

## Article: Complications

# Effects of genetic vs. environmental factors on cardiovascular autonomic function: a twin study

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### Abstract

**Aims** Cardiovascular autonomic function is often assessed in patients with diabetes by measuring heart rate variability and baroreflex sensitivity, the heritability of which is not fully understood. The present study was aimed to determine the effects of genetic and environmental factors on heart rate variability and baroreflex sensitivity in monozygotic and dizygotic adult healthy twin pairs.

**Methods** A total of 101 (63 monozygotic, 38 dizygotic) adult twin pairs ( $n = 202$ ; mean age 44.3 years) were investigated. Anthropometric variables and serum metabolic markers were measured, while environmental characteristics were evaluated by questionnaires. Linear and spectral indices of heart rate variability and baroreflex sensitivity were determined by non-invasive methods. All measurements were adjusted for age and gender (model 1) and for all significantly relevant covariates (model 2). Heritability A-C-E structural equation models were used for characterizing the proportion of additive genetic, shared and unshared environmental influences.

**Results** Genetic influence of different cardiovascular autonomic indices was estimated between 10.3 and 39.4%, common environmental influence was found between 0.0 and 33.2%, while unshared environmental influence was observed between 60.6 and 81.4% in model 1 analysis. In multivariable-adjusted heritability estimates (model 2), the magnitude of the genetic effects decreased to 0.0%, common environmental influence was nearly unchanged (values between 4.4 and 14.5%), while unshared environmental influence slightly increased (values between 85.5 and 96.5%).

**Conclusions** Unshared environmental but not genetic factors have substantial influence on cardiovascular autonomic function, suggesting that appropriate treatment of all modifiable environmental factors is of importance in order to prevent or ameliorate cardiovascular autonomic neuropathy.

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**Keywords** baroreflex sensitivity, cardiovascular autonomic function, cardiovascular autonomic neuropathy, heart rate variability, twin study

### Introduction

Autonomic nervous system exerts the principal physiological control over heart rate, through parasympathetic and sympathetic innervations. Diabetes mellitus is known to impair cardiovascular autonomic function, leading to clinical symptoms and signs, commonly termed as cardiovascular autonomic neuropathy [1,2].

Cardiovascular autonomic function can be assessed by determining heart rate variability and the dysfunction of autonomic innervations can be described by time and frequency domain alterations of parameters of heart rate variability. Besides, the assessment of baroreflex sensitivity evaluates cardiac autonomic control in response to blood pressure changes [3].

The clinical significance of cardiovascular autonomic dysfunction is widely investigated both by diabetologists and cardiologists. Previous studies have indicated that autonomic dysfunction (reduced heart rate variability and depressed baroreflex sensitivity) is associated with increased cardiovascular morbidity and mortality; this was observed in patients with previous myocardial infarction or diabetes [4–6].

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Several clinical studies were conducted in patients with diabetes mellitus for characterizing the alterations of heart rate variability and baroreflex sensitivity. In addition, different treatment options were investigated in order to influence diabetic neuropathy, including cardiovascular autonomic neuropathy [7]. Obviously, understanding the heritability of cardiovascular autonomic function would provide insights into the pathomechanism of cardiovascular autonomic neuropathy. Moreover, the effectiveness of cardiovascular autonomic neuropathy treatment might be anticipated more correctly knowing the influence of genetic and environmental factors on cardiovascular autonomic nervous function.

We performed a classical twin study in order to determine the genetic and environmental influences on cardiovascular autonomic function. Both heart rate variability and baroreflex sensitivity were assessed in monozygotic and dizygotic adult twin pairs without diabetes.

## Subjects and methods

In this classical twin study, 101 adult twin pairs ( $n = 202$ ; women 72.3%; mean age 44.3 years, range 18–81 years) were investigated. As no twin registry is available in Hungary at present, participants were recruited from national twin meetings and through advertisements published in local newspapers. Exclusion criteria included pregnancy, diabetes mellitus, myocardial infarction or regular alcohol consumption (more than 2 units daily) in the past medical history, conditions possibly interfering with compliance during test procedures and acute infection within 3 weeks of measurement. All subjects were asked to suspend taking drugs that potentially affect heart rate 36–48 h prior to study procedure. Anthropometric measurements (recording weight, height and waist circumference) were carried out and a complete physical examination, including blood pressure measurement in sitting position, was performed. Body mass index (BMI) was calculated from the values of weight and height. Waist circumference was measured using the standard method. Physical activity level was assessed by the standardized method: subjects reported the amount of time spent on five different intensity levels of physical activity on an average weekday as a total 24 h, then values of daily metabolic equivalent score were derived and used for statistical analysis. Smoking habit was assessed as smoking years, while alcohol consumption was evaluated as unit per week. Fasting venous blood samples were taken from twin pairs and routine laboratory methods were used for measuring blood glucose, lipids and serum creatinine.

