Heritability of Indices for Cardiac Contractility in Ambulatory Recordings

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Heritability of Cardiac Contractility. ***Introduction:*** **Overactivity of the sympathetic nervous system (SNS) plays a pivotal role in the development of cardiovascular disease. This involvement suggests that the genetic susceptibility to adverse cardiovascular events may derive in part from individual differences in SNS activity.**

Methods and Results: **To establish a genetic contribution to SNS activity, we measured sympathetic effects on cardiac contractility in 755 healthy adult twins and their singleton siblings. The preejection period (PEP) and the ratio of PEP to the left ventricular ejection time (PEP/LVET ratio) were derived from ambulatory recordings of the ECG and thorax impedance. During this type of prolonged recordings in a real life setting, the extent of cardiac sympathetic activity will vary with the demands of daily activities. Therefore, the genetic architecture of both indices was examined separately across three daytime periods (morning, afternoon, evening), and during nighttime sleep. Results showed significant genetic contribution to PEP (48–62%) over all daily periods. Heritability estimates for PEP/LVET ratio ranged between 35% and 58%. Cardiac sympathetic activity during the waking and sleep periods was largely influenced by genetic factors that were common to the entire 24-hour period. During sleep, additional genetic influences emerged that accounted for 8% of the variance in PEP.**

Conclusion: **Impedance-derived measures of sympathetic effects on cardiac contractility show substantial heritability across all periods of the day and during sleep.** *(J Cardiovasc Electrophysiol, Vol. 17, pp. 877-883, August 2006)*

impedance cardiography, genetics, twins, sympathetic nervous system, contractility, ambulatory

Introduction

Chronic autonomic imbalance is well recognized as a potent risk factor for cardiovascular morbidity and mortality.[1] As many studies have shown, increased sympathetic nervous system (SNS) activity plays a pivotal role in the development of hypertension,[2-4] myocardial infarction (MI),[5] and tachycardia, the latter favoring arrhythmias.[6,7] Finally, SNS activity strongly influences the clinical progression of heart failure.[8-10] All these adverse cardiovascular events have a strong genetic component.[11-14] Individual differences in SNS activity may well account for this genetic susceptibility. There is an unfortunate lack of information, however, on the influence of hereditary factors on individual differences in SNS activity. Such information might provide new angles for future linkage and association studies attempting to unravel the genetic etiology of cardiovascular disease.

The preejection period (PEP) is a widely used, valid index of beta-adrenergic effects on cardiac contractility.[15,16] Because PEP is sensitive to changes in preload, the ratio of PEP to left ventricular ejection time (LVET) has been proposed as an alternative measure, although the relative merit of this ratio over PEP remains controversial.[17,18] A shortened PEP and an increase in the PEP/LVET ratio both signal increased inotropic control, i.e., larger sympathetic drive to the left ventricle. In the present study, impedance cardiography[17,19] was used to measure the systolic time intervals (PEP, LVET) across a 24-hour period in a large group of healthy twin families. Repeated ambulatory 24-hour measurements have shown that individual differences in ambulatory PEP and LVET are very stable.[20] An extended twin design (twins and siblings) was used to estimate the genetic contribution to these stable individual differences in cardiac sympathetic activity. The ambulatory and long-term nature of the measurements offers the opportunity to examine potential sleep-wake differences in the genetic architecture of cardiac SNS activity.

Methods

Subjects

All participants were registered with the Netherlands Twin Register. Their families were originally invited for a genetic linkage study searching for genes influencing anxiety and depression, which has been described elsewhere.[21,22] Of the

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first 1,332 offspring who returned a DNA sample for this study, 1,008 were successfully contacted for a cardiovascular ambulatory monitoring study. Of these, 174 subjects refused and 18 were excluded for various reasons (pregnancy, heart transplantation, presence of a pacemaker and known ischemic heart disease, congestive heart failure, or diabetic neuropathy). A final 816 subjects were eligible and willing to participate in cardiovascular ambulatory monitoring. Data from 34 participants on cardioactive medication (including all beta-blockers) were discarded. For 14 additional subjects, recordings were unavailable due to equipment failures, while 13 subjects had either a noisy ECG or impedance cardiogram (ICG) signal, and were therefore excluded. The final sample consisted of 215 identical twins (77 men), 296 fraternal twins (107 men), and 244 of their siblings (94 men) from 339 families. Zygosity of the twins was determined by DNA typing. The Ethics Committee of the Vrije Universiteit approved the study protocol and all subjects gave written consent before entering the study.

