases with the availability of genetically correlated repeated measurements, we believe ambulatory HRV to be an important asset in the search for genetic variation influencing cardiovascular disease risk.

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