Due to the lack of genotyping data of subjects, we used a multiple self-reported question approach to assess zygosity in order to maximize the accuracy of classification. The most likely zygosity was assigned based on the seven self-reported responses [8]. In this way, 63 monozygotic and 38 dizygotic twin pairs were investigated. All participants provided informed consent. The investigation was approved by the National Research Ethics Committee (ETT TUKEB, Budapest) and was conducted

according to the principles expressed in the Declaration of Helsinki.

## Measuring heart rate variability and baroreflex sensitivity

Heart rate variability and baroreflex sensitivity were investigated in the early afternoon hours under standardized conditions, in a quiet room at a comfortable temperature. All individuals refrained from smoking and fasted at least 2 h before testing. In addition, all subjects were asked to abstain from strenuous activity or drinking alcohol or caffeinated beverages for 24 h before the investigation. Subjects were equipped with the appropriate devices and then rested in the supine position for approximately 15 min until baseline conditions for heart rate and mean blood pressure were reached. Heart rate (expressed in R-R intervals) was determined from the second lead of a standard electrocardiograph (ECG) recording. Blood pressure fluctuations were monitored on the right hand middle finger by Finapres (Finapres Medical Systems, Amsterdam, the Netherlands). Systolic and diastolic blood pressure was measured on the brachial artery by automatic sphygmomanometry. To improve the reliability of the measurements, breathing rate was paced at 0.25 Hz. Blood pressure and ECG recordings were digitized and stored on a PC for subsequent offline analysis. Heart rate variability and baroreflex sensitivity were determined according to the international guidelines [9].

At investigation of heart rate variability, time and frequency domain parameters from 10-min recordings of R-R intervals were calculated using the WinCPRS program (WinCPRS Absolute Aliens Oy, Turku, Finland) [10]. Non-sinus beats were semi-automatically removed and corrected using interpolation of preceding beats. The number of analysed heartbeats varied, based on the heart rate of each subject. During a session, on the average, 707 beats were sampled (ranging from 478 to 1143). The following parameters were determined: the root mean square of successive differences (termed RMSSD) and the percentage of successive R-R intervals that differed by 50 ms (termed pNN50) as well as low-frequency (0.05–0.15 Hz) and high-frequency (0.15–0.4 Hz) power of R-R interval variability (termed LF and HF, respectively).

At investigation of baroreflex sensitivity, the coupling between spontaneous fluctuations in systolic blood pressure and heart rate was determined by the sequence method and by spectral analysis. The WinCPRS software detected ECG R-wave peaks and computed R-R interval and systolic blood pressure time series and identified spontaneously occurring sequences in which systolic blood pressure and R-R interval concurrently increased and decreased over three or more consecutive beats. Minimal accepted change was 1 mmHg for systolic blood pressure and 5 ms for R-R interval. Baroreflex sensitivity sequence indices were calculated from up–up (BRSseq+) and down–down (BRSseq–) sequences as the slope of the regression line between systolic blood pressure and R-R interval. Only sequences with a correlation coefficient > 0.85 were considered. To quantify spectral indices, the power spectra of systolic blood

pressure and R-R interval signals were determined using fast Fourier transformation-based methods. The low-frequency transfer function gain (LFGain) was determined, which expresses R-R interval and systolic blood pressure cross-spectral magnitude in the frequency range of 0.05–0.15 Hz, where coherence is  $> 0.5$ .