Procedure

The ambulatory recording procedure has been described previously.[23] Briefly, participants were visited at home and the VU-AMS ambulatory ECG/ICG device was attached together with an ambulatory blood pressure monitor (Spacelabs Medical, Inc.). Subjects were instructed to wear the VU-AMS for 24 hours and the blood pressure monitor until going to sleep and to keep a detailed diary during this period. Every 30 ($\pm$10) minutes the VU-AMS device produced an audible alarm to prompt them to write down a chronological account of activity, posture, location, and social situation during the past 30 minutes.

Impedance Cardiography

The VU-AMS (version 4.6) measures the ECG, the thorax impedance (Z_0), the changes in impedance (ΔZ), and the ICG continuously from a six-electrode configuration.[24-26] In addition, it measures vertical acceleration, which is used as a proxy for gross body movement. The technical specifications of the recording technique have been published previously.[24-26] The obtained dZ/dt signal of each 60-second period was ensemble averaged with reference to the R-wave.[27] This assembled dZ/dt waveform will be referred to as a "60-second ensemble average." Ensemble averaging reduces the impact of single-beat fluctuations in the ICG through respiration and slow thorax movement. Systolic time intervals scored in the 60-second ensemble-averaged ICG correspond very closely to the mean systolic time intervals obtained over the (reliable) single-beat ICG waves in that same minute.[27-30]

Data Reduction

Using the diary entries combined with the vertical accelerometer signal and the heart rate, the entire recording was divided into periods that were defined by posture, activity, location, and social situation. To reduce the amount of visual inspection needed, the same ensemble averaging strategy used to obtain 60-second averages from single-beat waveforms was applied to obtain large-scale ensemble averages (LSEA) across these periods. A previous ambulatory study by Riese et al.[25] showed that such a LSEA validly recaptures the information in the original 60-second ensemble averages, while substantially reducing the total amount of visual inspection time needed.

Postural changes and physical activity, affecting among others preload, afterload, and the electrical axis of the heart, may influence the duration of the PEP and LVET.[31,32] The present study, therefore, only included periods during which subjects were either sitting (daytime) or lying (nighttime).

ICG Waveform Scoring

Systolic time intervals and the dZ/dt_{min} were manually scored with a VU-AMS interactive software program (www.psy.vu.nl/vu-ams) that graphically displayed both the 60-second ensemble averages and the LSEA of the dZ/dt signal. Three time points were scored: the upstroke, the dZ/dt_{min} point, and the incisura (see Fig. 1). Occasional fragments of the dZ/dt, where it was not feasible to identify the three ICG waveform characteristics with certainty, were removed from the final daily period averages. Less than 2% of these ensemble averages had to be excluded for any of the subjects.

The PEP is defined as the time between the onset of the electromechanical systole (Q-wave onset) and the onset of left ventricular ejection at the opening of the aortic valves. As a proxy for the Q-wave onset we used the R-wave plus 48 msec, the rationale for this approach to PEP has been reported elsewhere.[25,26,31] We further calculated the ratio of PEP/LVET, which may be less dependent on preload.[18] Impedance-derived PEP and PEP/LVET ratio have convincingly been shown to be similar to their echocardiographic-derived counterparts.[33-35]

