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Heritability of cardiac vagal control in 24-h heart rate variability recordings: Influence of ceiling effects at low heart rates

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Abstract

This study estimated the heritability of 24-h heart rate variability (HRV) measures, while considering ceiling effects on HRV at low heart rates during the night. HRV was indexed by the standard deviation of all valid interbeat intervals (SDNN), the root mean square of differences between valid, successive interbeat intervals (RMSSD), and peak-valley respiratory sinus arrhythmia (pvRSA). Sleep and waking levels of cardiac vagal control were assessed in 1,003 twins and 285 of their non-twin siblings. Comparable heritability estimates were found for SDNN (46%–53%), RMSSD (49%–54%), and pvRSA (48%–57%) during the day and night. A nighttime ceiling effect was revealed in 10.7% of participants by a quadratic relationship between mean pvRSA and the interbeat interval. Excluding these participants did not change the heritability estimates. The genetic factors influencing ambulatory pvRSA, RMSSD, and SDNN largely overlap. These results suggest that gene-finding studies may pool the different cardiac vagal indices and that exclusion of participants with low heart rates is not required.

Descriptors: Parasympathetic nervous system, Ambulatory monitoring, RSA, RMSSD, Twins

Reduced heart rate variability (HRV) is a predictor for all-cause mortality and adverse cardiovascular events, including atrial fibrillation, myocardial infarction, congestive heart failure, and coronary artery disease in premorbid populations and samples of cardiac patients (Bigger, Jr., Fleiss, Rolnitzky, & Steinman, 1993; Bigger, Jr., et al., 1992; Bigger, Jr., Hoover, Steinman, Rolnitzky, & Fleiss, 1990; Buccelletti et al., 2009; Dekker et al., 1997, 2000; Kleiger, Miller, Bigger, Jr., & Moss, 1987; Singer et al., 1988; Tsuji et al., 1996; Vikman et al., 2003). A proposed mechanism that can explain the risk conveyed by low HRV is that it reflects a decrease in cardiac vagal activity, which increases the chance of arrhythmic events (La Rovere et al., 2001; Schwartz, Billman, & Stone, 1984; Schwartz et al., 1988).

A useful noninvasive measure of vagal activity is the HRV in the respiratory frequency range (0.15–0.4 Hz), also called respiratory sinus arrhythmia (RSA). RSA is generated when tonic firing of motor neurons in the nucleus ambiguous is modulated by phasic inhibition and excitation coupled to the respiratory cycle (Berntson, Cacioppo, & Quigley, 1993). This modulation is caused by connections between the nuclei that control the respiratory generator in the pre-Bötzinger and Bötzinger complexes and the vagal motor neurons, which lie in close proximity in the brainstem (Rekling & Feldman, 1998) and is further influenced by input from baro-, mechano-, and chemoreceptors. Respiration-autonomic nervous system (ANS) coupling yields an oscillatory pattern in the release of acetylcholine in the sinoatrial (SA) node, such that acetylcholine levels increase during expiration and decrease during inspiration. The effect of this respiratory "gating" (Eckberg, 2003) is that heart rate increases during inspiration and decreases during expiration. The effect of the respiratory-related changes in vagal gating on RSA shows relatively little sensitivity to sympathetic blockade but is affected in a dose-response way by muscarinic blockers in humans (Martinmaki, Rusko, Kooistra, Kettunen, & Saalasti, 2006) and vagal cooling in animals (Katona & Jih, 1975). This has led to the use of RSA as a proxy for individual differences in cardiac vagal activity (Berntson et al., 1997; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996), although it must be acknowledged that differences in the sensitivity of the muscarinic receptor signaling pathway and differences in respiratory behavior can influence RSA independently of true differences in cardiac vagal activity (Grossman & Kollai, 1993; Grossman, Wilhelm, & Spoerle, 2004; Ritz & Dahme, 2006). RSA, therefore, is more appropriately considered a measure of cardiac vagal control, rather than of vagal activity.

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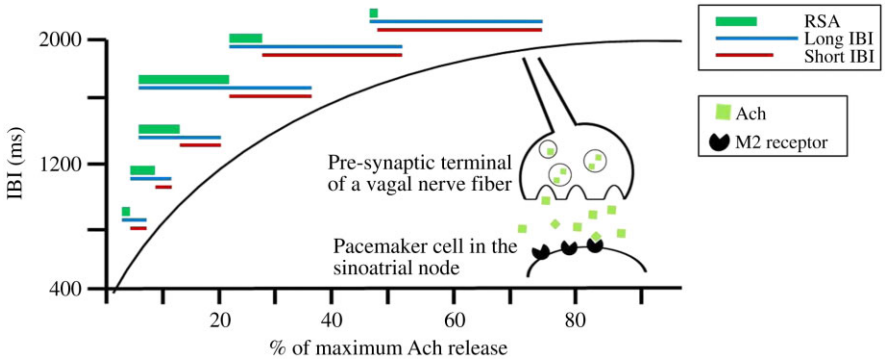

Figure 1. Graphic representation of the occurrence of a ceiling effect, showing that there is little room left for RSA at very long interbeat intervals (IBIs).

RSA can be measured directly from the combined electrocardiogram (ECG) and respiration signal as peak-valley RSA (pvRSA), but two HRV measures derived only from the interbeat interval (IBI) time series are also often used to index vagal control over the heart, namely, the standard deviation of all valid IBIs (SDNN) and the root mean square of differences between valid, successive IBIs (RMSSD). Age is a major source of interindividual differences in cardiac vagal control, with younger participants having higher RSA levels than older participants across the entire adolescent/adult age range (de Geus, Kupper, Boomsma, & Snieder, 2007; De Meersman & Stein, 2007; Quilliot, Fluckiger, Zannad, Drouin, & Ziegler, 2001; Vallejo, Marquez, Borja-Aburto, Cardenas, & Hermosillo, 2005). Sex, body mass index (BMI), and lifestyle variables like smoking and regular exercise have also been shown to affect RSA, although these effects are generally modest (de Geus et al., 2007; De Meersman & Stein, 2007; McNarry & Lewis, 2012; Sacknoff, Gleim, Stachenfeld, & Coplan, 1994; Umetani, Singer, McCraty, & Atkinson, 1998; Valentini & Parati, 2009; van Lien et al., 2011).

In contrast, a substantial portion of the interindividual variance in RSA appears to be due to genetic variation. Twin studies that record RSA in quiet resting conditions in the laboratory systematically reported a significant genetic contribution to RSA (Boomsma, van Baal, & Orlebeke, 1990; de Geus et al., 2007; Riese et al., 2006, 2007; Snieder, Boomsma, van Doornen, & de Geus, 1997; Tank et al., 2001; Tuvblad et al., 2010; Uusitalo et al., 2007; Wang et al., 2009; Wang, Thayer, Treiber, & Snieder, 2005; Zhang et al., 2007; Su et al., 2010). Heritability estimates at rest range from 25% to 71%. Ambulatory studies report heritabilities ranging from 35% to 65% (Busjahn et al., 1998; Kupper et al., 2004, 2005) and from 35% to 55% when confined to sleeping or sitting conditions only (Kupper et al., 2004, 2005). Estimates were very similar for European and African Americans (Wang et al., 2005), across the three different measures used (Goedhart, van der Sluis, Houtveen, Willemsen, & de Geus, 2007; Kupper et al., 2004), and none of these twin studies reported a sex difference in heritability or evidence for different genes being expressed in males and females.

Interestingly, three independent studies that measured cardiac vagal control at rest during a series of mental stressors all reported increased genetic variance in these measures under stress (de Geus et al., 2007; Riese et al., 2006; Wang et al., 2009). Compared to the heritability of resting baseline levels, the genetic contribution to the variance in measures of vagal control increased on average up to 10–20% when participants were exposed to various stress tasks. These findings have been taken to suggest that genetic influences on cardiac vagal control become more pronounced when the participant is challenged by mentally and emotionally "engaging" conditions, that is, they seem to provide evidence of Gene × Stress interaction (de Geus et al., 2007). This would lead to the prediction that RSA heritability estimates might vary across an ambulatory recording day, for instance, by being higher during daytime than at night. In addition, physical stressors may also give rise to higher genetic variance, as vagal withdrawal may not be as strong in each individual. Therefore, across the daytime, heritability of RSA measures may be lower during sitting conditions than during more physically active conditions.

Previous ambulatory twin studies have only indirectly addressed these questions and, importantly, failed to take into account the key observation that RSA can be paradoxically lowered at very low heart rates due to ceiling effects (Malik & Camm, 1993; van Lien et al., 2011). Normally, respiratory gating will result in a larger difference between the shortest IBI in inspiration and the longest IBI in expiration if tonic levels of vagal control over the heart are larger (Berntson et al., 1993; Eckberg, 2003). However, when cardiac vagal control is very high, a ceiling effect may prevent the lengthening of the IBI during expiration more than during inspiration (Malik & Camm, 1993). The biological basis of this ceiling effect is that high cardiac vagal control causes a large occupancy of the available muscarinic receptors on the SA node, and at this level of saturation any further increases in acetylcholine may no longer linearly increase the IBI as it would at low-to-moderate levels of cardiac vagal control (illustrated in Figure 1). As expiration is characterized by higher vagal control than inspiration, the beats during expiration suffer more strongly from the ceiling effect than beats during inspiration.

This ceiling effect is expected to cause a quadratic relationship between IBI and RSA at low heart rates. This quadratic shape of the IBI-RSA relationship has indeed been found in laboratory studies inducing variance in vagal control by phenylephrine and nitroprusside infusion (Goldberger, Challapalli, Tung, Parker, & Kadish, 2001). More recently, the occurrence of the quadratic shape was shown under naturalistic settings in 24-h recordings (van Lien et al., 2011). In a subset of 13 out of 52 participants, with half of them selected as being engaged in regular vigorous exercise, a ceiling effect on RSA was found during the nighttime. Based on these findings, it was hypothesized that cardiac vagal control in conditions of low heart rate levels will be underestimated by RSA in participants with a quadratic shape of the IBI-RSA relationship

compared to participants with a linear shape of the IBI-RSA relationship (van Lien et al., 2011). Here, we further hypothesize that the variation in the shape of the IBI-RSA curve may lead to an underestimation of the heritability of nighttime cardiac vagal control when it is based on RSA measures.