### Statistical analysis

#### *Assessment of the sample*

Because of the lack of central twin registry, we did not have the opportunity to match the participants on key traits of interest. Therefore, assessing similarities and differences between the monozygotic and dizygotic subsamples is of great importance in order to understand possible sources of bias in the results. To achieve this, we used a parametric difference test with cluster sampling correction [11]. The correction was needed because the observations in the sample are not completely independent of each other. The correction accounts for the difference in trait variance within and between clusters (families). Parametric tests were sufficient as all raw or log-transformed continuous traits were within acceptable parameters of normality and non-parametric tests do not offer such clustering corrections. Values are given as means  $\pm$  SD. Differences between parametric tests were considered significant at  $P < 0.05$  level. For dichotomous predictors, the proportions are presented and the hypothesis test was performed using a log-link function [12].

#### *Risk factors*

To better understand the role of risk factors, age- and gender-corrected bivariate correlations were derived between the dependent variables and risk factors. Bivariate correlations also utilized a cluster sampling standard errors for correct hypothesis tests. Bivariate relationships were considered significant at  $P < 0.05$  level.

We used a maximum likelihood regression model with robust standard errors (using the sandwich estimator) and cluster sample correction implemented in Mplus clustering by families to overcome the lack of independence between family members. The regression models always included the dependent and the independent variable of interest and age and sex. Under correlations we reported the standardized regression coefficients for the key independent variables of interest.

#### *Genetic and environmental impact*

The heritability model for twins raised together capitalizes on monozygotic twins sharing 100% of their genome while dizygotic twins sharing only 50% on average. The distribution of shared environmental components is assumed to be identical for monozygotic and dizygotic twins. Using this information, we used a structural equation model, often called the A-C-E model, where three latent variables, additive genetic effects (A), common (or shared) environment (C) and unshared (or unique) environment (E) drive the variance in the phenotype for each twin [13]. A is perfectly (1.0) correlated across monozygotic twins

and 0.5 correlated across dizygotic twins. C is perfectly correlated independently of zygosity. E is uncorrelated across co-twins. As measurement error in the phenotype is also uncorrelated across measurements, it appears as part of the unique environmental component. Considering the well-established, reliable measures used in this study, this property of the model is of little concern.

For each phenotype, two A-C-E models were estimated. The model 1 corrects for the twins' age and gender. The correction for age and gender is justified by gender being 100% genetic and age being 100% environmental. All other predictors could carry both a genetic and an environmental component. The results from model 1 tell us the total genetic and environmental impact on the dependent variable. Model 2, in addition to age and gender, also corrects for all significant risk factors based on the bivariate correlations. These results tell us the impact of genes and the environment after the impact of known risks are corrected for. Empirically derived bootstrapped confidence intervals are presented for the heritability and environmental proportion estimates [14]. In addition to the full A-C-E model, we also present a reduced C-E model where the impact of A is assumed to be zero. Standard hypothesis tests are inappropriate for these as proportions are bounded, can never be negative and therefore central limit theorem assumptions of traditional standard error based hypothesis tests are inappropriate. All inferential statistics were estimated using full information maximum likelihood with the software Mplus version 6 [15].

## Results

The zygosity-specific demographic characteristics and distribution of cardiovascular risk factors as well as cardiovascular autonomic function indices are presented in Table 1. Although a significant difference between ages of monozygotic vs. dizygotic twin pairs occurred, there were no significant differences between gender, BMI, smoking and drinking habits, as well as physical activity. As for traditional cardiovascular risk factors, serum total cholesterol and triglyceride values were higher in monozygotic vs. dizygotic twin pairs. No significant differences were found between cardiovascular autonomic function indices when monozygotic and dizygotic twins were compared.

The correlations of cardiovascular autonomic function indices with anthropometric measurements and cardiovascular risk factors are shown in Table 2. On the one hand, an inverse correlation was observed between some cardiovascular autonomic function indices and anthropometric measurements, blood pressure and serum glucose values. On the other hand, no association (one exception: BRSseq- and physical activity) was observed between cardiovascular autonomic function indices and serum lipid values, creatinine, smoking and drinking habits and physical activity.