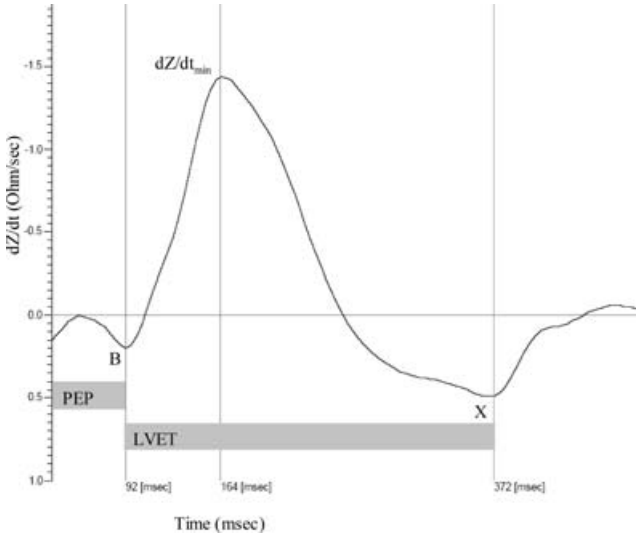

Figure 1. *Large-scale ensemble average of the impedance cardiogram (ICG) signal. The change in impedance across the heart cycle is plotted (Ohm/second, Y-axis) as a function of time (X-axis, msec). The first vertical line indicates the location of the B-point that represents the opening of the aortic valves at which blood starts to flow into the aorta (corresponding to the first heart sound). The second vertical line, at the top of the waveform, is the dZ/dt_{min} point corresponding to the time point of maximum velocity of the ejected blood. The third vertical line is the X-point or incisura, representing closure of the aortic valves (corresponding to the second heart sound). The PEP, or preejection period, is computed as the time from the R-wave to the B-point plus 48 msec (estimate of the fairly constant duration of the Q-R interval). The LVET, or left ventricular ejection time, is computed as the time between the B and X points.*

Statistical Analyses

Structural equation modeling

Genetic models were fit to the data on PEP and PEP/LVET ratio with the use of the structural equation modeling program Mx.[36] These models use the known difference in the genetic relationship between monozygotic (MZ) and dizygotic (DZ) twins/sibs to estimate to what extent additive and dominant genetic effects and shared environmental and nonshared environmental effects contribute to the variance in a trait. Shared environment (C) includes all effects on the trait shared by members of a family, e.g., diet, neighborhood, and family health practices. Nonshared, or unique environment (E), represents the environmental effects that are unique to each member of a family, plus measurement error. Additive genetic (A) effects derive from genes whose allelic effects combine additively. Nonadditive genetic effects include dominance (D), the interaction between alleles at the same locus, and epistasis, the interaction of alleles at different loci.

In a twin design that includes identical twins, fraternal twins and sibling pairs, estimates of C and D are confounded. The covariances between twins provide the sufficient information to test either a model with A, C, and E, or a model with A, D, and E. Inspection of the pattern of twin and sibling correlations was used to guide the most appropriate model for further analyses. The basic principles of structural equation modeling of twin data have been outlined elsewhere.[37,38] A detailed treatise on the statistical testing procedure is found in Neale and Cardon[39] and in Neale et al.[36]

A number of steps were taken to reduce complexity of the final genetic modeling. Because each additional sibling increases the complexity of the specified covariance matrices, we discarded data from seven siblings (1 male, 6 female) in a few families with more than four additional siblings. A series of increasingly constrained univariate models were fit for each period of the day and for each variable separately to test the homogeneity of means and variances for MZ twins, DZ twins, and siblings and for males and females. If homogeneity is found (e.g., the variance is the same in all sex by zygosity subgroups), a single parameter can be estimated instead of many parameters without loss of information. For the same purpose, homogeneity of male and female correlations, and of DZ twins and sibling pair correlations was tested.

After establishing the most parsimonious model (ACE or ADE, AE, CE, or E) for each daily period (univariate analysis), we used a full four-variate triangular decomposition to test whether the same or different genetic and environmental factors influenced cardiac contractility at each of the four periods of the day (morning, afternoon, evening, and night). The triangular decomposition imposes a structure of stratification on the shared latent factors (A, C or D, and E) such that there is a main factor that loads on, e.g., PEP at each of the four periods of the day, followed by a second factor that loads on all but the first period, followed by a third latent factor that loads on the final two periods. The final and fourth factor only loads on the last period. A priori, we expected a single genetic factor to underlie most of the variance throughout the day and night, with smaller genetic influences unique to each of the four periods of the day. The adequacy of the genetic one-factor model to describe the observed data was tested by contrasting it against the full triangular decomposition. Significance tests of the individual path coefficients were carried out by constraining paths to zero and applying likelihood ratio tests. Akaike's Information Criterion (AIC)[40] was used throughout to evaluate the relative fit of the various models.

Results

On average, the ambulatory monitoring period had a duration of 21 hours and 20 minutes (±4:14 hours), which included an average of 43 (±12) LSEA of the dZ/dt signal. Of these, 50.5% were recorded either during sitting or lying posture. Mean age of this twin population was 30.6 years (SD = 10.4). Means for PEP and PEP/LVET ratio for all periods during which subjects were sitting (daytime) or lying (nighttime) are presented in Table 1. Families were selected for participation based on the requirement that at least two members of a family scored extremely discordant or concordant on a factor score that indicated genetic vulnerability for *anxious depression*. Because of the recruitment of additional siblings in the selected families independent of their anxious depression scores, the distribution of the factor score approximated the normal distribution found in the population at large.[21] Yet, a small number of subjects attained clinical cut-offs for depression (n = 32) at the time of their ambulatory measurement. To test whether the sample could still be considered unselected for cardiovascular risk factors, the degree of their association to the subjects' anxious depression vulnerability score was computed. Only nonsignificant correlations were found.