In this study, we aim to test whether heritability of RSA at night differs from that of daytime RSA during sitting and physically active conditions. For this, we use the largest sample with 24-h ambulatory cardiac recordings to date. We assessed the shape of the IBI-RSA relationship in 24-h ambulatory recordings of 486 monozygotic (MZ) twins, 517 dizygotic (DZ) twins, and 285 of their singleton siblings. Based on the scatter plots of the mean of RSA and IBI in 10-min bins, we made a qualitative distinction between linear IBI-RSA shapes showing no evidence of ceiling/saturation effects and quadratic IBI-RSA shapes, suggesting reduction of RSA at long IBIs through ceiling/saturation effects. First, heritability of three often-used RSA measures were computed during sleep and daytime by comparing MZ and DZ/sibling resemblance using the established twin methodology (Neale & Cardon, 1992). Next, heritability estimates of these RSA measures were re-estimated after removing the participants with evidence of ceiling effects. Furthermore, heritability of pvRSA was estimated after replacing the nighttime pvRSA values by (a) the maximal observed pvRSA at the peak of their quadratic IBI-RSA curve, and (b) the pvRSA value at the longest nighttime IBI obtained from extrapolating from the linear part of their IBI-RSA curve. Although these virtual maxima may still underestimate the true level of cardiac vagal control, they preserve all the participants for the analysis and may better capture the total genetic variance. The rationale behind this approach is akin to the correction of blood pressure in medicated participants by adding the average effect of the antihypertensive medication to the observed blood pressure. This was shown to preserve genetic variance compared to removing participants with medication (Cui, Hopper, & Harrap, 2003).

Our foremost goal was to establish the heritability of RSA measures across the 24-h period while accounting for ceiling effects on RSA at low heart rate levels during the night. We expected the heritability estimates for the nighttime RSA measures to be significantly moderated by the IBI-RSA shape, such that heritability of cardiac vagal control is underestimated unless the ceiling effect is taken into account. As a secondary goal, we tested the degree of overlap in the genetic factors influencing pvRSA, RMSSD, and SDNN, and their potential sensitivity to the ceiling effects. As these measures are all used to capture individual differences in cardiac vagal control, the expectation is that the genetic factors influencing them are highly correlated.

Method

Participants

Participants were all registered in the Netherlands Twin Register and took part in a large cardiac ambulatory monitoring project in which 24-h recordings were collected in three data collection waves. A priori reasons for exclusion were pregnancy, heart transplantation, presence of a pacemaker, and known ischemic heart disease, congestive heart failure, or diabetic neuropathy. We excluded data of 8 participants showing many arrhythmias. Valid ambulatory HRV recordings were available for 1,373 participants, of which 797 participated in the first two waves between 1998 and 2003. Sixty-seven of these participants took part in both waves, as part of a study on temporal stability of the ambulatory recordings (Goedhart, Kupper, Willemsen, Boomsma, & de Geus, 2006). In Wave 3, running between 2010 to 2012, the sample was further expanded with 576 new participants.

Data for 71 participants (75 recordings) were excluded due to the use of cardiovascular medication (beta blockers, ATC C07; cardiac therapy, ATC C01), or antidepressants (ATC N06A) at the time of the ambulatory assessment. Of the remaining 1,302 participants, 63 had duplicate ambulatory recordings from which we selected a single recording only. When the difference in the duration between the duplicate recordings was greater than or equal to 200 min, the shorter recording was excluded (12 recordings). Next, preference was given to the recordings of the data collection wave in which both twins participated, by excluding the recordings for the wave when only one of the twins participated (25 recordings excluded). For the remaining duplicate recordings, the recording of the wave in which most family members participated was retained (26 recordings excluded).

To simplify genetic modeling, we excluded the third member of triplets and included a maximum of two singleton brothers and two singleton sisters per family. Therefore, eleven participants from larger families were additionally excluded: the third member of a triplet ($N = 1$), nine siblings when more than two brothers and two sisters participated, and one twin who belonged to a second twin pair in the family. For the latter, we selected the siblings who were closest in age to the twins. We additionally excluded three participants who took part in the first two waves while all other family members participated in the third wave.

The final sample comprised 1,288 participants belonging to 624 families, with 486 MZ twins (210 complete pairs), 517 DZ twins (205 complete pairs), and 285 non-twin siblings. Mean age was 33.5 years ($SD = 9.2$ years), and 61.6% of the sample was female. Zygosity of the twins was determined by DNA typing for 97.9% of the same-sex twin pairs. For the remaining same-sex pairs, zygosity was based on survey questions on physical similarity and the frequency of confusion of the twins by parents, other family members, and strangers. Agreement between zygosity based on these items and zygosity based on DNA is 96.1% (Willemsen et al., 2013). The Medical Ethics Committee of the VU University Medical Center approved of the study protocol, and all participants gave written consent before entering the study.

Ambulatory Measurements of Heart Rate and Heart Rate Variability

For the first two data collection waves, the VU University Ambulatory Monitoring System (VU-AMS) version 4.6 was used (VU University, Amsterdam, The Netherlands, www.vu-ams.nl). This version of the VU-AMS continuously recorded the ECG and changes in thoracic impedance (dZ) from a six-electrode configuration (de Geus & van Doornen, 1996; de Geus, Willemsen, Klaver, & van Doornen, 1995; Riese et al., 2003). The device automatically detects each R wave in the ECG signal, at which it reads out and resets a millisecond counter to obtain the heart period time series. The thoracic impedance (Z), assessed against a constant current of 50 KHz, 350 microamperes, was amplified and led to a precision rectifier. The rectified signal was filtered at 72 Hz (low-pass) to give basal impedance Z. Filtering Z at 0.1 Hz (high-pass) supplied the dZ signal, which was band-pass filtered with 0.1 and 0.4 Hz cutoffs, after tapering with $(\sin(x))^2$, to yield the respiration signal.

The IBI time series was obtained from the ECG by an online automated R-wave peak detector, where IBI is the interval in milliseconds between two adjacent R waves of the ECG. Artifact

processing was performed on the IBI data offline. When the IBI deviated more than 3 *SD* from the moving mean of a particular period, it was automatically coded as an artifact, and the IBI was either rejected during visual inspection or new IBIs were created by summing too short IBIs, or too long IBIs were split in two IBIs of equal length.

For the third wave, the 5fs version of the VU-AMS was used, which improved on the 4.6 version in that it stores the entire ECG for offline analysis rather than online R-wave peak-detection (van Dijk et al., 2013). The ECG signal was imported into the VU-DAMS software (version 3.2, VU University Amsterdam, www.vu-ams.nl). After automated detection of bad ECG signal fragments (artifacts), R-wave peak detection was done using a modified version of the algorithm by Christov (Christov, 2004). From the R-wave peaks, the IBI time series was again constructed and visually displayed for interactive correction of missed or incorrect R-wave peaks. In addition to the ECG, the 5fs version also stores the entire dZ at 1000 Hz to obtain the respiration signal. The dZ signal is filtered using a second order band-pass filter that passes all frequencies in the range of 0.1 to 0.4 Hz. An exponential smoothing average technique is then applied on the filtered DZ signal, which acts as an additional low-pass filter. The output of this filter is a weighted combination of previous smoothed value and the newest measured data, or in formula:

$$S_t = \alpha * S_{t-1} + (1-\alpha) * x_t$$

where S_t is the smoothed average, α is the tunable smoothing factor (which is in the range of 0 to 1), x_t is the observation at time t, and S_{t-1} is the previous smoothed value.

Computation of the RSA measures was done in the same way for all three waves. Combining the IBI time interval series with the respiration signal extracted from the thorax impedance signal (dZ), the "peak-valley" RSA method was used to assess pvRSA (de Geus et al., 1995; Grossman, van Beek, & Wientjes, 1990; Grossman & Wientjes, 1986). In this method, RSA is scored from the combined respiration and IBI time series by detecting the shortest IBI during inspiration and the longest IBI during expiration on a breath-to-breath basis according to the procedures detailed elsewhere (de Geus et al., 1995; Houtveen, Groot, & de Geus, 2005; van Lien et al., 2011). Breathing cycles that showed irregularities like gasps, breath holding, and coughing were considered invalid and were removed from further processing. If no shortest or longest IBI could be detected in inspiration and expiration, respectively, the breath was either set to missing or to zero when computing the average per condition for pvRSA. Similar results were found for pvRSA computed either way, and we employed only one (breaths set to missing) in further statistical analyses. The two other measures of RSA were derived from the IBI time series by taking the standard deviation of all valid IBIs (SDNN) and the root mean square of differences between valid, successive IBIs (RMSSD):

$$RMSSD = \sqrt{\frac{1}{n-1}\sum_{i=2}^{i=n}\left(IBI_i - IBI_{(i-1)}\right)}$$

Procedure

Participants were visited at home, before starting their normal daily activities. During a short interview, information on health status and current medication use was obtained. The VU-AMS was attached and its operation explained. Participants were instructed to wear the device the entire day and night up until awakening the next morning. Instructions were supplied that explained how to respond to potential alarm beeps (e.g., on loose electrode contacts), and telephone assistance was available during waking hours. Participants were requested to keep a diary and to write down a chronological account of activity, posture, location, and social situation over the time period. For Wave 1 and 2, this was done every 30 min, for Wave 3 every 60 min. Participants were instructed to refrain from vigorous exercise during the ambulatory recording day.

Data Reduction

Using the activity diary entries in combination with a visual display of the output of an inbuilt accelerometer (measuring movement), the entire 24-h recording was divided into fixed periods. These periods were coded for posture (supine, sitting, standing, walking, bicycling), activity (e.g., desk work, dinner, meetings, watching TV), and physical load (no load, light, intermediate, and heavy). Minimum duration of periods was 5 min and maximum duration was 1 h. If periods with similar activity and posture lasted more than 1 h (e.g., during sleep), they were divided into multiple periods of maximally 1 h. All periods were classified into three main ambulatory conditions: (1) lying asleep, (2) sitting during the day, or (3) mild physical activity (e.g., standing/walking) based on the dominant posture/activity reported in that period; the exact timing of changes in posture/activity was verified using the accelerometer signal from the ambulatory device.

To determine the shape of the relationship between IBI and pvRSA, we divided the entire 24-h recording into bins no longer than 10 min, thereby making a distinction between waking and sleeping periods. For the majority of the bins (83%), condition within the bin was uniform. The other bins did not fall within a single condition because it was not always determined for the entire bin, or the bin covered more than one condition. The mean IBI and pvRSA were determined per bin and the correlation across these IBI and pvRSA means was depicted in a separate scatter plot for each of the participants in the study. Four examples of the IBI-RSA relationship are shown in Figure 2 (full set of scatter plots available upon request from the corresponding author). Significance of the regression weights ($\beta 1$ and $\beta 2$) in the linear and quadratic terms was tested by the SPSS CURVEFIT procedure. First, automated classification of the shape was used. To be classified as quadratic, the $\beta 2$ parameters had to be significantly different from zero, the quadratic solution had to explain 20% of the variance in RSA, and the quadratic solution had to improve on the linear solution by at least 10% additional explained variance. Two human raters (MN and GW) independently verified this algorithmic classification of the scatter plots by visual inspection; a third rater (EG) resolved remaining discrepancies. For all participants, the intercept and standardized beta (daytime_slope) of the IBI-RSA curve was assessed in the waking part of the data, where it was found to be nonquadratic in all participants.