Using a structural equation model (Table 3), heritability (genetic influence) of different autonomic indices was estimated between 10.3 and 39.4%, common (shared) environmental influence was found between 0.0 and 33.2%, while unshared

**Table 1** Clinical/laboratory characteristics and cardiovascular autonomic function indices of 63 monozygotic and 38 dizygotic twin pairs

Characteristic	Monozygotic ( <i>n</i> = 126)	Dizygotic ( <i>n</i> = 76)	<i>P</i> -value
Female (%)	73.0	71.1	0.832
Age (years)	47.4 ± 15.5	38.3 ± 13.5	0.000
Weight (kg)	71.1 ± 14.5	72.4 ± 17.6	0.827
Body mass index (kg/m <sup>2</sup> )	25.9 ± 4.9	25.8 ± 5.9	0.576
Waist circumference (cm)	88 ± 14	88 ± 16	0.757
Systolic blood pressure (mmHg)	130.2 ± 14.8	125.3 ± 14.1	0.026
Diastolic blood pressure (mmHg)	74.7 ± 10.3	72.6 ± 9.8	0.152
Heart rate (min <sup>-1</sup> )	70.7 ± 12.1	70.2 ± 8.4	0.928
Fasting blood glucose (mmol/l)	5.01 ± 0.75	4.81 ± 0.63	0.064
Serum total cholesterol (mmol/l)	5.36 ± 1.23	5.00 ± 1.07	0.038
Serum triglycerides (mmol/l)	1.33 ± 0.86	1.07 ± 0.80	0.004
Serum HDL cholesterol (mmol/l)	1.60 ± 0.39	1.60 ± 0.36	0.755
Serum creatinine (μmol/l)	70.1 ± 9.9	72.3 ± 11.4	0.214
Smoking years	4.8 ± 9.5	4.8 ± 9.6	0.829
Alcohol units/week	1.1 ± 2.2	1.9 ± 3.4	0.553
Physical activity (daily MET)†	65.8 ± 22.2	60.3 ± 21.1	0.094
RMSSD (ms)‡	4.0 ± 2.5	4.4 ± 2.9	0.563
pNN50 (%)§	11.2 ± 15.6	15.3 ± 18.3	0.188
LF (ms <sup>2</sup> )¶	377.1 ± 410.7	511.2 ± 599.2	0.118
HF (ms <sup>2</sup> )††	421.7 ± 589.1	716.8 ± 1087.2	0.165
BRSseq+ (ms/Hgmm)‡‡	10.3 ± 5.2	12.2 ± 6.1	0.102
BRSseq- (ms/mmHg)§§	10.0 ± 5.5	11.6 ± 5.6	0.102
LFgain (ms/mmHg)¶¶	5.8 ± 4.0	6.8 ± 4.4	0.094

Values are given as means ± SD.

†Metabolic equivalent score.

‡Root mean square of successive R-R interval differences.

§Percentage of R-R intervals that differ > 50 ms.

¶Low-frequency (0.05–0.15 Hz) power of R-R interval variability.

††High-frequency (0.15–0.4 Hz) power of R-R interval variability.

‡‡Baroreflex sensitivity sequence index calculated from up–up sequences.

§§Baroreflex sensitivity sequence index calculated from down–down sequences.

¶¶Cross-spectral transfer gain in the low-frequency range.

(unique) environmental influence was observed between 60.6 and 81.4% in the crude model (model 1 A-C-E, no significant difference in comparison with C-E), where autonomic indices were adjusted for age and gender only. In multivariable-adjusted heritability estimates (model 2 A-C-E), the magnitude of the genetic effects decreased to 0.0%, common (shared) environmental influence was nearly unchanged (values between 4.4 and 14.5%) while unshared (unique) environmental influence slightly increased (values between 85.5 and 96.5%) (no significant differences in comparison with C-E). The difference of unshared (unique) environmental influence on autonomic function indices in model 2 A-C-E vs. model 1 A-C-E varied from 0.113 to 0.350, indicating that at least 11.3–35.0% of the environmental influence is explained by the combined effects of anthropometric parameters and traditional cardiovascular risk factors.

## Discussion

Our classical twin study documented that environmental factors have substantial influence, while heritability has no or negligible effect on cardiovascular autonomic function.

The sample size of our twin study was modest but comparable with other studies investigating the heritability of cardiovascular autonomic function in clinical settings [16]. The distribution of men and women and monozygotic and dizygotic twins in our study followed the distributions observed in volunteer samples [17].