The lower triangle of Table 2 contains the stability of PEP across the four periods of the day; the upper triangle likewise for the PEP/LVET ratio. Both PEP and PEP/LVET ratio appear very stable across the four periods of the day. As expected, PEP and PEP/LVET were highly correlated throughout the day (morning $r = 0.87$, afternoon $r = 0.88$, evening $r = 0.86$, and night $r = 0.92$).

Twin and Sibling Correlations

To determine the extent to which MZ twin pairs are more similar than DZ or sibling pairs, age-adjusted Pearson's correlation coefficients were calculated per zygosity, and per sex. All possible MZ and DZ/sibling pairs were used. The correlations are shown in Table 3. Throughout, a larger MZ than

TABLE 1

Means (SD) for Preejection Period (PEP), Left Ventricular Ejection Time (LVET), and PEP/LVET Ratio for Each Period of Day

	Number of Subjects	PEP (ms)	LVET (msec)	PEP/ LVET
Morning				
Men	265	96.4 (14.9)	292.4 (34.1)	0.34 (0.07)
Women	457	99.6 (18.5)	289.0 (35.7)	0.35 (0.09)
Afternoon				
Men	271	95.9 (14.2)	284.6 (32.7)	0.34 (0.07)
Women	468	98.2 (17.1)	285.0 (33.9)	0.35 (0.09)
Evening				
Men	264	95.7 (13.6)	293.2 (33.6)	0.33 (0.07)
Women	456	98.0 (16.2)	297.0 (35.2)	0.34 (0.08)
Night				
Men	289	105.4 (15.4)	334.7 (26.6)	0.32 (0.06)
Women	505	104.8 (15.3)	331.5 (27.2)	0.32 (0.06)

TABLE 2

Correlation of PEP and PEP/LVET Ratio Across the Four Periods of the Day

		PEP/LVET			
PEP		Morning	Afternoon	Evening	Night
	Morning		0.93	0.86	0.67
	Afternoon	0.96		0.89	0.69
	Evening	0.90	0.93		0.74
	Night	0.72	0.74	0.79	

Lower triangle: PEP; Upper triangle PEP/LVET ratio. Correlations were corrected for influences of age on the mean; all correlations are significant at $P < 0.000$.

DZ/sibling correlation is evident, suggesting the presence of additive genetic and unique environmental influences. For both PEP and the PEP/LVET ratio the majority of MZ twin correlations is more than twice as large as the DZ correlations, indicating the possible presence of dominance genetic effects. We, therefore, opted to model only A, D, and E effects, and no C effects. In addition, the opposite sex correlations for these variables are near zero, suggesting that different genes may be acting in males and females.

Structural Equation Modeling

There were no sex differences for mean values of PEP and PEP/LVET. Variances of PEP and PEP/LVET ratio significantly differed, however, between males and females. Further model fitting employed a scalar sex limitation[36,39] to account for these differences. With increasing age, the PEP/LVET ratio significantly decreased during all daily periods, and the PEP itself decreased with age during the night. The effects of age on the mean were taken into account in all further models.

For all variables, intrapair correlations of all same-sex non-MZ siblings, i.e., DZ twin-cotwin, sibling-twin, and sibling-sibling correlations were similar for all daily periods. This meant that these correlations could be estimated by a single parameter (denoted *r*DZ/sib in Table 3). As can be gauged from Table 3, the intrapair correlation of opposite sex pairs could be constrained at zero for PEP and PEP/LVET ratio. This means that different genetic effects operate in males and females.

