Original Article

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Heritability and genetic correlations of heart rate variability at rest and during stress in the Oman Family Study

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Introduction: Individual differences in heart rate variability (HRV) can be partly attributed to genetic factors that may be more pronounced during stress. Using data from the Oman Family Study (OFS), we aimed to estimate and quantify the relative contribution of genes and environment to the variance of HRV at rest and during stress; calculate the overlap in genetic and environmental influences on HRV at rest and under stress using bivariate analyses of HRV parameters and heart rate (HR).

Methods: Time and frequency domain HRV variables and average HR were measured from beat-to-beat HR obtained from electrocardiogram recordings at rest and during two stress tests [mental: Word Conflict Test (WCT) and physical: Cold Pressor Test (CPT)] in the OFS – a multigenerational pedigree consisting of five large Arab families with a total of 1326 participants. SOLAR software was used to perform quantitative genetic modelling.

Results: Heritability estimates for HRV and HR ranged from 0.11 to 0.31 for rest, 0.09–0.43 for WCT, and 0.07–0.36 for CPT. A large part of the genetic influences during rest and stress conditions were shared with genetic correlations ranging between 0.52 and 0.86 for rest-WCT and 0.60–0.92 for rest-CPT. Nonetheless, genetic rest–stress correlations for most traits were significantly smaller than 1 indicating some stress-specific genetic effects.

Conclusion: Genetic factors significantly influence HRV and HR at rest and under stress. Most of the genetic factors that influence HRV at rest also influence HRV during stress tests, although some unique genetic variance emerges during these challenging conditions.

Keywords: family study, genetic correlation, heart rate variability, heritability, Oman Family Study

Abbreviations: CPT, Cold Pressor Test; HR, heart rate; HRV, heart rate variability; IBI, interbeat intervals; OFS, Oman Family Study; PNS, parasympathetic nervous system; RMSSD, root mean square of the successive differences of normal-to-normal intervals; RSA, respiratory sinus arrhythmia; SDNN, standard deviation of normal-to-normal intervals; SNS, sympathetic nervous system; VLF, very low frequency; WCT, Word Conflict Test

INTRODUCTION

Heart rate variability (HRV) measures the beat-to-beat fluctuations in heart rate (HR) over time and reflects sinoartial responsiveness to fluctuations in parasympathetic input [1–4]. Prior studies have shown that a reduced HRV is associated with an increased risk of coronary heart disease [5], hypertension [6], cardiac mortality [7], and mortality from all causes [8].

HRV can be measured noninvasively using time-domain or frequency-domain methods. Both use the sequence of time intervals between heartbeats, that is, the time series of interbeat intervals (IBI) or normal-to-normal intervals to quantify the variability in the timing of the heartbeat. Two commonly used time-domain HRV measures, both reflecting principally parasympathetic modulation, are standard deviation of normal-to-normal intervals (SDNN) and the root mean square of the successive differences of normal-to-normal intervals (RMSSD). The frequency-domain method uses power spectral analysis of the IBIs to generate indirect estimates of both vagal and sympathetic neural

Journal of Hypertension 2018, 36:1477–1485

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Received 8 March 2017 **Revised** 10 January 2018 **Accepted** 14 February 2018

DOI:10.1097/HJH.0000000000001715

contributions to HR spectral power [1,2,4]. Frequency-domain HRV measures include: high frequency (0.15–0.40 Hz), low frequency (0.04–0.15 Hz), very-low frequency (VLF; 0.003–0.04 Hz), and total power [4,9].

Studies in healthy individuals have reported substantial individual differences for HRV that could partly be explained by demographic factors. For example, women in general have a higher HRV than men, and HRV is strongly inversely related to age [10–16]. Apart from demographic influences, genetic factors are another source of individual differences in HRV as observed in a limited number of family studies [17,18]. Sinnreich *et al.* based their study on 5-min Holter recordings from 451 kibbutz members and reported heritability estimates (h^2) of 41 and 39% for SDNN and RMSSD, respectively. Singh and colleagues investigated HRV from 2-h ambulatory recordings among siblings and found heritability estimates ranging from 13 to 19% for high frequency, low frequency, VLF, total power, and SDNN. In twin studies, HRV heritability estimates of up to 74% have been reported depending on the HRV trait analyzed [19,20]. Moreover, twin studies have indicated that genetic influences on HRV may be more pronounced during mental stress [19,21–23]. By applying a bivariate modelling approach to twin data De Geus *et al.* [22] found that heritability estimates of respiratory sinus arrhythmia (RSA) at rest in both adolescents ($h^2_{rest} = 31\%$) and middle-aged individuals ($h^2_{rest} = 32\%$) were augmented by mental stress tests (adolescents: $h^2_{stress} = 54\%$ and middle-aged: $h^2_{stress} = 44\%$) through the amplification of the same genetic factors already present at rest.

Most published studies regarding genetic influences on HRV have focused on individuals of European descent with only a few recent twin studies investigating the heritability of HRV in other ethnic groups [12,20,23]. For example, Wang *et al.* [23] found that heritability estimates of HRV both at rest and under stress in African Americans were similar to those of European Americans. In the current study, we estimated heritabilities and genetic correlations in the Oman Family Study (OFS) [24], a large study of multigenerational homogeneous Omani Arab pedigrees consisting of more than 1300 participants in which HRV was measured at rest and during stress. Our aims were to quantify the relative contribution of genes and environment to the variance of various HRV measurements by univariate analyses, calculate the overlap in genetic and environmental influences between HRV at rest and HRV under physical and mental laboratory stress tests, and discern between new (emerging) and preexisting (amplified/dampened) genetic and environmental influences during stress by using a bivariate quantitative genetic modelling approach of HRV at rest and under stress.

MATERIALS AND METHODS

Study population

The OFS was initiated in 2002 and consists of a homogeneous Arab population composed of five large, extended, and highly consanguineous families living in the Wilayat (state) of Nizwa, Oman [24]. Each family is currently living in separate villages. The geographical particularity of this area is that it is relatively isolated with a topography of mountains, oases, and seasonal river beds, which provides a more homogenous environmental exposure [25,26]. Furthermore, more than 50% of all marriages are first-cousin marriages with polygamy being widely practiced with some men marrying up to four wives. Supplementary Table 1, http://links.lww.com/HJH/A911 shows the total number of relative pairs in the five OFS pedigrees. A total of 1326 individuals were included in the OFS. A written and signed or thumb-printed rubber-stamped consent was obtained from each individual. The study was approved by the Medical Research and Ethics Committee of Sultan Qaboos University. A more detailed description of the OFS design can be found elsewhere [24].