For IBI, respiration rate, and the three HRV measures (SDNN, RMSSD, pvRSA), a mean value was computed across the sleep, sitting, and physically active periods in the recordings. In addition, two separate measures were computed to index maximal cardiac vagal control during the night. First, the median value of the six 10-min bins with the highest pvRSA value during the night (pvRSAmax) was obtained. For participants without ceiling effects, these bins occurred mostly around the end of the night. For participants with a ceiling effect, the highest pvRSA values were obtained in an earlier phase of the night, corresponding to the

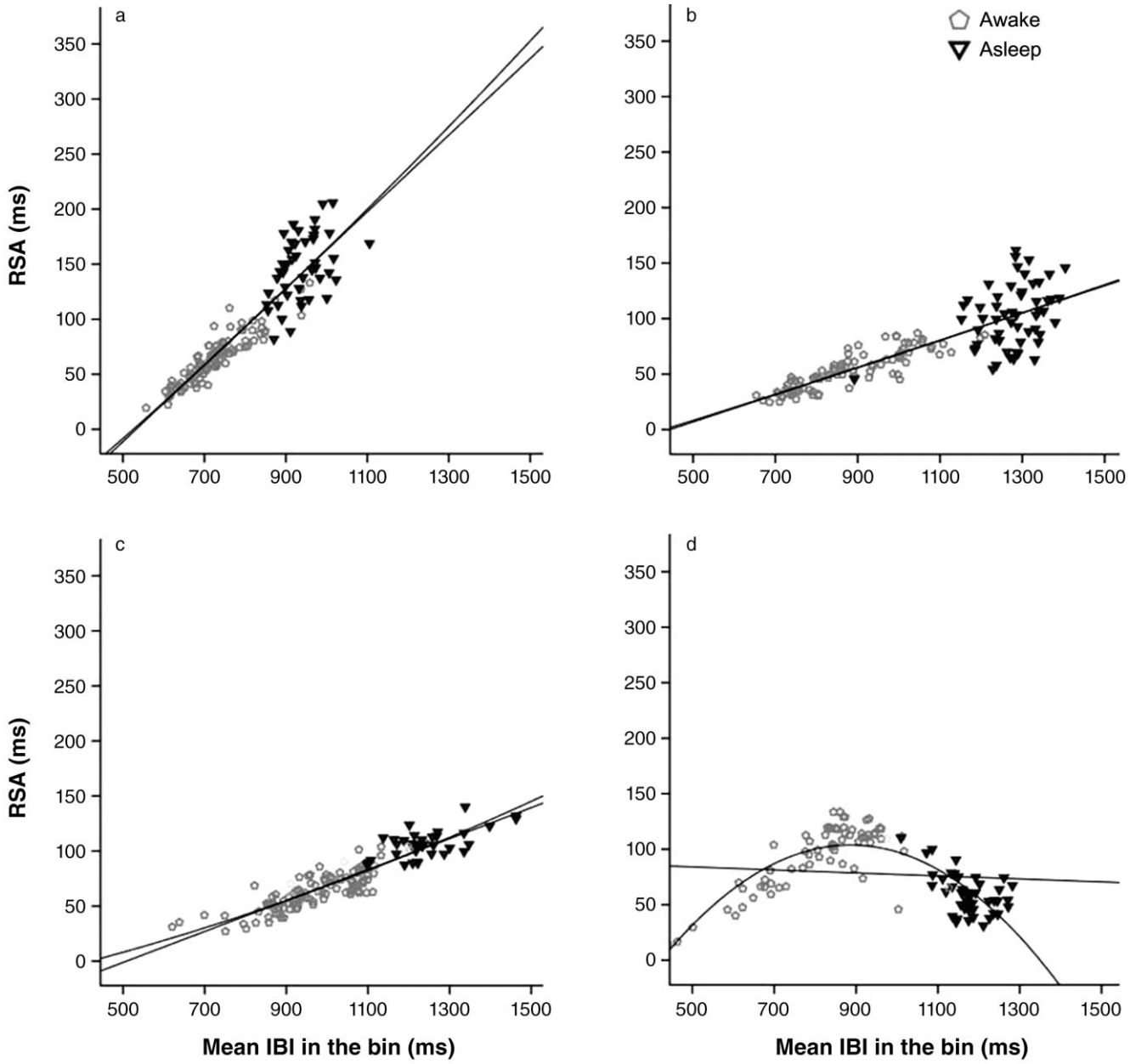

Figure 2. Representative IBI-RSA curves of three participants without ceiling effects (a, b, c) and one participant with ceiling effects (d). The lines represent the best fitting linear and quadratic function for each participant.

moment of occurrence of the peak in the quadratic IBI-RSA curve. Second, a virtual pvRSAmax (pvRSAmax_virtual) was calculated on the basis of the intercept and slope of the daytime IBI-RSA association during sitting activities, by extrapolating the pvRSA to the value that would have been obtained at the nighttime 10-min bin with the longest IBI value (IBImax in seconds) by the following formula:

$$\text{pvRSAmax\_virtual} = \text{intercept} + (\text{daytime\_slope} * \text{IBImax}).$$

The rationale behind this virtual value is that, by extrapolating the observed daytime IBI-RSA relationship to nighttime RSA, an RSA value may be obtained that may prove a valid alternative to excluding data of participants showing ceiling effects.

Statistical Analyses

SDNN and IBImax showed a continuous normal distribution. A logN (LN) transformation (for pvRSAmax, pvRSAmax_virtual, pvRSA, and RMSSD) or a squared transformation (for daytime_slope) was applied to obtain a normal distribution.

Group and Condition Effects

We used a mixed model ANOVA (IBM SPSS 20.0) and included age, sex, and respiration rate (the latter for pvRSA and RMSSD only) as covariates and family as a random factor. Respiration rate was only included as a covariate in the analyses for pvRSA and RMSSD, because SDNN does not specifically capture HRV related

to cardiorespiratory coupling. We tested the fixed effects of group (ceiling vs. no ceiling), ambulatory condition (sleep, sitting, active) and the Group × Condition interaction. Significant interaction was followed by post hoc tests of the ceiling effect within each of the ambulatory conditions. Mixed model ANOVA was also used to test the effects of group on the two alternative measures of maximal pvRSA at nighttime, pvRSAmax and pvRSAmax_virtual. Effects were considered significant when $p < .01$.

Genetic Analyses

In a twin study, the observed variance can be decomposed into four possible sources of variance: variance due to additive genetic effects (A), nonadditive genetic effects (D), common environment (C) shared by family members, and nonshared or unique environment (E) (Boomsma & Gabrielli, Jr., 1985). However, in a design that includes identical twins, fraternal twins, and sibling pairs, estimates of C and D are confounded, and the observed variances and covariances only provide sufficient information to model either an ACE model or an ADE model, but not both. Based on the pattern of twin and sibling correlations, we chose to model A, C, and E. For identical twins, fraternal twins, and sibling pairs alike, common environmental factors are correlated 1.0. Genetic factors are correlated 0.5 in siblings and DZ twins, and 1.0 in MZ twins. By definition, nonshared, or unique, environmental factors are uncorrelated in family members.

To answer the question to what extent A, C, and E factors contribute to the variance in the RSA measures, biometrical genetic models were fitted to the observed data using the structural equation modeling program Mx (Neale, Boker, Xie, & Maes, 2006). First, fully saturated models were fitted for each variable separately. In these fully parameterized models, means and variances were estimated freely for both sexes. Then, we tested for sex differences in means and variances by constraining these to be equal for males and females and tested whether these more constrained models led to a significant worse fit to the data. Next, we tested for heterogeneity of correlations of males versus females and of fraternal twins versus singletons. The resulting most parsimonious saturated model indicated to what extent we could limit the specification of the variance components models.

As the individual differences in the ambulatory RSA measures were expected to be sensitive to three main confounding variables, differences in age, sex, and respiration rate (de Geus et al., 2007; De Meersman & Stein, 2007; Eckberg, 2003; Quilliot et al., 2001; Umetani et al., 1998; Vallejo et al., 2005); the above models specifically regressed the effects of sex and age on RSA. Respiration rate was additionally included as a covariate for pvRSA and RMSSD.

Furthermore, we tested for effects of the version of the ambulatory recording device by comparing the means and variances of the three waves. In keeping with the highly similar strategies used to obtain the RSA measures, no device version effect was found so that all three waves were pooled during all genetic modeling. To examine whether the genetic architecture of the RSA measures changed from nighttime to daytime and, within the daytime, from sitting only to more physical active activities, full trivariate genetic ACE models in Cholesky decomposition were fitted to the mean values for the three RSA measures separately for the nighttime sleep, daytime sitting, and daytime physically active periods. Because the MZ twin correlations were always at least twice as high as the DZ and non-twin sibling correlations, it is more likely that familial resemblance derives from genetic factors rather than from shared environmental influences. The ACE model was therefore tested against the nested AE model only. The resulting most parsimonious model was used to further test the source of the observed covariance in the different HRV measures. Figure 3 depicts a schematic representation of the full trivariate genetic model that was fitted to the data.

The significance of A, C, and E factors was tested by comparing the fit of the more parsimonious nested models to the fit of the full model using the likelihood ratio (χ^2) test in which the difference in minus twice the logarithm of the likelihood (−2LL) was calculated. When the χ^2 test was significant ($p < .01$), the more parsimonious model was considered to fit significantly worse to the data than the fuller model it was tested against. In addition, Akaike's Information Criterion (AIC = χ^2 − 2df) (Akaike, 1987) was calculated for each model, which offers a quick approach to judging the fit of nested models. Those with lower values fit better than models with higher values. For more background information on the heritability estimation procedures, see de Geus (2010).

To test whether the heritability estimates were significantly affected by participants whose data showed ceiling effects, the trivariate genetic analyses were repeated excluding those participants. As an alternative to correct for a potential underestimation of the heritability of nighttime vagal control, two additional genetic analyses were performed on pvRSAmax and pvRSAmax_virtual, and it was tested whether the heritability estimates for these alternative measures were higher compared to the estimates obtained for uncorrected nighttime pvRSA.

Results

In 52 participants, no valid nighttime recording of the RSA measures was obtained for at least five 10-min bins. Analyses of the scatter plots of mean RSA and IBI across the 24-h period in the remaining 1,236 participants showed a significant quadratic relationship in 132 participants (10.7%). From Table 1, it can be seen that the nighttime pvRSA and RMSSD may be underestimated in these participants. Mixed ANOVA analysis with correction for family relatedness showed a significant Group × Condition interaction for pvRSA, $F(2,3061) = 36.20$, $R^2 = 0.64$, $p < .01$; and RMSSD, $F(2,3063) = 17.30$, $R^2 = 0.30$, $p < .01$; but not for SDNN, $F(2,3056) = 3.08$, $R^2 = 0.04$, $p = .046$. Whereas participants with a ceiling effect due to a quadratic IBI-RSA curve have a significantly longer IBI throughout the entire recording (hinting at higher cardiac vagal control) compared to the participants without a ceiling effect, their pvRSA and RMSSD are only higher during the two daytime conditions but not at night. Nighttime SDNN appears less affected by the ceiling effect, although the group differences in HRV values during the night were also less pronounced compared to the daytime conditions. Previously, a similar pattern was observed by our group (van Lien et al., 2011).