TABLE 3

Twin Correlations for Preejection Period (PEP) and PEP/LVET Ratio for Each Period of Day

		PEP		PEP/LVET	
		***r*MZ**	***r*DZ/sib**	***r*MZ**	***r*DZ/sib**
Morning	Male pairs	0.71	0.38	0.63	0.28
	Female pairs	0.72	0.23	0.62	0.25
	Opposite sex pairs	-	0.01	-	−0.03
Afternoon	Male pairs	0.70	0.42	0.64	0.41
	Female pairs	0.73	0.24	0.64	0.19
	Opposite sex pairs	-	−0.01	-	−0.09
Evening	Male pairs	0.69	0.32	0.80	0.31
	Female pairs	0.64	0.21	0.48	0.22
	Opposite sex pairs	-	−0.05	-	−0.07
Night	Male pairs	0.70	0.25	0.62	0.22
	Female pairs	0.46	0.13	0.50	0.20
	Opposite sex pairs	-	−0.08	-	−0.07

Twin correlations were corrected for influences of age on the mean.
MZ = monozygotic twins; DZ = dizygotic twins.

TABLE 4

Multivariate Model Fitting Results for Preejection Period (PEP) and PEP/LVET Ratio

	Model	Versus	$\Delta\chi^2$	Δdf	P value	AIC
	PEP					
1	AE triangular	Full	9.035	10	0.529	−10.965
2	E triangular	Full	72.737	10	0.000	52.737
3	AE common + four specifics	1	2.975	2	0.226	−1.025
4	AE common + one specific	3	4.063	3	0.255	−1.937
5	AE common	4	5.463	1	0.019	3.463
	PEP/LVET ratio					
1	AE triangular	Full	6.283	10	0.791	−13.717
2	E triangular	Full	57.534	10	0.000	37.534
3	AE common + four specifics	1	4.777	2	0.092	0.777
4	**AE common + one specific**	**3**	**0.103**	**3**	**0.991**	**−5.897**
5	**AE common**	**4**	**3.08**	**1**	**0.079**	**1.08**

The most parsimonious model is printed boldfaced. $\Delta\chi^2$ = increase in chi square; Δdf = difference in degrees of freedom between models; AIC = Akaike's information criterion; Full = most parsimonious unconstrained model, against which the triangular models are tested. When increase in χ^2 is not significant ($P > 0.01$), the most restrictive model is accepted.
Explanation of the models:
[1]*AE triangular:* triangular variance decomposition model in which variance is explained by additive (A) and nonshared environmental (E) factors;
[2]*E triangular*: triangular variance decomposition model in which variance is explained by nonshared environmental factors only;
[3]*AE common + four specifics:* apart from a common genetic factor, four period-specific genetic factors explained genetic variance during the four periods of day;
[4]*AE common + one specific*: apart from a common genetic factor, a period-specific factor explained genetic variance at night only; and
[5]*AE common:* a single common genetic factor explained genetic variance at all four periods of the day.

For each of the variables, univariate models including only additive genetic and unique environment factors (AE models) gave the best fit over all other possible models (ADE, CE, or E) on each of the four daily periods. Multivariate AE models (see Table 4), with the four daily periods as consecutive measurements, were used to test general and daytime-specific heritability each of the variables. For PEP and PEP/LVET ratio alike, one genetic factor was responsible for the genetic influences on the individual variation throughout the 24-hour recording. Heritability estimates for the final most parsimonious models are presented in Table 5. Although all variables showed a decrease in heritability during the evening and a further decrease during the night, these were not significant. For PEP, an additional genetic factor emerged during sleep that accounted for 8% of the variance in nighttime PEP.

Discussion

Based on prolonged measurements in a real life setting, obtained in a sample of 755 healthy adult twins and singleton siblings, the present study assessed the heritability of sympathetic effects on cardiac contractility, measured by the PEP, both with and without controlling for individual

TABLE 5
Heritability (±95%CI) Estimates for Preejection Period (PEP) and PEP/LVET Ratio

	PEP		
	Common h^2	**Specific h^2**	**Total h^2**
Morning	0.62 (0.49–0.72)	-	0.62
Afternoon	0.62 (0.48–0.72)	-	0.62
Evening	0.55 (0.41–0.66)	-	0.55
Night	0.40 (0.27–0.52)	0.08 (0.01–0.15)	0.48
	PEP/LVET ratio		
	Common h^2	Specific h^2	Total h^2
Morning	0.58 (0.43–0.69)	-	0.58
Afternoon	0.56 (0.41–0.68)	-	0.56
Evening	0.48 (0.32–0.61)	-	0.48
Night	0.35 (0.19–0.51)	-	0.35

Heritability estimates (h^2) were corrected for influences of age on the mean.

differences in LVET. Genetic modeling showed that the PEP is a significantly heritable trait. Correction for individual differences in LVET mildly decreased heritability estimates, but this was not significant.