Experimental procedure

Using the Task Force Monitor (TFM, CNSystems, Austria), six-lead ECG recordings were acquired in the supine position, in a quiet room with a temperature between 24 and 26 °C. Participants were made to rest for 10 min after which 10 min of resting beat-to-beat recordings were obtained, followed by a 3 min recording during the Word Conflict Test (WCT), and then 3 min of recovery or until the recording returned to baseline. The same procedure was then repeated for the Cold Pressor Test (CPT). Participants were monitored throughout to ensure that arrhythmias did not arise at rest or during interventions. Throughout the study, tests and measurements were administered by a research assistant of the same sex as the study participant [25,26]. See Supplementary for details, http://links.lww.com/HJH/A911.

ECG and HR data were available for 1223 individuals and HRV data for 1215 participants. Almost all had data for the CPT ($n = 1178$), but considerably fewer individuals participated in the WCT ($n = 666$) mainly because of the relatively high degree of illiteracy observed among the older OFS participants.

Heart rate variability and heart rate measurements

Beat-to-beat HR was obtained from lead II of the ECG [25,26,28] with sampling frequency of 1000 Hz. The algorithm used by the Task Force Monitor corrected for artefacts such as ventricular ectopy [29]. For the time-domain measurements, the normal-to-normal interval time series were processed in Microsoft Excel (Microsoft Corp., Redmond, Washington, USA) and only intervals between 300 and 2000 ms in length and with successive normal-to-normal interval ratios between 0.8 and 1.2 [23,30] were used to calculate SDNN and RMSSD. For the frequency domain measurements, the TFM used an adaptive autoregressive model [29] for power spectral analysis of ECG tracing free of ectopic beats to decompose the time series of normal-to-normal-intervals into its frequency components: high frequency (0.15–0.4 Hz), low frequency (0.04–0.15 Hz), VLF (0.003–0.04 Hz), and total power (approximately <0.4 Hz) according to standard guidelines [4].

Statistical analysis

To obtain better approximation of normal distributions, all HRV measurements and HR were transformed by natural logarithm. Participants ($n = 10$) that had more than 5% of

their ECG signals outside our criteria (i.e. normal-to-normal-intervals between 300 and 2000 ms and successive normal-to-normal-interval ratios between 0.8 and 1.2) were excluded. Measurements ($n = 19$) deviating more than 4 SD from the mean for a trait, were set to missing for the corresponding trait. A Student's *t*-test was used to calculate the significance of sex differences in means. A paired *t*-test (rest versus WCT or CPT) was used to test if trait levels were significantly different during stress compared with rest (i.e. stress reactivity).

SOLAR (v7.2.5), a Sequential Oligogenic Linkage Analysis Routines software package for genetic analysis [31], was used to perform univariate and bivariate analyses. See Supplementary for details, http://links.lww.com/HJH/A911. As sensitivity analyses, we also performed both univariate and bivariate analysis for rest and CPT only within the younger subsample of participants ($n = 666$) who participated in the WCT and had data on at least one outcome variable.

Bivariate quantitative genetic analyses were conducted to estimate the genetic and environmental correlations between rest and during each stress test (WCT and CPT) for each HRV trait and HR. With the additive genetic correlation, r_G, the extent of genetic effects common to the two traits being analyzed is determined. To determine the significance of shared genetic effects ($r_G > 0$), r_G was first estimated and subsequently fixed to zero in a nested sub-model allowing for a comparison of the two models using a likelihood ratio test. Similarly to determine the significance of incomplete overlap of genetic effects ($r_G < 1$), r_G was fixed to one and compared with the more general model in which it was freely estimated. An $r_G = 0$ means that two traits being analyzed are influenced by independent genetic factors. If $|r_G| = 1$, the genetic factors are completely shared, that is, complete pleiotropy [32,33].

Considering the OFS characteristic of high consanguinity between the five pedigrees, all participants in the cohort were considered as a single family pedigree [26] whenever calculating heritability. BMI, sex, age, and age^2 were included as covariates. A *P* value greater than 0.05 was considered statistically significant.

Linkage analysis

We performed a multipoint linkage analysis in SOLAR [31] on HRV and HR measurements at rest and during stress (i.e. WCT and CPT) in the OFS, similar to Hassan *et al.* [26]. The thresholds for significant (LOD $\geq$3.0) and suggestive (LOD $\geq$2.0) linkage were given by Lander and Kruglyak [34].

RESULTS

Study population

Table 1 shows the descriptive characteristics, HRV and HR measurements at rest of men and women. Overall, the total number of 1326 participants had a median age (interquartile range, IQR) of 28 [21–44] years. Male participants were significantly taller and weighed more compared to women, but there was no significant difference in age or BMI between the two sexes. At rest, men had significantly higher HRV values for all measures (but lower HR) compared with women. Figure 1 shows the difference between rest and stress (both WCT and CPT) for the HRV and HR measurements. For the WCT, HR showed a significant increase under stress compared with rest and significant decreases were observed for all HRV traits, except for VLF, which showed a nonsignificant increase. For CPT, under stress versus rest, RMSSD and high frequency showed a significant decrease, whereas SDNN, low frequency, VLF, total power, and HR showed a significant increase.

Heritability and genetic correlation analysis

Univariate narrow sense h^2 estimates for the HRV measurements ranged from 11 to 22% at rest; from 9 to 38% during WCT; and from 7 to 22% during CPT (Table 2). HR was consistently more heritable with estimates of $31 \pm 6\%$ at rest; $43 \pm 10\%$ during WCT; and $36 \pm 6\%$ during CPT. VLF h^2 failed to reach significance during WCT but it was significant at rest and during CPT. Overall the h^2 estimates of HRV for WCT are larger than the ones at rest, whereas those for CPT are similar to the ones at rest. However, if the analyses are limited to the younger sample of individuals who participated in the WCT, we also see a general increase in HRV heritability estimates for the CPT compared with

TABLE 1. Descriptive statistics of men and women of basic characteristics, heart rate variability measurements, and heart rate at rest