Cardiovascular autonomic function was assessed by investigating both heart rate variability and baroreflex sensitivity. Different methods can be used for evaluating alterations in heart rate variability. In patients with diabetes, the classical cardiovascular autonomic function tests, such as beat-to-beat variation, Valsalva ratio, 30:15 ratio, postural systolic blood pressure changes, were the method of choice for bedside evaluation of cardiovascular autonomic neuropathy more than two decades ago [18]. In recent years, the measurement of time and frequency domain parameters and the use of spectral analysis of heart rate variability became widely accepted [19]. As for baroreflex sensitivity, the non-invasive measurement of spontaneous baroreflex sensitivity is used in large, population-based studies, although vasoactive drugs such as phenylephrine are preferable in research [4]. In our study, both heart rate variability and baroreflex sensitivity were assessed and

**Table 2** Correlation of autonomic function indices with anthropometric parameters and traditional cardiovascular risk factors in 63 monozygotic and 38 dizygotic twin pairs ( $n = 202$ )

	RMSSD‡	pNN50§	LF¶	HF††	BRSseq+‡‡	BRSseq-§§	LFgain¶¶
Body mass index	-0.203*	-0.125	-0.082	-0.208*	-0.167*	-0.216*	-0.162*
Waist circumference	-0.149	-0.072	-0.059	-0.175*	-0.191*	-0.253*	-0.209*
Systolic blood pressure	-0.097	-0.173*	-0.072	-0.170*	-0.208**	-0.191*	-0.163*
Diastolic blood pressure	-0.160*	-0.195**	-0.045	-0.176*	-0.236**	-0.212**	-0.082
Smoking years	0.070	-0.063	0.136	0.069	-0.263	-0.245	-0.258
Alcohol units/week	0.083	0.065	0.239	0.128	-0.069	0.013	0.052
Physical activity (daily MET)†	-0.068	-0.393	-0.036	-0.119	-0.134	-0.151*	-0.072
Serum fasting glucose	-0.230**	-0.226**	-0.235***	-0.256**	-0.152*	-0.128	-0.196**
Serum total cholesterol	-0.070	0.022	0.062	0.018	-0.043	-0.011	0.101
Serum triglycerides	-0.035	0.030	0.013	-0.042	-0.112	-0.084	-0.106
Serum HDL cholesterol	-0.066	-0.087	0.006	-0.042	-0.014	0.011	0.071
Serum creatinine	0.052	0.112	-0.034	0.010	0.075	0.091	-0.114

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

†Metabolic equivalent score.

‡Root mean square of successive R-R interval differences.

§Percentage of R-R intervals that differ  $> 50$  ms.

¶Low-frequency (0.05–0.15 Hz) power of R-R interval variability.

††High-frequency (0.15–0.4 Hz) power of R-R interval variability.

‡‡Baroreflex sensitivity sequence index calculated from up–up sequences.

§§Baroreflex sensitivity sequence index calculated from down–down sequences.

¶¶Cross-spectral transfer gain in the low-frequency range.

several indices were recorded, which yielded results to a more complex evaluation of cardiovascular autonomic neural function.

As cardiovagal function is age- and gender-dependent [20], all of our parameters were corrected for age and gender (model 1). A negative correlation between some autonomic function indices and anthropometric measurements was documented in our study, which is in line with observations of others [20]. A negative correlation was documented between fasting blood glucose and autonomic measurements, which corresponds to previous observations that abnormal cardiovascular reflex tests are associated with higher HbA<sub>1c</sub> values in patients with diabetes [21]. In our model 2 analysis, all measurements were adjusted not only for age and gender but for all covariates associated significantly with autonomic function indices. In this way, genetic influence found in model 1 disappeared.

The first large clinical study to examine the heritability of heart rate variability was performed within the framework of the Framingham Heart Study, where first-degree relatives and unrelated subjects (spouse pairs) were investigated, and the findings suggested that genetic factors have a substantial contribution to the variance of heart rate [22]. In another study, inheritance of heart rate variability was investigated among kibbutzim family members and the results proved to be inconsistent [23]. In a subsequent twin study, ambulatory heart rate variability measures proved to be heritable [24]. In a small study with normal twins, genetic influence was found on baroreflex function [16]. Finally, a polymorphic variation in the choline transporter gene was found to be associated significantly with heart rate variability indices related to