Daytime generally is associated with relative sympathetic dominance while nighttime is characterized by parasympathetic dominance.[41-43] To allow for the possibility that different genetic factors would affect sympathetic control of the contractility of the heart during waking and sleeping hours or during leisure (evening) and work (morning, afternoon) periods, the entire ambulatory impedance recording was split into four daily periods. Total genetic influence on variance in cardiac sympathetic control was found to be higher during the daytime than during the evening and lowest during the night. A common set of genes, however, influenced the variables during all three daytime periods and at night. At night, significant new genetic variance emerged for PEP. This is in keeping with studies in rodents on diurnal variation in gene expression in the heart.[44,45] These studies found variation in diurnal gene expression to be driven in part by the central circadian pacemaker, but also by changes from light to dark phases. The presence of the night-specific genetic influences on cardiac contractility would support the proposition by Young[46] that the presence of night-specific gene expression in the hearts of rodents may be extrapolated to humans. Because the specific genetic effects disappeared after correcting for LVET, we cannot exclude the possibility that they are due to preload effects caused by the change to a supine posture.

Chronic sympathetic hyperactivity and its physiological sequelae play a vital role in the development of hypertension and subsequent adverse cardiovascular events.[2-10] Indeed, previous clinical studies have shown that hypertensive patients are characterized by a decrease in PEP.[47] In addition, PEP has been positively correlated with the incidence of MI, and even predicts future MI events.[48] Subjects with a genetic make-up that gives rise to increased cardiac sympathetic activity, evident in a shorter PEP, may be at larger risk to develop hypertension and subsequent adverse cardiovascular events than subjects lacking such genetic susceptibility. Genes affecting between-subject variance in sympathetic control of cardiac contractility may be found at different levels. They may reflect individual differences in state of arousal linked to a different rate of sympathetic nerve firing, for instance as part of personality characteristics.[49,50] Individual differences in sympathetic cardiac drive may also derive from genes at a (neuro)physiological regulatory level. In the heart, beta-adrenergic receptors modulate cardiac function by controlling chronotropic and inotropic responses to catecholamines of the SNS. Hence, genes controlling catecholamine metabolism, neuronal norepinephrine reuptake,[4] beta-adrenergic receptor function, and signal transduction may be involved in determining the observed heritability of the two cardiac sympathetic indices. Apart from genes in the noradrenergic signal transduction pathway, genes involved in the dopamine system might also be involved. Dopamine affects sympathetic cardiac drive by negatively modulating its activity. Polymorphisms in dopamine receptor genes have been associated with increased SNS activity, and elevated blood pressure levels.[51]

The DOS correlation for PEP was close to zero, indicating that different genes play a role in individual differences in SNS activity in men and women. The most likely explanation for this sex difference is an interaction between adrenoceptor signaling and male and female sex hormones. Several studies have shown the presence of such interaction. A role for testosterone in adrenoceptor regulation is found in adipose tissue metabolism.[52] Recently, it was reported that testosterone regulates gene expression of the major calcium regulatory proteins in isolated ventricular myocytes.[53] This indicates that testosterone may very well contribute to the sex differences in genetic influences on cardiac function. A further role for female sex hormones is supported by several studies showing that estrogen inhibits β_1-adrenergic receptor activation on the heart,[54,55] thereby reducing sympathetic cardiac drive and decreasing the risk for ischemic heart disease in women.[56]

Finding the actual causal genes for cardiovascular diseases has proven a very difficult task. It is increasingly appreciated that genetic epidemiological studies of complex diseases may benefit from the use of more narrowly defined risk factors, or endophenotypes, over broadly defined disease phenotypes.[57] For future studies, we would suggest to use PEP (or PEP/LVET ratio) as an endophenotype in the search for genetic susceptibility causing high SNS activity at a young premorbid age. Genetic variation in this index of sympathetic inotropic drive was largely explained by a common set of genes acting throughout the day and night. This is advantageous for gene finding on at least two accounts. First, using multiple highly genetically correlated traits provides higher statistical power to find genes in linkage analysis.[58] Secondly, these genes, by virtue of having a pervasive influence on cardiac contractility across all situations, will also have the largest clinical relevance. We, therefore, conclude that this ambulatory impedance-derived index of cardiac contractility provides a useful trait for future gene-finding studies targeting hypertension, MI, and arrhythmias.

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