Characteristics	Men		Women		*P* value
	N	Statistic[a]	*N*	Statistic[a]	
Age (years)	583	27.0 [20.0–42.0]	743	30.0 [22.0–45.0]	NS
Height (m)	571	1.66 (0.73)	729	1.52 (0.55)	<.001
Weight (kg)	571	67.0 [58.0–77.0]	729	56.0 [49.6–67.0]	<.001
BMI (kg/m^2)	571	24.6 [20.9–28.2]	729	24.7 [21.4–28.8]	NS
SDNN (ms)	540	64.8 [48.1–82.0]	675	51.0 [37.7–65.9]	<.001
RMSSD (ms)	539	39.5 [28.6–56.1]	675	34.1 [22.8–50.3]	<.001
lnHF (ms^2)	503	10.9 (1.4)	615	10.3 (1.5)	<.001
lnLF (ms^2)	504	11.1 (1.1)	614	10.3 (1.3)	<.001
lnVLF (ms^2)	504	10.4 (1.1)	614	9.6 (1.2)	<.001
lnTP (ms^2)	504	12 (1.1)	614	11.4 (1.2)	<.001
low frequency/high frequency	504	1.13 [0.73–1.64]	614	0.97 [0.65–1.48]	<.05
HR (beats/min)	543	66.8 [61.3–73.8]	680	72.5 [66.0–79.2]	<.001

HF, high frequency; HR, heart rate; LF, low frequency; ms, milliseconds; NS, not significant; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; TP, total power; VLF, very-low frequency.
[a]Data expressed as mean (SD) or median [IQR] in case of skewed distributions.

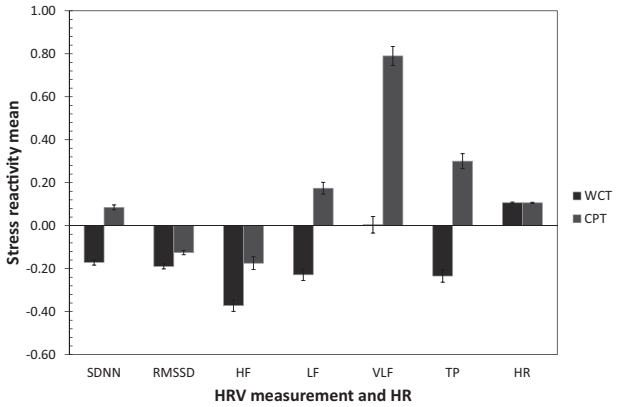

FIGURE 1 Stress reactivity of Word Conflict Test (WCT) and Cold Pressor Test (CPT) for log-transformed heart rate variability measurements and heart rate. HF, high frequency; HR, heart rate; LF, low frequency; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; TP, total power; VLF, very low frequency. Error bars show standard error. #Stress reactivity = ln(STRESS) − ln(REST) = ln(STRESS/REST).

rest, except for VLF and LFHF ratio (Supplementary Table 3, http://links.lww.com/HJH/A911).

The bivariate analyses showed that r_G were positive (0.52–0.86 between rest and WCT; 0.60–0.92 between rest and CPT) and significantly different from zero for all HRV measurements and HR between rest and both stress tests, except for VLF ($r_G = 0.52 \pm 0.30$), which was not significantly different from zero between rest and WCT (Table 3). Between rest and CPT, all HRV measurements and HR were significantly different from zero. In addition, r_G between HRV and HR at rest and under stress appeared to be significantly different from 1, except for low frequency ($r_G = 0.92 \pm 0.07$) and total power ($r_G = 0.82 \pm 0.18$) for CPT and VLF for WCT. The environmental correlation (r_E) was found to be positive and significant for all HRV measurements and HR between rest and WCT (0.58–0.87) and between rest and CPT (0.29–0.75). From the proportions of genetic and environmental factors contributing to the phenotypic correlations, it can be seen that for both stress conditions environmental factors contributed substantially more compared with genetic factors for all HRV measurements and HR, which is a reflection of the relatively large effect of the environmental variance components compared with the h^2 estimates. Results of the bivariate analyses in the smaller and younger subsample limited to participants who participated in the WCT were very similar (Supplementary Table 4, http://links.lww.com/HJH/A911).

We also performed bivariate analysis on both stress levels (i.e. WCT versus CPT) for all HRV measurements and HR and found the r_G significantly different from zero ranging from 0.60 to 1.00 (for low frequency, VLF, and total power: $r_G = 1$), and the r_E ranged from 0.30 to 0.81 (Table 4). The genetic correlations for HR, SDNN, and RMSSD were significantly different from 1 as well. Results of the bivariate analyses between WCT and CPT in the smaller and younger subsample limited to participants who participated in the WCT were very similar (Supplementary Table 5, http://links.lww.com/HJH/A911).

Linkage analysis

From the linkage analysis (Fig. 2) we found for SDNN and high frequency on chromosome 6 overlapping signals for rest, WCT and CPT – with at rest showing the highest LOD score (SDNN LOD = 2.52, marker between D6S12D05 and D6S1051; and high frequency LOD = 2.06, marker D6S032Z). For RMSSD on chromosome 12 (highest LOD score = 2.26, marker D12S1294) – and for low frequency on chromosome 7 (highest LOD = 2.3, marker between D7S2477 and D7SA119B), we observed single suggestive linkage signals specific for WCT and rest, respectively. The only significant linkage signal was for HR on chromosome 3 (LOD: 4.02, marker between D3S1766 and D3ST128), which was only found for WCT.

DISCUSSION

In this study, we quantified the contribution of genes to the variance of HRV and HR at rest and under mental and physical stress and the extent to which genes overlap

TABLE 2. Heritability estimates for log-transformed heart rate variability measurements and heart rate during rest and two stress tests (Word-Conflict Test and Cold Pressor Test)

HRV measurements and HR	REST			WCT			CPT		
	N	h^2 (SE)	Proportion of variance because of covariates	*N*	h^2 (SE)	Proportion of variance because of covariates	*N*	h^2 (SE)	Proportion of variance because of covariates
lnSDNN	1215	**0.14** (0.04)	0.13	666	**0.28** (0.09)	0.09	1178	**0.12** (0.04)	0.17
lnRMSSD	1214	**0.19** (0.05)	0.18	664	**0.36** (0.10)	0.03	1178	**0.18** (0.05)	0.16
lnHF	1118	**0.16** (0.05)	0.14	640	**0.17** (0.08)	0.05	1068	**0.13** (0.05)	0.13
lnLF	1118	**0.12** (0.04)	0.23	640	**0.21** (0.09)	0.10	1068	**0.16** (0.05)	0.25
lnVLF	1118	**0.11** (0.04)	0.13	640	0.09 (0.07)	0.14	1068	**0.09** (0.04)	0.12
lnTP	1118	**0.12** (0.04)	0.16	640	**0.13** (0.09)	0.12	1068	**0.07** (0.04)	0.15
Ln(low frequency/high frequency)	1115	**0.22** (0.05)	0.03	639	**0.38** (0.09)	0.07	1066	**0.22** (0.05)	0.04
lnHR	1223	**0.31** (0.06)	0.09	700	**0.43** (0.10)	0.08	1217	**0.36** (0.06)	0.16