parasympathetic (cholinergic) activity [25]. Our study was not confirmative in this respect because we clearly documented in a Hungarian twin cohort that environmental factors have a major influence on heart rate variability and baroreflex sensitivity. Namely, 85.5–96.5% of the total variance of autonomic indices is attributable to unique environmental factors. In addition, the comparison of the results of the model 1 and model 2 analyses indicated that at least 11.3–35.0% of the unique environmental influence is explained by the combined effects of anthropometric measurements and traditional cardiovascular risk factors. The discrepancies between the results of our and former studies can be explained by the diverse populations investigated and the different methods used. It is noteworthy that the Hungarian population is at increased risk of cardiovascular morbidity and mortality compared with other European countries, which is largely attributed to environmental factors operating regionally [26]. This phenomenon might contribute to the strong environmental influence detected in our study. Moreover, heritability can be assessed more appropriately in twin studies comparing monozygotic to dizygotic pairs, who share 100 and 50% of their genome, respectively. On the contrary, cohort studies with closely related and unrelated individuals rely on assumptions regarding the genetic background of the participants. In addition, a complex evaluation using a broad range of heart rate variability and baroreflex sensitivity indices in our study could yield a more comprehensive assessment of autonomic nervous function than measuring a single parameter or a few indices only. Although the genetic case–control association analysis studying the effect of a choline transporter gene polymorphism [25] is of interest, the results have to be

**Table 3** Parameter estimates for additive genetics (A), common environmental (C) and unique environmental (E) influences on autonomic function indices by structural equation modeling

		Parameter estimates				
		-2LL	P-value	A (95% confidence interval)	C (95% confidence interval)	E (95% confidence interval)
<b>RMSSD‡</b>						
Model 1	A-C-E	-1073.7	0.5645	0.244 (0.000–0.603)	0.088 (0.000–0.443)	0.668 (0.422–0.891)
	C-E	-1074.0				
Model 2	A-C-E	-3522.6	1.0000	0.000 (0.000–0.065)	0.095 (0.000–0.462)	0.905 (0.578–1.000)
	C-E	-3522.6				
<b>pNN50§</b>						
Model 1	A-C-E	-1380.3	1.0000	0.000 (0.000–0.582)	0.332 (0.031–0.551)	0.668 (0.454–0.889)
	C-E	-1380.3				
Model 2	A-C-E	-4046.0	1.0000	0.000 (0.000–0.127)	0.035 (0.000–0.345)	0.965 (0.667–1.000)
	C-E	-4046.0				
<b>LF¶</b>						
Model 1	A-C-E	-1327.6	1.0000	0.000 (0.000–0.231)	0.186 (0.000–0.425)	0.814 (0.503–1.000)
	C-E	-1327.6				
Model 2	A-C-E	-2120.9	1.0000	0.000 (0.000–0.253)	0.073 (0.000–0.425)	0.927 (0.666–1.000)
	C-E	-2120.9				
<b>HF††</b>						
Model 1	A-C-E	-1344.9	0.8034	0.103 (0.000–0.533)	0.208 (0.000–0.472)	0.689 (0.443–0.916)
	C-E	-1344.9				
Model 2	A-C-E	-5539.7	1.0000	0.000 (0.000–0.000)	0.062 (0.000–0.374)	0.938 (0.672–1.000)
	C-E	-5539.7				
<b>BRSseq+‡‡</b>						
Model 1	A-C-E	-995.9	1.0000	0.000 (0.000–0.429)	0.317 (0.000–0.529)	0.683 (0.504–0.837)
	C-E	-995.9				
Model 2	A-C-E	-5195.6	1.0000	0.000 (0.000–0.000)	0.145 (0.000–0.445)	0.855 (0.580–1.000)
	C-E	-5195.6				
<b>BRSseq-§§</b>						
Model 1	A-C-E	-1025.0	0.3159	0.394 (0.000–0.715)	0.000 (0.000–0.494)	0.606 (0.362–0.789)
	C-E	-1026.0				
Model 2	A-C-E	-5515.7	1.0000	0.000 (0.000–0.416)	0.044 (0.000–0.453)	0.956 (0.631–1.000)
	C-E	-5515.7				
<b>LFgain¶¶</b>						
Model 1	A-C-E	-1113.6	0.6143	0.221 (0.000–0.586)	0.024 (0.000–0.407)	0.756 (0.489–0.993)
	C-E	-1113.6				
Model 2	A-C-E	-4545.3	1.0000	0.000 (0.000–0.726)	0.101 (0.000–0.451)	0.899 (0.457–1.000)
	C-E	-4545.3				

Model 1, crude model, adjusted only for age and gender.

Model 2, adjusted for all covariates associated significantly with autonomic function indices (see Table 2).