Using the maximal observed pvRSA at night still suggests that the participants with a ceiling effect have comparable cardiac vagal control ($F_{group} = 0.29$, $R^2 = 0.10$, $p = .588$) in spite of their longer nighttime IBI. Only when the linear relationship between RSA and IBI was extrapolated to the maximal IBI (pvRSAmax_virtual) was a higher value found in the ceiling group ($F_{group} = 10.47$, $R^2 = 0.84$, $p < .01$). Daytime_slope for the participants with a ceiling effect was steeper compared to the daytime_slope of the participants without a ceiling effect ($F_{group} = 12.19$, $R^2 = 0.73$, $p < .01$).

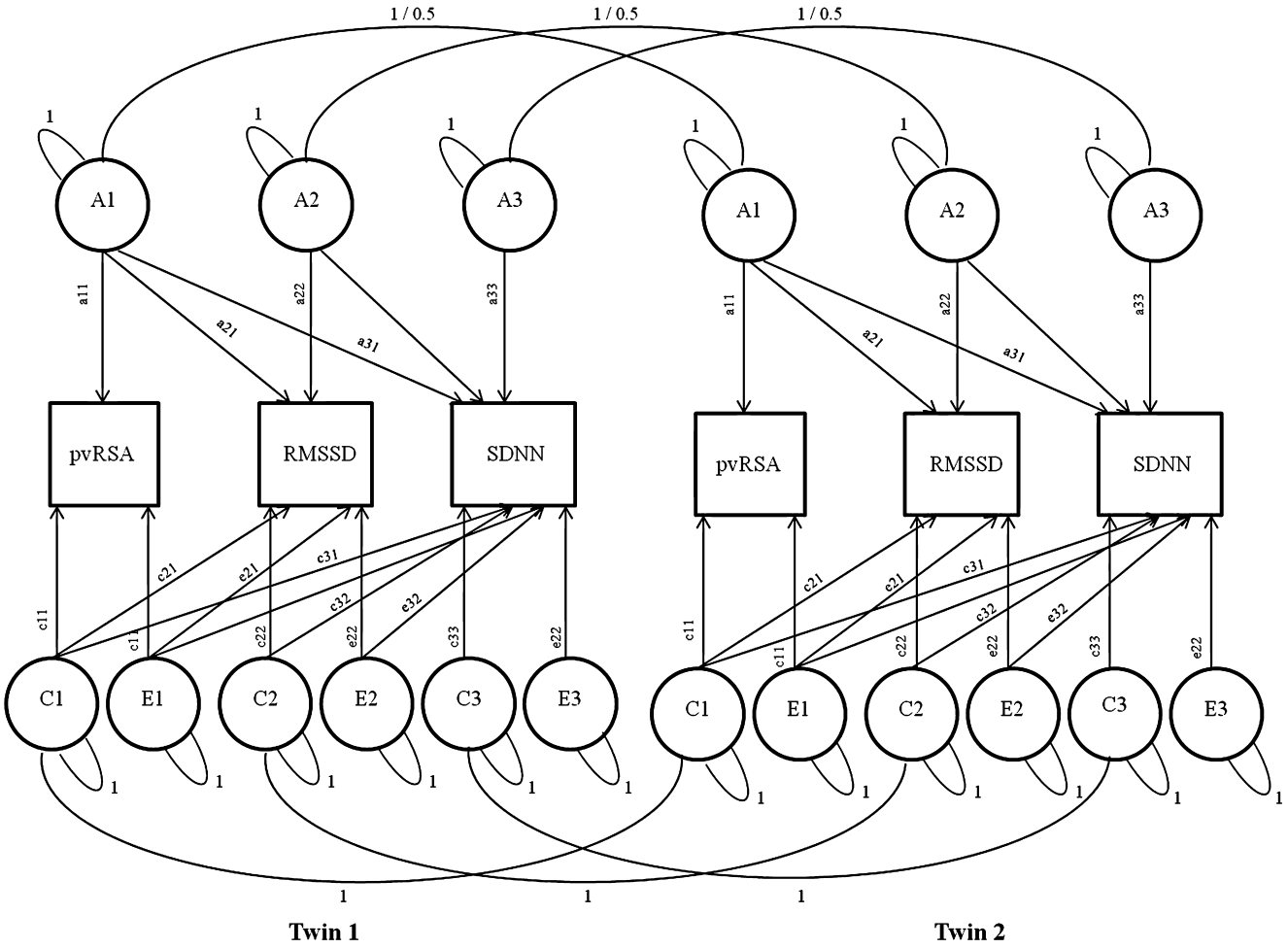

Figure 3. Example of a path model decomposing trait variance into additive genetic (A), and shared (C) and unique environmental (E) factors in one twin pair. Unique A, C and E factors load on all three HRV measures. Additionally, A, C and E factors can be shared between the phenotypes. This is depicted by the "a," "c" and "e" paths running from the former A, C and E factors of a HRV phenotype to the next (a21, a31, a32, c21, c31, c32, and e21, e31 and e32). With these paths, the genetic and environmental covariance can be studied. Finally, MZ twins correlate 1.0 regarding the A factor scores because they are assumed to share all of their genetic material, whereas DZ or sibling pairs correlate 0.5.

Twin-Sibling Resemblance and Heritability

Table 2 shows the twin and sibling correlations for all measures. From the pattern of the correlations, it is clear that there is a substantial genetic contribution to all measures. The overarching pattern seen is that DZ and sibling correlations are about half of the MZ correlations, with the exception of the male DZ correlations, which are low and suggestive of nonadditivity. Formal testing, however, showed that the DZ twin and non-twin sibling correlations were homogeneous (all *p*s > .01) and could be equalized in further model fitting. Correlations of MZ males and MZ females also did not differ significantly, nor did the same-sex DZ and opposite-sex DZ twin and non-twin sibling correlations (all *p*s > .01), so no quantitative or qualitative sex differences were modeled.

Table 3a shows the best trivariate models for pvRSA, RMSSD, and SDNN separately for the sleep, sitting, and physically active conditions. An AE model provided the best fit. Heritability of pvRSA was lower at night than during the day, but, as can be seen from the confidence intervals, the heritability estimates were not significantly different during sleep, sitting, and active periods. For RMSSD and SDNN, heritability was also very comparable across all periods.

Genetic and Unique Environmental Correlations

Table 4a shows significant phenotypic correlations between the various HRV measures in all three periods. Generally, the phenotypic, the genetic, and the unique environmental correlations between pvRSA and RMSSD, and RMSSD and SDNN, were higher compared to the phenotypic, genetic, and unique environmental correlations between pvRSA and SDNN for the entire recording time. A single genetic and unique environmental factor influenced all three RSA measures, but RMSSD and SDNN were also influenced by independent genetic and unique environmental factors that did not affect pvRSA. The observed correlation between the three possible dyads of the RSA measures was for 51% to 56% attributable to shared genetic factors, and for 44% to 49% attributable to shared unique environmental factors.

Correcting for Ceiling Effects on Nighttime RSA

To test the impact of the ceiling effects on the RSA measures on the heritability of cardiac vagal control, we repeated the above trivariate analysis after excluding participants with the ceiling effects. Results are depicted in Table 3b. No significant changes in

Table 1. *Means (Standard Deviation) for IBI, pvRSA, RMSSD, SDNN, daytime_slope, pvRSAmax, and pvRSAmax_virtual by Ceiling Status and Ambulatory Condition*

Variable	No ceiling effects (1,100 < *N* < 1,104)	Ceiling effects (*N* = 132)	Group difference
IBI (ms)			
Sleep	975.34 (125.42)	1053.08 (165.02)	77.74*
Sitting	804.93 (102.13)	844.62 (115.90)	39.69*
Active	712.60 (87.53)	741.08 (100.49)	28.48*
pvRSA (ms)			
Sleep	56.10 (24.43)	54.05 (26.32)	–2.05
Sitting	44.49 (17.22)	58.18 (21.98)	13.69*
Active	35.24 (12.63)	44.96 (14.72)	9.72*
RMSSD (ms)			
Sleep	53.08 (25.58)	60.31 (31.88)	7.23
Sitting	36.03 (16.61)	51.24 (24.83)	15.21*
Active	29.54 (12.85)	40.24 (18.65)	10.70*
SDNN (ms)			
Sleep	91.49 (26.90)	104.21 (31.37)	12.72*
Sitting	68.92 (19.80)	86.82 (25.01)	17.90*
Active	81.73 (21.34)	98.26 (24.89)	16.53*
daytime_slope	108.50 (64.17)	110.84 (65.86)	2.34*
pvRSAmax (ms)	86.03 (34.83)	89.21 (33.42)	3.18
pvRSAmax_virtual (ms)	73.31 (32.45)	89.62 (39.92)	16.31*

Note. All variables were corrected for family relatedness, age, and sex. pvRSA, RMSSD, pvRSAmax, and pvRSAmax_virtual values were additionally corrected for respiration rate. IBI = interbeat interval; pvRSA = peak-valley respiratory sinus arrhythmia; RMSSD = root mean square of differences between valid successive IBIs; SDNN = standard deviation of all valid IBIs.
*Significant main effect of ceiling (within ambulatory condition) ($p < .01$).

heritability estimates were noticeable after excluding participants with ceiling effects.

Likewise, excluding the participants with ceiling effects did not significantly change the phenotypic, the genetic, and the unique environmental associations between the RSA measures, nor was a meaningful change observed in the genetic and unique environmental contribution to their covariance (see Table 4b).

Alternative Measures of Nighttime Cardiac Vagal Control

As an alternative measure of nighttime cardiac vagal control, which might be less sensitive to the impact of ceiling effects, we used the median value of the six 10-min bins with the highest RSA value in all participants (Table 5a). This led to heritability estimates that were higher (5%), but not significantly different from those for the pvRSA during the entire sleep period (Table 3a).