Covariates BMI, sex, age, and age^2 were included in the analyses. Bold numbers are statistically significant: *P* value less than 0.05. CPT, Cold Pressor Test; HF, high frequency; HR, heart rate; LF, low frequency; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; TP, total power; VLF, very-low frequency; WCT, Word Conflict Test.

TABLE 3. Bivariate quantitative analyses of log-transformed heart rate variability measurements and heart rate examining the genetic (r_G), environmental (r_E) and phenotypic (r_P) correlations between rest and the Word Conflict Test (WCT) and Cold Pressor Test (CPT)

		WCT						CPT					
		h^2 (SE)						h^2 (SE)					
HRV measurements and HR	N_{max}	REST	WCT	Genetic correlation r_G (SE)	Environmental correlation r_E (SE)	Phenotypic correlation r_P	Proportions of r A/E[a]	REST	CPT	Genetic correlation r_G	Environmental correlation r_E	Phenotypic correlation r_P	Proportions of r A/E[a]
lnSDNN	1214	0.14 (0.05)	0.25 (0.07)	**0.62**[b] (0.14)	0.71 (0.03)	0.69	0.17/0.83	0.14 (0.05)	0.12 (0.04)	**0.60**[b] (0.15)	0.60 (0.03)	0.60	0.13/0.87
lnRMSSD	1214	0.19 (0.05)	0.27 (0.07)	**0.75**[b] (0.09)	0.83 (0.02)	0.81	0.21/0.79	0.19 (0.05)	0.18 (0.05)	**0.77**[b] (0.09)	0.75 (0.02)	0.75	0.19/0.81
lnHF	1120	0.17 (0.05)	0.19 (0.07)	**0.86**[b] (0.06)	0.87 (0.02)	0.87	0.18/0.82	0.16 (0.05)	0.13 (0.05)	**0.82**[b] (0.08)	0.74 (0.02)	0.75	0.16/0.84
lnLF	1119	0.14 (0.05)	0.20 (0.07)	**0.86**[b] (0.08)	0.78 (0.02)	0.79	0.18/0.82	0.13 (0.04)	0.17 (0.05)	**0.92** (0.07)	0.59 (0.03)	0.64	0.21/0.79
lnVLF	1119	0.11 (0.04)	0.07 (0.05)	0.52 (0.30)	0.60 (0.04)	0.59	0.07/0.93	0.11 (0.04)	0.09 (0.04)	**0.66**[b] (0.22)	0.29 (0.04)	0.32	0.20/0.80
lnTP	1119	0.11 (0.04)	0.10 (0.06)	**0.72**[b] (0.16)	0.78 (0.02)	0.77	0.10/0.90	0.11 (0.04)	0.07 (0.04)	**0.82** (0.18)	0.48 (0.03)	0.51	0.15/0.85
Ln(low frequency/high frequency)	1119	0.22 (0.05)	0.31 (0.07)	**0.72**[b] (0.11)	0.58 (0.05)	0.61	0.30/0.70	0.21 (0.05)	0.22 (0.05)	**0.72**[b] (0.10)	0.48 (0.04)	0.53	0.29/0.71
lnHR	1224	0.32 (0.06)	0.41 (0.07)	**0.85**[b] (0.06)	0.79 (0.03)	0.81	0.38/0.62	0.31 (0.06)	0.36 (0.06)	**0.79**[b] (0.06)	0.73 (0.03)	0.75	0.35/0.65

Covariates: BMI, age, sex, age^2. HF, high frequency; HR, heart rate; LF, low frequency; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; TP, total power; VLF, very-low frequency. Bold numbers are genetic correlation significantly different from zero

[a]A/E is the percentage of the phenotypic correlation that is caused by genes (A) or environment (E), based on the following equation [39]. $r_P = (\sqrt{h^2_{rest}} \times r_G \times \sqrt{h^2_{stress}}) + (\sqrt{e^2_{rest}} \times r_E \times \sqrt{e^2_{stress}})$.

[b]Genetic correlations are significantly different from 1.

between rest and stress conditions in a large homogeneous Arab population. We found that genetic factors significantly influence HRV and HR at rest in this Arab population and that the heritability estimates increased particularly under mental stress, but not under physical stress. Furthermore, most traits showed large shared genetic influences between rest and stress conditions, but in comparison with rest, there were also modest stress-specific genetic effects.

This is the first family study aiming at disentangling genetic and environmental factors that influence HRV at rest and during stress using bivariate analysis. We showed that there is a significant overlap of genetic factors between rest and both WCT and CPT for all HRV measurements except for VLF during the WCT. Furthermore, genetic correlations significantly smaller than 1 were found for most traits, which means that there is evidence of new genetic effects emerging during stress that do not influence HRV or HR at rest. Only for low frequency and total power between rest and CPT, and VLF between rest and WCT genetic correlations not significantly different from 1 were found suggesting evidence of complete pleiotropy. We also found a significant overlap of genetic factors under physical and mental stress for all HRV traits and HR with SDNN, RMSSD, and HR showing incomplete overlap indicating that for these latter traits, there are unique sets of genes that are operational in the two stress conditions. For the SDNN, high frequency, low frequency, VLF, total power and LFHF ratio a larger genetic overlap between the two stress conditions was observed than for each of the stress conditions compared with rest. However, genetic correlations for RMSSD and HR between the stress conditions appeared to be smaller than the genetic correlations between rest and stress suggesting that there is less overlap of genes influencing HR and RMSSD between the two stress conditions than between rest and stress. Comparatively, the genetic correlation between HR during rest and CPT was 0.79 in our study and with 0.75 very similar in a twin study by Zhang *et al.* [35].