‡Root mean square of successive R-R interval differences.

§Percentage of R-R intervals that differ > 50 ms.

¶Low-frequency (0.05–0.15 Hz) power of R-R interval variability.

††High-frequency (0.15–0.4 Hz) power of R-R interval variability.

‡‡Baroreflex sensitivity sequence index calculated from up–up sequences.

§§Baroreflex sensitivity sequence index calculated from down–down sequences.

¶¶Cross-spectral transfer gain in the low-frequency range.

corroborated in a different population or by at least a family-based transmission–disequilibrium analysis.

It is widely accepted that reduced heart rate variability and baroreflex sensitivity reflects a shift in cardiac sympathovagal balance from parasympathetic to sympathetic control over heart rhythm and this alteration could contribute to the increase of cardiovascular morbidity and mortality. Several clinical studies documented that cardiovascular autonomic neuropathy, assessed either by using classical cardiovascular function tests

[18] or by measuring time and frequency domain parameters of heart rate variability [19], might contribute to adverse cardiac outcome in patients with diabetes mellitus [5]. Our study documented that unshared environmental factors have considerable effect on cardiovascular autonomic function, whereas genetic background has no or minimal influence. It is noteworthy that this was observed in twin pairs with normal physiologic function of the autonomic nervous system. Therefore, our findings should be interpreted cautiously in

patients with signs or symptoms of cardiovascular autonomic neuropathy. Clearly, the generalizability of our findings needs further investigation in patients with autonomic dysfunction. Nevertheless, it can be assumed that improvement in cardiovascular autonomic neuropathy is more achievable, or deterioration of heart rate variability can be easily prevented with appropriate medical intervention or lifestyle changes, as environmental but not genetic factors have substantial influences on the phenotypic variation of heart rate variability. Accordingly, a significant improvement in cardiovascular autonomic dysfunction was observed in patients with Type 1 diabetes treated with intensive vs. conventional insulin treatment in the Diabetes Control and Complications Trial (DCCT) cohort and, interestingly, the benefits of former intensive therapy extend to measures of cardiovascular autonomic neuropathy up to 14 years after the close of the DCCT [27]. In patients with Type 2 diabetes, a multifactorial treatment approach resulted in improvement of autonomic neuropathy in the STENO-2 study [28]. Besides appropriate blood glucose control and cardiovascular risk management, specific drugs, such as  $\alpha$ -lipoic acid, proved to be, to some extent, beneficial for treating cardiovascular autonomic neuropathy in patients with Type 2 diabetes [7].

Our study has some limitations. The sample size was modest. The zygosity in our twin cohort was classified according to validated questionnaires. Nevertheless, this method is widely accepted in clinically oriented twin studies [8]. Our results were derived from a healthy twin population with normal cardiac autonomic function, therefore the extrapolation of our findings to patients with cardiovascular diseases or diabetes and autonomic dysfunction has some limitations. Although diabetes mellitus was considered as an exclusion criterion, only fasting blood glucose values were investigated and an oral glucose tolerance test was not carried out in our cohort. Our heritability model assumes equal common environmental influences on both monozygotic and dizygotic twin pairs. If this assumption does not hold, the estimate of heritability may be biased. Although short-term heart rate variability measurements are subject to day-to-day variations, random error represents a limited part of the between-subjects variability and observed differences between individuals mostly reflect differences in the subjects' error-free value rather than random error [29]. For improving reliability of heart rate variability measurements, breathing rate was paced in our cohort [29]. The strengths of our study should be also pointed out. For evaluating cardiovascular autonomic function, a broad range of indices was evaluated. Both heart rate variability and baroreflex sensitivity measurements were performed by the same investigators (JO and TH) using the same equipment. To the best of our knowledge, this is the first classical twin study documenting that alterations of heart rate variability and baroreflex sensitivity are primarily related to unique environmental rather than inheritable factors.

In conclusion, our study documented that unshared environmental but not inheritable factors have substantial influence on cardiovascular autonomic function. Our data

should be considered suggestive for prevention or treatment of autonomic cardiac dysfunction. Undoubtedly, proper management of all modifiable environmental factors by lifestyle changes or medical therapy is of importance in order to prevent or ameliorate cardiovascular autonomic neuropathy in patients with different diseases, including diabetes mellitus.

## Competing interests

Nothing to declare.

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