A second alternative replaced the nighttime pvRSA values by the estimated pvRSAmax (pvRSAmax_virtual) value based on the daytime linear association of RSA and IBI and extrapolating to the bin with the longest IBI at night (Table 5b). The heritability estimate now increased 7% compared to the uncorrected nighttime pvRSA measure, but this increase was again not significant as confidence intervals still overlapped. For completeness, Table 5c

Table 2. *Twin and Sibling Correlations as Estimated from the Full Saturated Model*

	MZ twins		DZ/sibs male		DZ/sibs female		Opposite sex sibs	
	MZM	MZF	DZM	twin/sib-sib	DZF	twin/sib-sib	DOS	twin/sib-OS sib
SDNN/sleep[1]	0.70	0.56	0.17	0.09	0.17	0.21	0.45	0.19
SDNN/sitting[1]	0.64	0.55	–0.07	0.24	0.49	0.30	0.27	0.38
SDNN/active[1]	0.65	0.56	0.01	0.31	0.46	0.36	0.35	0.32
RMSSD/ sleep[2]	0.74	0.60	0.15	0.31	0.17	0.27	0.43	0.29
RMSSD/sitting[2]	0.58	0.61	0.01	0.34	0.40	0.32	0.26	0.31
RMSSD/active[2]	0.57	0.50	0.10	0.40	0.27	0.31	0.28	0.30
pvRSA/sleep[2]	0.69	0.56	0.22	0.26	0.31	0.34	0.34	0.29
pvRSA/sitting[2]	0.60	0.65	0.07	0.35	0.44	0.33	0.28	0.23
pvRSA/active[2]	0.68	0.63	0.07	0.46	0.37	0.40	0.35	0.19
pvRSAmax[2]	0.64	0.64	0.25	0.24	0.30	0.35	0.25	0.28
pvRSAmax_virtual[2]	0.63	0.72	0.19	0.32	0.44	0.29	0.33	0.34
IBImax[1]	0.53	0.55	0.17	0.27	0.38	0.27	0.31	0.27
daytime_slope[1]	0.46	0.48	–0.06	0.21	0.26	0.25	0.20	0.24

Note. SDNN = standard deviation of all valid interbeat intervals (IBIs); RMSSD = root mean square of differences between valid successive IBIs; pvRSA = peak-valley respiratory sinus arrhythmia; pvRSAmax = median value of the six 10-min bins with the highest pvRSA value during the night; IBImax = nighttime 10-min bin with the longest IBI value; daytime_slope = slope of the IBI-RSA curve in the waking part of the data; pvRSAmax_virtual = nonobserved experimental variable that extrapolates the pvRSA from the intercept and slope of the daytime IBI-RSA association during sitting activities to the value that would have been obtained at the longest IBI value (IBImax); MZM = monozygotic male, MZF = monozygotic female; DZM = dizygotic male, DZF = dizygotic female, DOS = dizygotic opposite sex, sib = non-twin sibling.
[1]corrected for age, sex. [2]corrected for age, sex, respiration rate.

Table 3. *Proportion of Variance and Confidence Intervals (CI) Due to A and Due to E*

3a. *Full sample* (1,232 < *N* < 1,288)

Condition	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	Phenotype	A	E
Sleep	1	3669	ACE	3632.224	10970.224							
	2	3675	AE	3620.358	10970.358	1	0.135	6	1	pvRSA	0.48 (0.37–0.57)	0.52 (0.43–0.63)
										RMSSD	0.53 (0.43–0.61)	0.47 (0.39–0.57)
										SDNN	0.46 (0.36–0.56)	054 (0.44–0.65)
Sitting	1	3831	ACE	2488.129	10150.129							
	2	3837	AE	2477.869	10151.869	1	1.740	6	.942	pvRSA	0.53 (0.44–0.62)	0.47 (0.38–0.56)
										RMSSD	0.54 (0.45–0.62)	0.46 (0.38–0.55)
										SDNN	0.48 (0.39–0.57)	0.52 (0.43–0.61)
Active	1	3834	ACE	2830.663	10498.663							
	2	3840	AE	2823.792	10503.792	1	5.129	6	.527	pvRSA	0.57 (0.48–0.65)	0.43 (0.35–0.52)
										RMSSD	0.49 (0.40–0.57)	0.51 (0.43–0.60)
										SDNN	0.53 (0.45–0.60)	0.47 (0.40–0.55)

Note. *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; phenotype = specification of the HRV parameter; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model; pvRSA = peak-valley respiratory sinus arrhythmia; RMSSD = root mean square of differences between valid successive interbeat intervals (IBIs); SDNN = standard deviation of all valid IBIs.

3b. *Excluding the Participants with a Ceiling Effect (1,100 < N < 1,104)*

Condition	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	Phenotype	A	E
Sleep	1	3273	ACE	3144.299	9690.299							
	2	3279	AE	3132.299	9690.299	1	0	6	1	pvRSA	0.53 (0.42–0.63)	0.47 (0.37–0.58)
										RMSSD	0.53 (0.42–0.63)	0.47 (0.38–0.58)
										SDNN	0.47 (0.35–0.57)	053 (0.43–0.65)
Sitting	1	3282	ACE	2017.696	8581.696							
	2	3288	AE	2006.158	8582.158	1	0.462	6	.998	pvRSA	0.57 (0.46–0.66)	0.43 (0.34–0.54)
										RMSSD	0.52 (0.41–0.61)	0.48 (0.39–0.59)
										SDNN	0.46 (0.35–0.56)	0.54 (0.44–0.65)
Active	1	3282	ACE	2327.274	8891.274							
	2	3288	AE	2327.386	8903.386	1	12.112	6	.060	pvRSA	0.57 (0.47–0.66)	0.43 (0.34–0.53)
										RMSSD	0.46 (0.35–0.55)	0.54 (0.45–0.65)
										SDNN	0.54 (0.44–0.62)	0.46 (0.38–0.56)

Note. *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; phenotype = specification of the HRV parameter; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model; pvRSA = peak-valley respiratory sinus arrhythmia; RMSSD = root mean square of differences between valid successive interbeat intervals (IBIs); SDNN = standard deviation of all valid IBIs.

and 5d also present the heritability of the daytime_slope and (nighttime) IBImax.

Discussion

Using a twin family design, this paper shows that genetic factors explain around half of the individual differences in ambulatory cardiac vagal control as measured by pvRSA, RMSSD, or SDNN. Our expectation that the heritability estimates for the nighttime RSA measures would be significantly affected by the IBI-RSA shape, such that heritability of cardiac vagal control is underestimated at low heart rates at nighttime, was not supported by the data. In spite of significant changes in mean RSA values across the 24-h period, there was no evidence for differences in heritability of cardiac vagal control at night compared to daytime conditions, and, during daytime conditions, heritability was very comparable between sitting-only conditions and conditions in which participants were physically active. The genetic factors influencing ambulatory pvRSA, RMSSD, and SDNN largely overlapped, providing support for the idea that pvRSA, RMSSD, and SDNN capture the same biological phenomenon. Nonetheless, the overlap in genetic factors influencing pvRSA and RMSSD was higher than the overlap in the genetic factors shared between pvRSA and SDNN.

Earlier twin studies on the heritability of HRV were mainly based on laboratory recordings, and the heritability estimates that were found varied substantially between conditions and across the different HRV measures that were used. Laboratory studies reported heritability estimates of pvRSA in resting conditions between 25% to 39% (Boomsma et al., 1990; de Geus et al., 2007; Snieder et al., 1997; Tuvblad et al., 2010). Estimates for resting RMSSD values in the laboratory ranged from 36% to 71% (Uusitalo et al., 2007; Wang et al., 2005, 2009). Only one laboratory study researched resting SDNN and estimated heritability to be 66% (Wang et al., 2005). These resting laboratory levels can be best approximated by the sitting-only condition in our sample. Heritability of sitting-only pvRSA was estimated at 53%, RMSSD at 54%, and SDNN at 48%.

During (mental) stress, laboratory studies reported increases in heritability of pvRSA and RMSSD of 8% to 23% when compared to heritability estimates of resting levels (de Geus et al., 2007; Wang et al., 2009), suggesting that more genetic variance is expressed under conditions in which participants were aroused. This Gene × Stress interaction that was observed in controlled

Table 4. *Phenotypic and Genetic Correlation Between Dyads of RSA Measures and % of Covariance Explained by Overlapping Genetic Factors*

4a. *Full sample (1,232 < N < 1,288)*

	Phenotypic correlation	Genetic correlation	Contribution of A to the phenotypic covariance	Unique environmental correlation	Contribution of E to the phenotypic covariance
Sleep					
pvRSA–RMSSD	0.82 (0.80–0.84)	0.85 (0.80–0.90)	52% (41–62%)	0.79 (0.74–0.83)	48% (38–59%)
RMSSD–SDNN	0.84 (0.82–0.85)	0.87 (0.82–0.91)	51% (40–61%)	0.81 (0.76–0.85)	49% (39–60%)
pvRSA–SDNN	0.57 (0.53–0.61)	0.63 (0.50–0.74)	52% (37–65%)	0.51 (0.42–0.60)	48% (35–63%)
Sitting					
pvRSA–RMSSD	0.89 (0.88–0.91)	0.94 (0.91–0.96)	56% (47–65%)	0.85 (0.81–0.87)	44% (35–53%)
RMSSD–SDNN	0.86 (0.84–0.87)	0.89 (0.84–0.92)	53% (43–62%)	0.83 (0.79–0.86)	47% (38–57%)
pvRSA–SDNN	0.70 (0.67–0.73)	0.74 (0.65–0.81)	54% (42–64%)	0.66 (0.59–0.72)	46% (36–58%)
Active					
pvRSA–RMSSD	0.86 (0.84–0.87)	0.91 (0.87–0.94)	56% (46–64%)	0.80 (0.76–0.84)	44% (36–54%)
RMSSD–SDNN	0.81 (0.79–0.83)	0.84 (0.79–0.88)	53% (43–61%)	0.78 (0.73–0.82)	47% (39–57%)
pvRSA–SDNN	0.68 (0.65–0.71)	0.66 (0.58–0.73)	53% (42–63%)	0.71 (0.65–0.77)	47% (37–58%)

Note. pvRSA = peak-valley respiratory sinus arrhythmia; RMSSD = root mean square of differences between valid successive interbeat intervals (IBIs); SDNN = standard deviation of all valid IBIs.

4b. *Participants with Ceiling Effects Excluded (1,100 < N < 1,104)*

	Phenotypic correlation	Genetic correlation	Contribution of A to the phenotypic covariance	Unique environmental correlation	Contribution of E to the phenotypic covariance
Sleep					
pvRSA–RMSSD	0.82 (0.80–0.84)	0.85 (0.78–0.89)	55% (43–65%)	0.79 (0.73–0.83)	45% (35–57%)
RMSSD–SDNN	0.83 (0.81–0.85)	0.87 (0.81–0.92)	52% (40–63%)	0.80 (0.74–0.84)	48% (37–60%)
pvRSA–SDNN	0.57 (0.53–0.61)	0.63 (0.50–0.74)	55% (39–69%)	0.52 (0.41–0.61)	45% (31–61%)
Sitting					
pvRSA–RMSSD	0.90 (0.89–0.91)	0.94 (0.91–0.96)	57% (45–66%)	0.85 (0.81–0.89)	43% (34–55%)
RMSSD–SDNN	0.85 (0.83–0.86)	0.88 (0.83–0.92)	51% (39–61%)	0.81 (0.77–0.85)	49% (39–61%)
pvRSA–SDNN	0.70 (0.66–0.73)	0.75 (0.65–0.82)	55% (41–66%)	0.65 (0.57–0.73)	45% (34–59%)
Active					
pvRSA–RMSSD	0.86 (0.84–0.87)	0.92 (0.88–0.95)	55% (43–65%)	0.80 (0.75–0.84)	45% (35–57%)
RMSSD–SDNN	0.80 (0.78–0.82)	0.82 (0.76–0.87)	51% (39–61%)	0.79 (0.73–0.83)	49% (39–61%)
pvRSA–SDNN	0.68 (0.64–0.71)	0.63 (0.54–0.71)	52% (39–63%)	0.73 (0.66–0.79)	48% (37–61%)

Note. pvRSA = peak-valley respiratory sinus arrhythmia; RMSSD = root mean square of differences between valid successive interbeat intervals (IBIs); SDNN = standard deviation of all valid IBIs.

laboratory situations was not recaptured by a nighttime–daytime effect. Instead, the genetic variance was rather stable across the 24-h period. This finding was supported by previous analyses within a subset of the present sample in which we did not observe significant differences in heritability estimates across different times of day (Kupper et al., 2004, 2005). This raises the important but possibly misleading question of which of these two paradigms is "right." The ambulatory situation is by definition a less standardized setting compared to the laboratory setting in which physical activity and postures can be carefully controlled. On the other hand, ambulatory 24-h measurements in real life may give a better reflection of the actual day-to-day situation of participants and thereby capture daily life better. Cardiac recordings in an ambulatory setting presumably also contribute more reliably to risk prediction compared to a recording in the laboratory, which is generally shorter and more sensitive to momentary or lab-specific influences (e.g., the white coat effect; Zanstra & Johnston, 2011). Although not tested here, it would be interesting to elaborate on this topic and explore different operationalizations of real-life reactivity in future research.