Heritability estimates for HRV and HR at rest from this study were similar to those from two earlier family studies [17,36], but for SDNN and RMSSD at rest, they were slightly lower than those by Sinnreich *et al.* [18,37] and for HR at rest, it was higher than found by Singh *et al.* [17]. Differences between studies could be caused by the different beat-to-beat measurement methods used: we used 3–10 min ECG recordings, Singh *et al.* [17] used 2-h ambulatory ECG recordings and Sinnreich *et al.* [18] used 5-min Holter recordings. Also, differences could be because of sample sizes, pedigree structure and complexity, and covariates used.

HR heritability estimates were higher in comparison with overall HRV components for rest, WCT, and CPT. This is similar to the family study by Singh *et al.* [17] who also found resting HR heritability to be moderately higher compared with high frequency, low frequency, VLF, total power, and SDNN. However, our resting, WCT and CPT HR heritability estimates are lower compared with those typically found by twin studies during rest and both mental and physical stress [22,35]. This could be because of the different study design, their smaller population, and/or ethnically mixed sample size.

TABLE 4. Bivariate quantitative analyses of log-transformed heart rate variability measurements and heart rate examining the genetic (r_G), environmental (r_E), and phenotypic (r_P) correlations between the Word Conflict Test and Cold Pressor Test

HRV measurements and HR	*N*	h^2 (SE) WCT	h^2 (SE) CPT	Genetic correlation r_G (SE)	Environmental correlation r_E (SE)	Phenotypic correlation r_P	Proportions of r_P A/E[a]
lnSDNN	1188	0.22 (0.07)	0.12 (0.04)	**0.78**[a] (0.14)	0.61 (0.04)	0.60	0.20/0.80
lnRMSSD	1188	0.29 (0.08)	0.18 (0.05)	**0.60**[a] (0.13)	0.79 (0.03)	0.74	0.19/0.81
lnHF	1096	0.12 (0.06)	0.14 (0.05)	**0.99** (0.08)	0.81 (0.02)	0.83	0.16/0.84
lnLF	1096	0.13 (0.06)	0.17 (0.05)	**1.00** (nc)	0.74 (0.02)	0.78	0.19/0.81
lnVLF	1096	0.09 (0.05)	0.10 (0.04)	**1.00** (nc)	0.30 (0.05)	0.36	0.26/0.74
lnTP	1096	0.09 (0.06)	0.08 (0.04)	**1.00** (nc)	0.54 (0.04)	0.58	0.15/0.85
Ln(low frequency/high frequency)	1092	0.30 (0.08)	0.54 (0.21)	**0.89** (0.09)	0.59 (0.04)	0.66	0.34/0.66
lnHR	1220	0.43 (0.08)	0.36 (0.06)	**0.72**[a] (0.07)	0.72 (0.04)	0.72	0.39/0.61

BMI, sex, age, and age^2 were included as covariates in the analyses. Bold numbers are genetic correlation significantly different from zero. CPT, Cold Pressor Test; HF, high frequency; HR, heart rate; LF, low frequency; nc, not computable; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; TP, total power; VLF, very-low frequency; WCT, Word Conflict Test.
[a]Genetic correlations are significantly different from 1.

In this study, heritability estimates were in general higher under mental stress compared with those at rest, but this pattern was not seen under physical stress. These results are consistent with those from previous twin studies that observed a similar pattern for RSA [22,38] and for RMSSD and high frequency [20,23]. This may suggest that genetic influences on cardiac vagal activity become more pronounced whenever the participant is 'engaged' by mental and emotional challenges [27,40].

Moreover, in our study, we did not consider the underlying genetics of normalized units (nu) for high frequency, low frequency, and LFHF ratio because relevant genetic influences captured by the magnitude of total power's dynamic range – which could be of several orders of magnitude – would disappear with such normalization. In other words, individual 1 (who is young and healthy) has a resting low frequency of 4000 ms^2 and a high frequency of 5000 ms^2; and individual 2 (who is older but healthy and still with considerable sinus arrhythmia) has resting low frequency and high frequency values of 40 and 50 ms^2, respectively. Both individuals would then have the same normalized units, but with vastly different magnitude of oscillations.

We also observed striking differences in stress reactivity patterns to the two tests amongst the different HRV

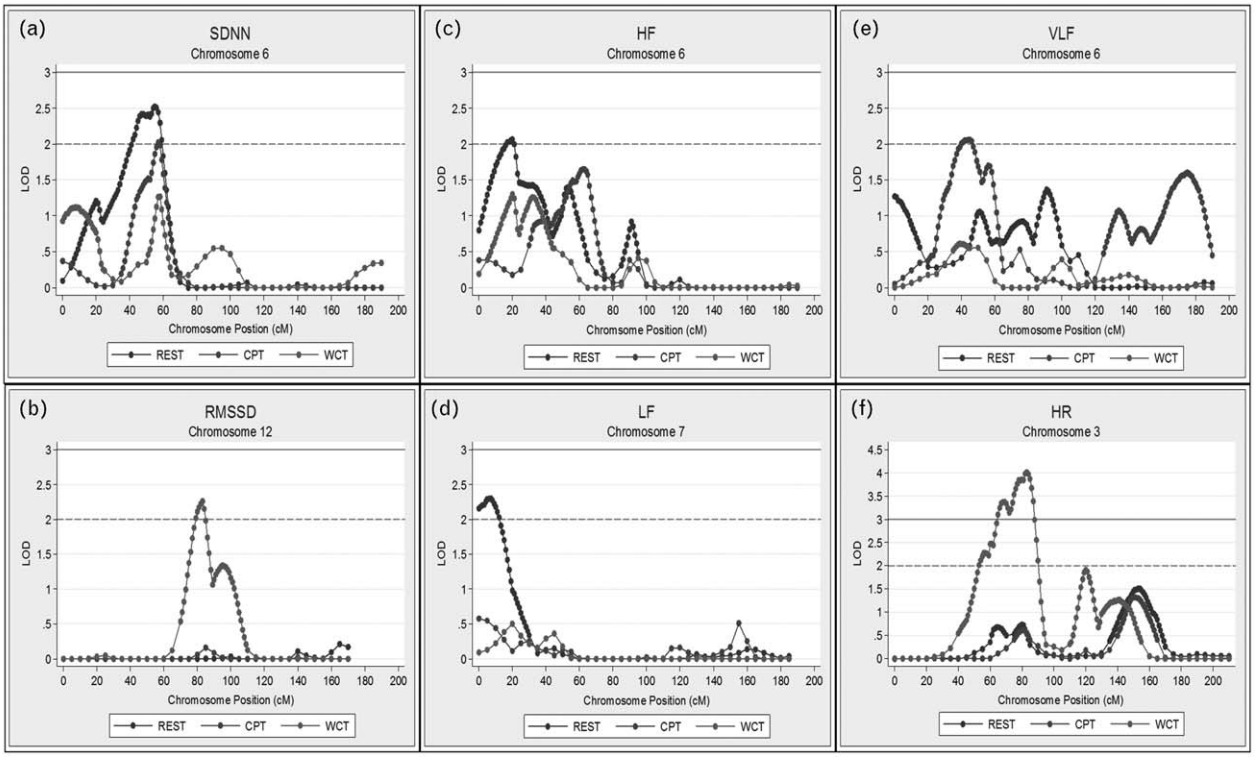

FIGURE 2 (a–f) Multipoint variance component-based linkage analysis plots for HRV parameters and HR with suggestive (≥2.0) and significant (≥3.0) logarithm of the odds (LOD) scores. Reference line: dashed line is for suggestive LOD scores; orange straight line is for significant LOD scores. Chr, chromosome; cM, centimorgan; CPT, Cold Pressor Test; HF, high frequency; HR, heart rate; LF, low frequency; LOD, logarithm of the odds; marker(s), nearest marker/flanking markers; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal intervals; VLF, very-low frequency; WCT, Word Conflict Test.

measurements in contrast to an almost identical HR reactivity to mental and physical stress. SDNN, low frequency, and total power significantly decreased in response to the WCT, but increased in response to the CPT, whereas RMSSD, high frequency, and HR showed directionally consistent and significant effects to both stressors. The clustering in reactivity patterns among HRV variables might be expected because both SDNN and total power reflect total variability in HR encompassing both short-term high frequency and lower frequency components [4,41], which are influenced by both the parasympathetic (PNS) and sympathetic (SNS) nervous system [42]. In contrast, RMSSD and high frequency encompass short-term HRV changes only [4,41] and reflect only the PNS [42]. Therefore, based on our HRV data, the reactivity to mental stress appears predominantly because of vagal withdrawal, whereas the reactivity to physical stress appears characterized by a decrease in PNS activity accompanied by a simultaneous increase in SNS activity. These diverging response patterns eventually resulted in virtually identical reactivity of HR to mental and physical stress. Our results confirm those from Snieder *et al.* [19] who also observed a stronger reduction in respiratory sinus arrhythmia (i.e. vagal withdrawal) in response to mental stress as compared with the CPT and those from Fonkoue and Carter [43] who, conversely, observed a stronger increase in muscle sympathetic nerve activity in response to the CPT than in response to mental stress.

Interestingly, our bivariate modeling results were confirmed by the genome-wide multipoint linkage analyses. We found significant linkage for HR on chromosome 3 and suggestive linkage on chromosomes 6, 7, and 12 for some HRV traits. Moreover, we observed indeed that some loci were shared between rest and stress whereas others were specific for either rest or stress. However, a downside of using linkage analysis for a complex phenotype such as HRV, is that the effect sizes of the individual causal variants are likely too small to allow for detection via co-segregation [44]. Therefore, the power to detect genes for complex traits with linkage analysis is minimal [45] and mapping resolution is low [46], which could explain why most of our linkage results for HRV were only suggestive. A more suitable approach that we recently used for gene identification of HRV is the hypothesis-free genome-wide association study [47].

Notably, our study is the first to use an Arab population to examine the genetic contribution to HRV variables and HR at rest and stress in a family design using univariate and bivariate analyses. The OFS cohort has a number of major strengths: it is geographically isolated, which provides a more homogenous environmental exposure; the socioeconomic status is similar among OFS participants; the participants have similar health-related habits (e.g. religious abstinence from alcohol); and genealogical records are authentic and well accessible. At the same time, this homogeneity may limit the generalizability of our results. Further investigation in families of other ethnicities could be warranted.

In our analyses, we limited our covariate adjustment to sex, age, age^2, and BMI (Table 2), which we found are the most important in terms of explained variance. This has been confirmed in our ongoing [48] work using more than 150 000 participants from the Lifelines Cohort Study and Biobank [49] where we found that age and sex were the major contributors to variance explained of HRV. In as far as (non-included) potential covariates are primarily environmental, their effects will end up in the environmental variance component, which will not have biased our heritability estimates in any way. Similarly to our recently published large meta-analyses of genome-wide association for HRV ($N = 53\,174$ individuals) [47], which only used sex and age as covariates, we did not want to adjust away the pleiotropic effects on HRV heritability by including additional heritable covariates. Regarding commonly used HRV covariates of smoking and/or alcohol use, at the time of our analyses, we did not have this information available to us. This is because it is deemed offensive to ask participants for their smoking and alcohol habits as this is religiously and socially prohibited in this religious part of Oman. However, the numbers are likely to be small. Nonetheless, we did ask participants to refrain from smoking and alcohol use for 2 days prior to their measurements.

Another limitation of our study is that we only investigated one type of mental and one type of physical stress. For future studies, using a variety of stress tests to represent mental and physical stress could be of interest such as a bicycle exercise challenge to represent physical stress and a virtual reality car driving challenge [23] to represent mental stress. Using CPT as a representative of physical stress could be considered a drawback because its stress response is largely driven by the experience of pain in response to exposure to cold, which is influenced by an individual's pain tolerance. Individual variations in pain tolerance have been shown in other studies [50].

Addressing the full complexity of the neural underpinnings of HRV was beyond the scope of our study, given that each RR interval (or IBI) depends on the interaction of vagal and sympathetic efferent activity within its humoral environment and molecular structures [51] and RR variability does not provide direct measures of autonomic (parasympathetic/sympathetic) activity (i.e. neural firing) but only indices of regulatory modulation [52,53]. However, we do believe that gene-finding studies of HRV may provide novel insights into vagal heart rhythm regulation as shown in our recent meta-analyses of genome-wide association studies for HRV ($N = 53\,174$) [47].