Previous research has already shown that RSA can be reliably measured under naturalistic conditions with the use of ambulatory monitoring (de Geus et al., 1995; Wilhelm, Roth, & Sackner, 2003). Such recordings yield large individual differences in RSA that appear to reflect stable trait characteristics. For the average 24-h levels of RSA, high test-retest correlations ($.63 < r < .90$) have been found after 3 to 65 days in both healthy individuals and cardiac patients (Bigger, Jr., et al., 1992; Hohnloser, Klingenheben, Zabel, Schroder, & Just, 1992; Kleiger et al., 1991; Sinnreich, Kark, Friedlander, Sapoznikov, & Luria, 1998; Stein, Rich, Rottman, & Kleiger, 1995), and moderate-to-high long-term temporal stability ($.58 < r < .76$) has been shown over periods of 7 months to 3.4 years (Goedhart et al., 2007; Pitzalis et al., 1996). Our results corroborate the stability of these individual differences and suggest that they have a genetic basis.

In keeping with the substantial genetic contribution found across different samples and the proven reliability of the assessment of RSA over time, several studies have tried to identify the actual genetic variants underlying RSA heritability. These mainly concerned candidate gene studies, in which a few genes were selected based on their known involvement in processes leading to differences in HRV levels. Candidate gene studies have found the angiotensin-converting enzyme (ACE; Busjahn et al., 1998), alpha-kinase anchoring protein 10 (AKAP 10; Neumann et al., 2009; Tingley et al., 2007), methylene-tetrahydrofolate reductase (MTHFR; Baccarelli et al., 2008), the circadian clock gene PERIOD3 (PER3) (Viola, James, Archer, & Dijk, 2008), and the brain-derived neurotrophic factor (BDNF; Yang et al., 2010) to be

Table 5. *Model Fit Statistics and Variance Decomposition (CI) for the Univariate pvRSA Measure, Correcting for Ceiling Effects*

5a. *Maximal pvRSAmax Obtained During the Night*

Phenotype	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	A	E
pvRSAmax	1	1230	ACE	−1641.008	818.992						
	2	1231	AE	−1643.008	818.992	1	0	1	1	0.53 (0.42–0.62)	0.47 (0.38–0.58)
	3	1232	E	−1570.323	893.677	1	74.685	2	.000		

Note. pvRSAmax = median value of the six 10-min bins with the highest pvRSA value during the night; *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model.

5b. *Virtual pvRSAmax Extrapolated from the Daytime IBI-RSA Intercept and Slope to the Maximal IBI Obtained During the Night*

Phenotype	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	A	E
pvRSAmax	1	1230	ACE	−1376.033	1083.967						
_virtual	2	1231	AE	−1378.033	1083.967	1	0	1	1	0.55 (0.45–0.64)	0.46 (0.36–0.55)
	3	1232	E	−1296.892	1167.108	1	83.141	2	.000		

Note. pvRSAmax_virtual = nonobserved experimental variable that extrapolates the pvRSA from the intercept and slope of the daytime IBI-RSA association during sitting activities to the value that would have been obtained at the nighttime 10-min bin with the longest IBI value (IBImax); *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model.

5c. *Maximal IBI Obtained During the Night*

Phenotype	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	A	E
IBImax	1	1282	ACE	−3918.594	−1354.594						
	2	1283	AE	−3920.412	−1354.412	1	0.182	1	.670	0.52 (0.43–0.60)	0.48 (0.40–0.57)
	3	1284	E	−3814.549	−1246.549	1	108.45	2	.000		

Note. IBImax = nighttime 10-min bin with the longest IBI value; *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model.

5d. *Daytime RSA-IBI Slope*

Phenotype	Model	*df*	Model	AIC	−2LL	vs.	$\Delta\chi^2$	Δ *df*	*p*	A	E
daytime	1	1282	ACE	−1113.303	1450.697						
_slope	2	1283	AE	−1115.303	1450.697	1	0	1	1	0.35 (0.25–0.44)	0.65 (0.56–0.75)
	3	1284	E	−1072.076	1495.924	1	45.227	2	.000		

Note. daytime_slope = slope of the IBI-RSA curve in the waking part of the data; *df* = degrees of freedom; Model = specification of the model that is tested; AIC = Akaike's Information Criterion; −2LL = minus twice the logarithm of the likelihood; vs. = the model against which this submodel is tested; $\Delta\chi^2$ = model fit statistic: difference in −2LL of two nested models; Δ*df* = difference in the number of parameters between the two models; *p* = *p* value; A and E = proportions of variance explained by additive and unique environmental effects for the most parsimonious AE model.

associated with RMSSD and/or SDNN levels. Candidate gene studies do, however, need to be interpreted with caution before they have been confirmed in independent replication (Sullivan, 2007), and, ideally, functional studies exist that confirm a plausible pathway through which the genetic variant can influence RSA. In that sense, of all candidates found, the evidence of AKAP10 to be involved in HRV levels is strongest as its involvement was confirmed in animal research, as well (Tingley et al., 2007). Candidate gene studies are by definition confined to current knowledge, and are selected from the biological mechanisms already expected to be involved in heart rate regulation. In genome-wide association studies (GWAS), no a priori assumptions concerning biological mechanisms are made, and the entire genome is considered to be a "candidate" (Manolio et al., 2009; Tabor, Risch, & Myers, 2002). To our knowledge, the Framingham Heart Study is the only group that has performed a GWAS on HRV thus far, but none of the associations reached genome-wide significance (Newton-Cheh et al., 2007). The sample studied was, however, small ($N = 548$). In a secondary analysis, this group did find significant candidate gene associations between the alpha-adrenergic receptor type 1A (ADDRA1A) and the alpha-adrenergic receptor type 1B (ADRA1B) genes and SDNN at $p < .05$. However, to be able to find genome-wide significant hits, analyses on tens of thousands of participants are needed for which whole genome single nucleotide polymorphism and HRV data is available. Because no single research group can mount such numbers, the Genetic Variability in Heart Rate Variability (VgHRV) consortium was set up with the aim to share association results between different research groups and perform across-study meta-analyses on HRV (Nolte et al., 2011). This solves the problem of small sample sizes but may

introduce yet another pitfall, namely, the lack of unity regarding the HRV measures that are used across different studies. With this study, we show that the genetic overlap between pvRSA and RMSSD, and RMSSD and SDNN, is high (genetic correlations are estimated at .94 and .89 in resting ambulatory sitting conditions) while the genetic overlap between pvRSA and SDNN, albeit lower, was still .74. From this, it follows that HRV studies that assessed pvRSA and RMSSD, or RMSSD and SDNN, can be safely pooled as the genetic architecture is expected to be highly similar.

A major limitation of this study is that exercise status was not obtained for all participants at the time of testing. Therefore, we cannot rule out that exercise status may explain part of the heritability that is found for HRV, since previous work has shown that exercise behavior and RSA are genetically correlated (de Geus, Boomsma, & Snieder, 2003). A second limitation is that we used a crude approach in pooling all 24-h HRV data in only three ambulatory conditions (sleep, sitting, or physically active), thereby potentially introducing heterogeneity within conditions, considering the wide range of activities that meet these criteria. This may be particularly pertinent for physical activity, where the range of variety is largest. On the other hand, ambulatory activities need to be generalized to some extent to make data of different participants comparable. Finally, as nighttime HRV recordings in twins and siblings, to our knowledge, have only been performed by our group, we cannot compare our results to those of independent twin studies.

The major strength of this study is that this is the largest 24-h ambulatory cardiac monitoring sample to date in which the heritability of three widely accepted HRV measures is studied, including pvRSA, which is taken to be the most "pure" HRV measure. Additionally, for the first time, within-participant IBI-RSA associations were inventoried to assess ceiling status, and the impact of this potential confounding factor on the heritability estimates has now been thoroughly tested.

We conclude that about half of the variation that is seen in the levels of the three HRV measures that are currently used most in the fields of cardiology and psychophysiology is genetically determined in the healthy adult population. The heritability estimates were robust against confounding by IBI-RSA ceiling effects that were observed in a subgroup of participants that took part in the study. There is no pressing need to exclude these participants, who may be overrepresented among healthy exercisers, in genetic studies of HRV. The genetic overlap between the three RSA measures studied is large, especially for pvRSA and RMSSD and RMSSD and SDNN, thereby implicating that these measures can be pooled in future GWASs to obtain larger sample sizes and increase power to find the actual genetic variants being responsible for individual differences in cardiac vagal control.

References

Akaike, H. (1987). Factor analysis and AIC. *Psychometrika*, *52*, 317–332. doi: 10.1007/BF02294359

Baccarelli, A., Cassano, P. A., Litonjua, A., Park, S. K., Suh, H., Sparrow, D., . . . Schwartz, J. (2008). Cardiac autonomic dysfunction: Effects from particulate air pollution and protection by dietary methyl nutrients and metabolic polymorphisms. *Circulation*, *117*, 1802–1809. doi: 10.1161.CIRCULATIONAHA.107.726067

Berntson, G. G., Bigger, J. T., Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., . . . van der Molen, M. W. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology*, *34*, 623–648. doi: 10.1111/j.1469-8986.1997.tb02140.x

Berntson, G. G., Cacioppo, J. T., & Quigley, K. S. (1993). Respiratory sinus arrhythmia: Autonomic origins, physiological mechanisms, and psychophysiological implications. *Psychophysiology*, *30*, 183–196. doi: 10.1111/j.1469-8986.1993.tb01731.x

Bigger, J. T., Jr., Fleiss, J. L., Rolnitzky, L. M., & Steinman, R. C. (1993). Frequency domain measures of heart period variability to assess risk late after myocardial infarction. *Journal of the American College of Cardiology*, *21*, 729–736. doi: 10.1016/0735-1097(93)90106-B

Bigger, J. T., Jr., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M., Kleiger, R. E., & Rottman, J. N. (1992). Correlations among time and frequency domain measures of heart period variability two weeks after acute myocardial infarction. *American Journal of Cardiology*, *69*, 891–898. doi: 10.1016/0002-9149(92)90788-Z

Bigger, J. T., Jr., Hoover, C. A., Steinman, R. C., Rolnitzky, L. M., & Fleiss, J. L. (1990). Autonomic nervous system activity during myocardial ischemia in man estimated by power spectral analysis of heart period variability. The Multicenter Study of Silent Myocardial Ischemia Investigators. *American Journal of Cardiology*, *66*, 497–498. doi: 10.1016/0002-9149(90)90712-A

Boomsma, D. I., & Gabrielli, W. F., Jr. (1985). Behavioral genetic approaches to psychophysiological data. *Psychophysiology*, *22*, 249–260. doi: 10.1111/j.1469-8986.1985.tb01596.x

Boomsma, D. I., van Baal, G. C., & Orlebeke, J. F. (1990). Genetic influences on respiratory sinus arrhythmia across different task conditions. *Acta Geneticae Medicae et Gemellologiae (Roma)*, *39*, 181–191.

Buccelletti, E., Gilardi, E., Scaini, E., Galiuto, L., Persiani, R., Biondi, A., . . . Silveri, N. G. (2009). Heart rate variability and myocardial infarction: Systematic literature review and metanalysis. *European Review for Medical and Pharmacological Sciences*, *13*, 299–307.

Busjahn, A., Voss, A., Knoblauch, H., Knoblauch, M., Jeschke, E., Wessel, N., . . . Luft, F. C. (1998). Angiotensin-converting enzyme and angiotensinogen gene polymorphisms and heart rate variability in twins. *American Journal of Cardiology*, *81*, 755–760. doi: 10.1016/S0002-9149(97)01019-9

Christov, I. I. (2004). Real time electrocardiogram QRS detection using combined adaptive threshold. *Biomedical Engineering Online*, *3*, 28. doi: 10.1186/1475-925X-3-28

Cui, J. S., Hopper, J. L., & Harrap, S. B. (2003). Antihypertensive treatments obscure familial contributions to blood pressure variation. *Hypertension*, *41*, 207–210. doi: 10.1161/01.HYP.0000044938.94050.E3

de Geus, E. J. C. (2010). Quantitative genetics in behavioral medicine. In A. Steptoe (Ed.), *Handbook of behavioral medicine* (pp. 399–422). London, UK: Springer. doi: 10.1007/978-0-387-09488-5-28

de Geus, E. J., Boomsma, D. I., & Snieder, H. (2003). Genetic correlation of exercise with heart rate and respiratory sinus arrhythmia. *Medicine and Science in Sports and Exercise*, *35*, 1287–1295. doi: 10.1249/01.MSS.0000079073.20399.11

de Geus, E. J., Kupper, N., Boomsma, D. I., & Snieder, H. (2007). Bivariate genetic modeling of cardiovascular stress reactivity: Does stress uncover genetic variance? *Psychosomatic Medicine*, *69*, 356–364. doi: 10.1097/PSY.0b013e318049cc2d

de Geus, E. J., Willemsen, G. H., Klaver, C. H., & van Doornen, L. J. (1995). Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. *Biological Psychology*, *41*, 205–227. doi: 10.1016/0301-0511(95)05137-6

de Geus, E. J. C., & van Doornen, L. J. P. (1996). Ambulatory assessment of parasympathetic/sympathetic balance by impedance cardiography. In J. Fahrenberg & M. Myrtek (Eds.), *Ambulatory assessment: Computer-assisted psychological and psychophysiological methods in monitoring and field studies* (pp. 141–163). Gottingen, Germany: Hogrefe & Huber.

De Meersman, R. E., & Stein, P. K. (2007). Vagal modulation and aging. *Biological Psychology*, *74*, 165–173. doi: 10.1016/j.biopsycho.2006.04.008

Dekker, J. M., Crow, R. S., Folsom, A. R., Hannan, P. J., Liao, D., Swenne, C. A., . . . Schouten, E. G. (2000). Low heart rate variability in a 2-minute rhythm strip predicts risk of coronary heart disease and mortality from several causes: The ARIC Study. Atherosclerosis Risk in Communities. *Circulation*, *102*, 1239–1244. doi: 10.1161/01.CIR.102.11.1239

Dekker, J. M., Schouten, E. G., Klootwijk, P., Pool, J., Swenne, C. A., & Kromhout, D. (1997). Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged

and elderly men. The Zutphen Study. *American Journal of Epidemiology*, *145*, 899–908. doi: 10.1093/oxfordjournals.aje.a009049

Eckberg, D. L. (2003). The human respiratory gate. *Journal of Physiology*, *548*, 339–352. doi: 10.1113/jphysiol.2002.037192

Goedhart, A. D., Kupper, N., Willemsen, G., Boomsma, D. I., & de Geus, E. J. (2006). Temporal stability of ambulatory stroke volume and cardiac output measured by impedance cardiography. *Biological Psychology*, *72*, 110–117. doi: 10.1016/j.biopsycho.2005.08.004

Goedhart, A. D., van der Sluis, S., Houtveen, J. H., Willemsen, G., & de Geus, E. J. (2007). Comparison of time and frequency domain measures of RSA in ambulatory recordings. *Psychophysiology*, *44*, 203–215. doi: 10.1111/j.1469-8986.2006.00490.x

Goldberger, J. J., Challapalli, S., Tung, R., Parker, M. A., & Kadish, A. H. (2001). Relationship of heart rate variability to parasympathetic effect. *Circulation*, *103*, 1977–1983. doi: 10.1161/01.CIR.103.15.1977

Grossman, P., & Kollai, M. (1993). Respiratory sinus arrhythmia, cardiac vagal tone, and respiration: Within- and between-individual relations. *Psychophysiology*, *30*, 486–495. doi: 10.1111/j.1469-8986.1993.tb02072.x

Grossman, P., van Beek, J., & Wientjes, C. (1990). A comparison of three quantification methods for estimation of respiratory sinus arrhythmia. *Psychophysiology*, *27*, 702–714. doi: 10.1111/j.1469-8986.1990.tb03198.x

Grossman, P., & Wientjes, K. (1986). Respiratory sinus arrythmia and parasympathetic cardiac control: Some basic issues concerning quantification, applications and implications. In R. Grossman, K. H. L. Janssen, & D. Vital (Eds.), *Cardiorespiratory and cardiosomatic psychophysiology* (pp. 117–138). New York, NY: Plenum Press. doi: 10.1007/978-1-4757-0360-3_8

Grossman, P., Wilhelm, F. H., & Spoerle, M. (2004). Respiratory sinus arrhythmia, cardiac vagal control, and daily activity. *American Journal of Physiology, Heart and Circulatory Physiology*, *287*, H728–H734. doi: 10.1152/ajpheart.00825.2003

Hohnloser, S. H., Klingenheben, T., Zabel, M., Schroder, F., & Just, H. (1992). Intraindividual reproducibility of heart rate variability. *Pacing and Clinical Electrophysiology*, *15*, 2211–2214. doi: 10.1111/j.1540-8159.1992.tb03049.x

Houtveen, J. H., Groot, P. F., & de Geus, E. J. (2005). Effects of variation in posture and respiration on RSA and pre-ejection period. *Psychophysiology*, *42*, 713–719. doi: 10.1111/j.1469-8986.2005.00363.x

Katona, P. G., & Jih, F. (1975). Respiratory sinus arrhythmia: Noninvasive measure of parasympathetic cardiac control. *Journal of Applied Physiology*, *39*, 801–805.

Kleiger, R. E., Bigger, J. T., Bosner, M. S., Chung, M. K., Cook, J. R., Rolnitzky, L. M., . . . Fleiss, J. L. (1991). Stability over time of variables measuring heart rate variability in normal subjects. *American Journal of Cardiology*, *68*, 626–630. doi: 10.1016/0002-9149(91)90355-O

Kleiger, R. E., Miller, J. P., Bigger, J. T., Jr., & Moss, A. J. (1987). Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *American Journal of Cardiology*, *59*, 256–262. doi: 10.1016/0002-9149(87)90795-8

Kupper, N., Willemsen, G., Posthuma, D., de Boer, D., Boomsma, D. I., & de Geus, E. J. (2005). A genetic analysis of ambulatory cardiorespiratory coupling. *Psychophysiology*, *42*, 202–212. doi: 10.1111/j.1469-8986.2005.00276.x

Kupper, N. H., Willemsen, G., van den Berg, M., de Boer, D., Posthuma, D., Boomsma, D. I., . . . de Geus, E. J. C. (2004). Heritability of ambulatory heart rate variability. *Circulation*, *110*, 2792–2796. doi: 10.1161/01.CIR.0000146334.96820.6E

La Rovere, M. T., Pinna, G. D., Hohnloser, S. H., Marcus, F. I., Mortara, A., Nohara, R., . . . Schwartz, P. J. (2001). Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening arrhythmias: Implications for clinical trials. *Circulation*, *103*, 2072–2077. doi: 10.1161/01.CIR.103.16.2072

Malik, M., & Camm, A. J. (1993). Components of heart rate variability—What they really mean and what we really measure. *American Journal of Cardiology*, *72*, 821–822. doi: 10.1016/0002-9149(93)91070-X

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., . . . Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, *461*, 747–753. doi: 10.1038/nature08494

Martinmaki, K., Rusko, H., Kooistra, L., Kettunen, J., & Saalasti, S. (2006). Intraindividual validation of heart rate variability indexes to measure vagal effects on hearts. *American Journal of Physiology, Heart and Circulatory Physiology*, *290*, H640–H647. doi: 10.1152/ajpheart.00054.2005

McNarry, M. A., & Lewis, M. J. (2012). Interaction between age and aerobic fitness in determining heart rate dynamics. *Physiological Measurement*, *33*, 901–914. doi: 10.1088/0967-3334/33/6/901

Neale, M. C., Boker, S. M., Xie, G., & Maes, H. H. (2006). *Mx: Statistical Modeling* (7th ed.) Richmond, VA: VCU.

Neale, M. C., & Cardon, L. R. (1992). *Methodology for genetic studies of twins and families*. Dordrecht, The Netherlands: Kluwer Academic Publishers. doi: 10.1007/978-94-015-8018-2

Neumann, S. A., Tingley, W. G., Conklin, B. R., Shrader, C. J., Peet, E., Muldoon, M. F., . . . Manuck, S. B.(2009). AKAP10 (I646V) functional polymorphism predicts heart rate and heart rate variability in apparently healthy, middle-aged European-Americans. *Psychophysiology*, *46*, 466–472. doi: 10.1111/j.1469-8986.2009.00802.x

Newton-Cheh, C., Guo, C. Y., Wang, T. J., O'Donnell, C. J., Levy, D., & Larson, M. G. (2007). Genome-wide association study of electrocardiographic and heart rate variability traits: The Framingham Heart Study [Supplement 1]. *BMC Medical Genetics*, *8*, S1–S7. doi: 10.1186/1471-2350-8-S1–S7

Nolte, I., Tragante do Ó, V., Whitsel, E., Penninx, B. W., Oldehinkel, A., Bis, J., . . . de Geus, E. J. C. (2011). Meta-analysis of genome wide association studies on heart rate variability. *Behavior Genetics*, *41*, 927.