In conclusion, we showed that genetic factors significantly influence HRV measurements and HR; the heritability estimates of HRV under mental stress, in particular, for SDNN and RMSSD, are higher than those at rest, whereas those during physical stress are similar to the ones at rest; there is evidence for a large overlap of genetic factors influencing HRV at rest and during mental or physical stress; SDNN, RMSSD, and HR also show genetic effects that are specific to one of the stress conditions; and environmental factors contribute more than genetic factors in explaining the phenotypic correlation between HRV and HR at rest and under stress conditions. In addition, our linkage-based gene-finding approach suggested some loci to be shared between HRV and HR at rest and under stress, whereas others were specific for either rest or stress, which confirmed our bivariate modelling results.

Perspectives

Our results provide more information regarding the contribution of the genetic factors of HRV at rest and under physical and mental stress conditions and emphasize the importance of carefully defining the trait and standardizing its conditions in future gene-discovery efforts. Uncovering genes for HRV, for example, in GWASs, would facilitate investigation into their underlying function and impact on clinical outcomes.

ACKNOWLEDGEMENTS

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Acharya UR, Joseph KP, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. *Med Biol Eng Comput* 2006; 44:1031–1051.
2. Notarius CF, Floras JS. Limitations of the use of spectral analysis of heart rate variability for the estimation of cardiac sympathetic activity in heart failure. *Europace* 2001; 3:29–38.
3. Routledge HC, Chowdhary S, Townend JN. Heart rate variability – a therapeutic target? *J Clin Pharm Ther* 2002; 27:85–92.
4. Task Force of the European Society of Cardiology the North American Society of Pacing Electrophysiology. Heart Rate variability. standards of measurment, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 1996; 93:1043–1065.
5. Liao D, Cai J, Rosamond WD, Barnes RW, Hutchinson RG, Whitsel EA, *et al.* Cardiac autonomic function and incident coronary heart disease: a population-based case-cohort study. The ARIC study. Atherosclerosis Risk in Communities Study. *Am J Epidemiol* 1997; 145:696–706.
6. Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. Reduced heart rate variability and new-onset hypertension. Insights into pathogenesis of hypertension: The Framingham Heart Study. *Hypertension* 1998; 32:293–297.
7. de Bruyne MC, Kors JA, Hoes AW, Klootwijk P, Dekker JM, Hofman A, *et al.* Both decreased and increased heart rate variability on the standard 10-s electrocardiogram predict cardiac mortality in the elderly The Rotterdam Study. *Am J Epidemiol* 1999; 150:1282–1288.
8. Dekker JM, Schouten EG, Klootwijk P, Pool J, Swenne CA, Kromhout D. Heart rate variability from short electrocardiographic recordings predicts mortality from all causes in middle-aged and elderly men. The Zutphen Study. *Am J Epidemiol* 1997; 145:899–908.
9. Billman GE. Heart rate variability - a historical perspective. *Front Physiol* 2011; 2:86.
10. Antelmi I, De Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index and functional capactiy on heart rate variability in a cohort of subjects without heart disease. *Am J Cardiol* 2004; 93:381–385.
11. Bigger JT, Fleiss JL, Rolnitzky LM, Steinman RC. The ability of several short-term measures of RR variability to predict mortality after myocardial infarction. *Circulation* 1993; 88:927–934.
12. Liao D, Barnes RW, Chambless LE, Simpson RJ, Sorlie P, Heiss G. Age, race, and sex differences in autonomic cardiac function measured by spectral analysis of heart rate variability - the ARIC study. *Am J Cardiol* 1995; 76:906–912.
13. Tsuji H, Larson MG, Venditti JFJ, Manders ES, Evans GW, Feldman CL, *et al.* Impact of reduced heart rate variability of risk for cardiac events. The Framingham Heart Study. *Circulation* 1996; 94:2850–2855.
14. Tsuji H, Venditti FJ, Manders ES, Evans JC, Larson MG, Feldman CL, *et al.* Determinants of heart rate variability. *J Am Coll Cardiol* 1996; 28:1539–1546.
15. Umentani K, Singer DH, McCraty R, Atkinson M. Twenty-four hour time domain heart rate variability and heart rate: relations to age and gender over nine decades. *J Am Coll Cardiol* 1998; 31:593–601.
16. Snieder H, van Doornen LJP, Boomsma DI, Thayer JF. Sex differences and heritability of two indices of heart rate dynamics: a twin study. *Twin Res Hum Genet* 2007; 10:364–372.
17. Singh JP, Larson MG, O'Donnel CJ, Tsuji H, Evans JC, Ley D. Heritability of heart rate variability: the Framingham Heart Study. *Circulation* 1999; 99:2251–2254.
18. Sinnreich R, Friedlander Y, Sapoznikov D, Kark JD. Familial aggregation of heart rate variability based on short recordings - the kibbutzim family study. *Hum Genet* 1998; 103:34–40.
19. Snieder H, Boomsma DI, van Doornen LJP, de Geus EJC. Heritability of respiratory sinus arrhythmia: dependency on task and respiration rate. *Psychophysiology* 1997; 34:317–328.
20. Wang X, Thayer JF, Treiber F, Snieder H. Ethnic differences and heritability of heart rate variability in African- and European American youth. *Am J Cardiol* 2005; 96:1166–1172.
21. Boomsa DI, van Ball GC, Orlebeke JF. Genetic influences on respiratory sinus arrhythmia across different task conditions. *Acta Genet Med Gemellol (Roma)* 1990; 39:181–191.
22. de Geus EJC, Kupper N, Boomsma DI, Snieder H. Bivariate genetic modeling of cardiovascular stress reactivity: does stress uncover genetic variance? *Psychosom Med* 2007; 69:356–364.
23. Wang X, Ding X, Su S, Li Z, Riese H, Thayer J, *et al.* Genetic influences on heart rate variability at rest and during stress. *Psychophysiology* 2009; 46:458–465.
24. Hassan MO, Albarwani S, Al Yahyaee S, Al Haddabi S, Rizwi S, Jaffer A, *et al.* A family study in Oman: large, consanguineous, polygamous Omani Arab pedigrees. *Community Genet* 2005; 8:56–60.
25. Albarwani S, Munoz ML, Voruganti VS, Jaju D, Al-Yahyaee VS, Rizvi SG, *et al.* Heritability of ambulatory and beat-to-beat office blood pressure in large multigenerational Arab pedigrees: the 'Oman Family Study. *Twin Res Hum Genet* 2012; 15:753–758.
26. Hassan MO, Jaju D, Voruganti VS, Bayoumi RA, Albarwani S, Al-Yahyaee S, *et al.* Genome-wide linkage analysis of hemodynamic parameters under mental and physical stress in extended Omani Arab pedigrees: the Oman family study. *Twin Res Hum Genet* 2011; 14:257–267.
27. Neijts M, van Lien R, Kupper N, Boomsma D, Willemsen G, de Geus EJ. Heritability of cardiac vagal control in 24-h heart rate variability recordings: influence of ceiling effects at low heart rates. *Psychophysiology* 2014; 51:1023–1036.
28. Hassan MO, Bayoumi RA, Lopez-Alvarenga JC, Snieder H, Jaju D, Al-Yahyaee S, *et al.* Heritability of hemodynamic reactivity to laboratory stressors in a homogenous Arab population: an Oman family study. *Twin Res Hum Genet* 2009; 12:541–548.
29. Gratze G, Fortin J, Grasenick K, Pfurtscheller G, Wach P, Schönegger J, *et al.* A software package for noninvasive, real-time beat-to-beat monitoring of stroke volume, blood pressure, total peripheral resistance and for assessment of autonomic function. *Comput Biol Med* 1998; 28:121–142.
30. Timonen KL, Vanninen E, de Hartog J, Ibald-Mulli A, Brunekreef B, Gold DR, *et al.* Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: the ULTRA study. *J Expo Sci Environ Epidemiol* 2006; 16:332–341.
31. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998; 62:1198–1211.
32. Almasy L, Blangero J. Variance component methods for analysis of complex phenotypes. *Cold Spring Harb Protoc* 2010; 2010:pdb.top77.
33. Choh AC, Czerwinski SA, Lee M, Demerath EW, Wilson AF, Towne B, *et al.* Quantitative genetic analysis of blood pressure response during the cold pressor test. *Am J Hypertens* 2005; 18:1211–1217.
34. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 1995; 11:241–247.
35. Zhang K, Deacon DC, Rao F, Schork AJ, Fung MM, Waalen J, *et al.* Human heart rate: heritability of resting and stress values in twin pairs, and influence of genetic variation in the adrenergic pathway at a microribonucleic acid (microrna) motif in the 3'-UTR of cytochrome b561 [corrected]. *J Am Coll Cardiol* 2014; 63:358–368.
36. Singh JP, Larson MG, O'Donnell CJ, Levy D. Genetic factors contribute to the variance in frequency domain measures of heart rate variability. *Auton Neurosci Basic Clin* 2001; 90:122–126.
37. Sinnreich R, Friedlander Y, Luria MH, Sapoznikov D, Kark JD. Inheritance of heart rate variability: the kibbutzim family study. *Hum Genet* 1999; 105:654–661.
38. Neijts M, van Lien R, Kupper N, Boomsma D, Gonneke W, de Geus EJC. Heritability and temporal stability of ambulatory autonomic stress reactivity in unstructured 24-h recordings. *Psychosom Med* 2015; 77:870–881.

39. Wu T, Treiber FA, Snieder H. Genetic influence on blood pressure and underlying hemodynamics measured at rest and during stress. *Psychosom Med* 2013; 75:404–412.
40. de Geus E, van Lien R, Neijts M, Willemsen G. Genetics of autonomic nervous system activity. In: Canli T, editor. *The Oxford handbooks of molecular psychology*. United States of America: Oxford University Press; 2015.
41. Stein PK, Bosner MS, Kleiger RE, Conger BM. Heart rate variability: a measure of cardiac autonomic tone. *Am Heart J* 1994; 127: 1376–1381.
42. Malik M, Camm AJ. Components of heart rate variability – what they really mean and what we really measure. *Am J Cardiol* 1993; 72:821–822.
43. Fonkoue IT, Carter JR. Sympathetic neural reactivity to mental stress in humans: test-retest reproducibility. *Am J Physiol Regul Integr Comp Physiol* 2015; 309:R1380–R1386.
44. Visscher PM, Brown MA, McCarthy MI, Yang J. Five years of GWAS discovery. *Am J Hum Genet* 2012; 90:7–24.
45. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; 273:1516–1517.
46. Wang X, Prins BP, Siim S, Laan M, Snieder H. Beyond genome-wide association studies: new strategies for identifying genetic determinants of hypertension. *Curr Hypertens Rep* 2011; 13:442–451.
47. Nolte IM, Munoz ML, Tragante V, Amare AT, Jansen R, Vaez A, *et al*. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nat Commun* 2017; 8:15805.
48. Tegegne BS, van Roon A, Riese H, Snieder H. Demographic, lifestyle and psycho-social determinants of heart rate variability in the general population: a study from the Lifelines Cohort and Biobank Study. In: 62. Jahrestagung der Deutschen Gesellschaft für Medizinische Informatik, Biometrie und Epidemiologie e. V. (GMDS). German Medical Science GMS Publishing House; 2017.
49. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, *et al*. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol* 2015; 44:1172–1180.
50. Mourot L, Bouhaddi M, Regnard J. Effects of the cold pressor test on cardiac autonomic control in normal subjects. *Physiol Res Online* 2009; 58:83–91.
51. D'Souza A, Bucchi A, Johnsen AB, Logantha SJRJ, Monfredi O, Yanni J, *et al*. Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat Commun* 2014; 5:3775.
52. Pelliccia F, Kaski JC, Crea F, Camici PG. Pathophysiology of Takotsubo syndrome. *Circulation* 2017; 135:2426–2441.
53. Paton JFR, Boscan P, Pickering AE, Nalivaiko E. The yin and yang of cardiac autonomic control: vago-sympathetic interactions revisited. *Brain Res Rev* 2005; 49:555–565.

Reviewers' Summary Evaluations

Reviewer 1

This study has shown that resting heart rate and heart rate variability are influenced by genetic factors and that most of the genetic factors that influence heart rate variability at rest also influence heart rate variability during stressful situations. The peculiarity of this investigation is that the results were obtained in a homogeneous Arab population. Limitations of the study are that important confounding factors such as smoking and alcohol drinking could not be accounted for when establishing genetic associations and that these findings cannot be extended to other ethnic groups.

Reviewer 2

This paper has two major objectives: a genetic study and a neural one. They are both complex and require simplifications, but nevertheless convincingly show an interaction between genes and HRV (as a proxy of autonomic regulation).

In addition, simplifications carry the risk of becoming over-simplifications or impossible to understand, particularly in the case of issues still being debated, such as the mechanisms explaining HRV.