Pitzalis, M. V., Mastropasqua, F., Massari, F., Forleo, C., Di, M. M., Passantino, A., . . . Rizzon, P. (1996). Short- and long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. *Cardiovascular Research*, *32*, 226–233. doi: 10.1016/0008-6363(96)00086-7

Quilliot, D., Fluckiger, L., Zannad, F., Drouin, P., & Ziegler, O. (2001). Impaired autonomic control of heart rate and blood pressure in obesity: Role of age and of insulin-resistance. *Clinical Autonomic Research*, *11*, 79–86. doi: 10.1007/BF02322050

Rekling, J. C., & Feldman, J. L. (1998). PreBotzinger complex and pacemaker neurons: Hypothesized site and kernel for respiratory rhythm generation. *Annual Review of Physiology*, *60*, 385–405. doi: 10.1146/annurev.physiol.60.1.385

Riese, H., Groot, P. F., van den Berg, M., Kupper, N. H., Magnee, E. H., Rohaan, E. J., . . . de Geus, E. J. (2003). Large-scale ensemble averaging of ambulatory impedance cardiograms. *Behavior Research Methods, Instruments, and Computers*, *35*, 467–477. doi: 10.3758/BF03195525

Riese, H., Rijsdijk, F. V., Ormel, J., van Roon, A. M., Neeleman, J., & Rosmalen, J. G. (2006). Genetic influences on baroreflex sensitivity during rest and mental stress. *Journal of Hypertension*, *24*, 1779–1786. doi: 10.1097/01.hjh.0000242402.83709.27

Riese, H., Rosmalen, J. G., Ormel, J., van Roon, A. M., Oldehinkel, A. J., & Rijsdijk, F. V. (2007). The genetic relationship between neuroticism and autonomic function in female twins. *Psychological Medicine*, *37*, 257–267. doi: 10.1017/S0033291706009160

Ritz, T., & Dahme, B. (2006). Implementation and interpretation of respiratory sinus arrhythmia measures in psychosomatic medicine: Practice against better evidence? *Psychosomatic Medicine*, *68*, 617–627. doi: 10.1097/01.psy.0000228010.96408.ed

Sacknoff, D. M., Gleim, G. W., Stachenfeld, N., & Coplan, N. L. (1994). Effect of athletic training on heart rate variability. *American Heart Journal*, *127*, 1275–1278. doi: 10.1016/0002-8703(94)90046-9

Schwartz, P. J., Billman, G. E., & Stone, H. L. (1984). Autonomic mechanisms in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with healed myocardial infarction. An experimental preparation for sudden cardiac death. *Circulation*, *69*, 790–800. doi: 10.1161/01.CIR.69.4.790

Schwartz, P. J., Vanoli, E., Stramba-Badiale, M., De Ferrari, G. M., Billman, G. E., & Foreman, R. D. (1988). Autonomic mechanisms and sudden death. New insights from analysis of baroreceptor reflexes in conscious dogs with and without a myocardial infarction. *Circulation*, *78*, 969–979. doi: 10.1161/01.CIR.78.4.969

Singer, D. H., Martin, G. J., Magid, N., Weiss, J. S., Schaad, J. W., Kehoe, R., . . . Lesch, M. (1988). Low heart rate variability and sudden cardiac death [Supplement]. *Journal of Electrocardiology*, *21*, S46–S55. doi: 10.1016/0022-0736(88)90055-6

Sinnreich, R., Kark, J. D., Friedlander, Y., Sapoznikov, D., & Luria, M. H. (1998). Five minute recordings of heart rate variability for population

studies: Repeatability and age-sex characteristics. *Heart*, *80*, 156–162.

Snieder, H., Boomsma, D. I., van Doornen, L. J., & de Geus, E. J. (1997). Heritability of respiratory sinus arrhythmia: Dependency on task and respiration rate. *Psychophysiology*, *34*, 317–328. doi: 10.1111/j.1469-8986.1997.tb02402.x

Stein, P. K., Rich, M. W., Rottman, J. N., & Kleiger, R. E. (1995). Stability of index of heart rate variability in patients with congestive heart failure. *American Heart Journal*, *129*, 975–981. doi: 10.1016/0002-8703(95)90119-1

Su, S., Lampert, R., Lee, F., Bremner, J. D., Snieder, H., Jones, L., . . . Vaccarino, V. (2010). Common genes contribute to depressive symptoms and heart rate variability: The Twins Heart Study. *Twin Research and Human Genetics*, *13*, 1–9. doi: 10.1375/twin.13.1.1

Sullivan, P. F. (2007). Spurious genetic associations. *Biological Psychiatry*, *61*, 1121–1126. doi: 10.1016/j.biopsych.2006.11.010

Tabor, H. K., Risch, N. J., & Myers, R. M. (2002). Candidate-gene approaches for studying complex genetic traits: Practical considerations. *Nature Reviews Genetics*, *3*, 391–397. doi: 10.1038/nrg796

Tank, J., Jordan, J., Diedrich, A., Stoffels, M., Franke, G., Faulhaber, H. D., . . . Busjahn, A. (2001). Genetic influences on baroreflex function in normal twins. *Hypertension*, *37*, 907–910. doi: 10.1161/01.HYP.37.3.907

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *Circulation*, *93*, 1043–1065.

Tingley, W. G., Pawlikowska, L., Zaroff, J. G., Kim, T., Nguyen, T., Young, S. G., . . . Conklin, B. R. (2007). Gene-trapped mouse embryonic stem cell-derived cardiac myocytes and human genetics implicate AKAP10 in heart rhythm regulation. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 8461–8466. doi: 10.1073/pnas.0610393104

Tsuji, H., Larson, M. G., Venditti, F. J., Jr., Manders, E. S., Evans, J. C., Feldman, C. L., . . . Levy, D. (1996). Impact of reduced heart rate variability on risk for cardiac events. The Framingham Heart Study. *Circulation*, *94*, 2850–2855. doi: 10.1161/01.CIR.94.11.2850

Tuvblad, C., Isen, J., Baker, L. A., Raine, A., Lozano, D. I., & Jacobson, K. C. (2010). The genetic and environmental etiology of sympathetic and parasympathetic activity in children. *Behavior Genetics*, *40*, 452–466. doi: 10.1007/s10519-010-9346-0

Umetani, K., Singer, D. H., McCraty, R., & Atkinson, M. (1998). Twenty-four hour time domain heart rate variability and heart rate: Relations to age and gender over nine decades. *Journal of the American College of Cardiology*, *31*, 593–601. doi: 10.1016/S0735-1097(97)00554-8

Uusitalo, A. L., Vanninen, E., Levalahti, E., Battie, M. C., Videman, T., & Kaprio, J. (2007). Role of genetic and environmental influences on heart rate variability in middle-aged men. *American Journal of Physiology, Heart and Circulatory Physiology*, *293*, H1013–H1022. doi: 10.1152/ajpheart.00475.2006

Valentini, M., & Parati, G. (2009). Variables influencing heart rate. *Progress in Cardiovascular Diseases*, *52*, 11–19. doi: 10.1016/j.pcad.2009.05.004

Vallejo, M., Marquez, M. F., Borja-Aburto, V. H., Cardenas, M., & Hermosillo, A. G. (2005). Age, body mass index, and menstrual cycle influence young women's heart rate variability—A multivariable analysis. *Clinical Autonomic Research*, *15*, 292–298. doi: 10.1007/s10286-005-0272-9

van Dijk, A. E., van Lien, R., van, Eijsden, M., Gemke, R. J., Vrijkotte, T. G., & de Geus, E. J. (2013). Measuring cardiac autonomic nervous system (ANS) activity in children. *Journal of Visualized Experiments*,*74*, e50073. doi: 10.3791/50073

van Lien, R., Goedhart, A., Kupper, N., Boomsma, D., Willemsen, G., & de Geus, E. J. (2011). Underestimation of cardiac vagal control in regular exercisers by 24-hour heart rate variability recordings. *International Journal of Psychophysiology*, *81*, 169–176. doi: 10.1016/j.ijpsycho.2011.06.007

Vikman, S., Makikallio, T. H., Yli-Mayry, S., Nurmi, M., Airaksinen, K. E., & Huikuri, H. V. (2003). Heart rate variability and recurrence of atrial fibrillation after electrical cardioversion. *Annals of Medicine*, *35*, 36–42. doi: 10.1080/07853890310004110

Viola, A. U., James, L. M., Archer, S. N., & Dijk, D. J. (2008). PER3 polymorphism and cardiac autonomic control: Effects of sleep debt and circadian phase. *American Journal of Physiology, Heart and Circulatory Physiology*, *295*, H2156–H2163. doi: 10.1152/ajpheart.00662.2008

Wang, X., Ding, X., Su, S., Li, Z., Riese, H., Thayer, J. F., . . . Snieder, H. (2009). Genetic influences on heart rate variability at rest and during stress. *Psychophysiology*, *46*, 458–465. doi: 10.1111/j.1469-8986.2009.00793.x

Wang, X., Thayer, J. F., Treiber, F., & Snieder, H. (2005). Ethnic differences and heritability of heart rate variability in African- and European American youth. *American Journal of Cardiology*, *96*, 1166–1172. doi: 10.1016/j.amjcard.2005.06.050

Wilhelm, F. H., Roth, W. T., & Sackner, M. A. (2003). The lifeShirt. An advanced system for ambulatory measurement of respiratory and cardiac function. *Behavior Modification*, *27*, 671–691. doi: 10.1177/0145445503256321

Willemsen, G., Vink, J. M., Abdellaoui, A., den, Braber, A., van Beek, J. H., Draisma, H. H., . . . Boomsma, D. I. (2013). The adult Netherlands twin register: Twenty-five years of survey and biological data collection. *Twin Research and Human Genetics*, *16*, 271–281. doi: 10.1017/thg.2012.140

Yang, A. C., Chen, T. J., Tsai, S. J., Hong, C. J., Kuo, C. H., Yang, C. H., . . . Kao, K. P. (2010). BDNF Val66Met polymorphism alters sympathovagal balance in healthy subjects. *American Journal of Medical Genetics Part B–Neuropsychiatric Genetics*, *153B*, 1024–1030. doi: 10.1002/ajmg.b.31069

Zanstra, Y. J., & Johnston, D. W. (2011). Cardiovascular reactivity in real life settings: Measurement, mechanisms and meaning. *Biological Psychology*, *86*, 98–105. doi: 10.1016/j.biopsycho.2010.05.002

Zhang, L., Rao, F., Zhang, K., Khandrika, S., Das, M., Vaingankar, S. M., . . . O'Connor, D. T. (2007). Discovery of common human genetic variants of GTP cyclohydrolase 1 (GCH1) governing nitric oxide, autonomic activity, and cardiovascular risk. *Journal of Clinical Investigation*, *117*, 2658–2671. doi: 10.1172/JCI31093

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