## Online Supplementary

### Supplementary Materials and Methods

#### Supplementary Experimental Methods

The WCT is a mental stress test (also known as the Stroop test1) that evaluates the ability to distinguish between two conflicting stimuli (words and colors). The original English names of colors were translated into Arabic and the same incongruent colors were displayed on a monitor. The observer selected the words at a constant speed of one word/second for three minutes. The subjects were asked to say the color of the word and not to read the word. They were encouraged to respond fast and to concentrate during the stress test2.

The CPT is a physical stress test, which is based on inducing a cardiovascular reaction by stimulation of pain receptors3. The subject’s left foot up to the ankle joint was immersed in cold water with crushed ice (4°C) for three minutes or until the test was aborted due to intolerable pain. Recordings of less than two minutes were excluded. The foot rather than the hand was immersed in the cold water because both hands were used for blood pressure measurements2,4.

#### Supplementary Statistical Analysis

It uses a variance components decomposition-based method to analyze family-based quantitative data by decomposing the overall phenotypic variance into genetic and environmental components using the observed covariance in the trait among family members. It then estimates narrow sense heritability (h2) by the proportion of the phenotypic variance attributed to additive genetic effects5. The h2 significance was determined by using a likelihood ratio test where the log-likelihood of the estimated model is compared to the nested model in which the genetic effect is fixed to zero6.

#### Supplementary Linkage Analysis

SOLAR uses, as a default, the Kosambi mapping function and an approximate multipoint identity by descent (IBD) estimating method of which the advantage is that it is not limited by the pedigree size7. SOLAR’s multipoint analysis is an extension of Fulker’s sib-pair method8. This method generates estimates of the IBD probabilities at any point on a chromosome using a constrained linear function of observed IBD probabilities at genetic markers at known locations within the region9,10.

### Supplementary Reference

1. Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol 1935;18:643–622.

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3. Wolf S, Hardy JD. Studies on pain.  Observations on pain due to local cooling and on factors involved in the ‘cold pressor’ effect. J Clin Invest 1941;20:521–33.

4. 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.

5. Almasy L, Blangero J. Variance component methods for analysis of complex phenotypes. Cold Spring Harb Protoc 2010;2010:pdb.top77.

6. 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.

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8. Fulker DW, Cherny SS, Cardon LR. Multipoint interval mapping of quantitative trait loci, using sib pairs. Am J Hum Genet 1995;56:1224–33.

9. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–211.

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### Supplementary Tables

**Supplementary Table 1:** Relative pairs in the five pedigrees in the Oman Family Study.

|  |  |
| --- | --- |
| **Relationship** | **N** |
| Parent-offspring | 2482 |
| Siblings | 1278 |
| Grandparent-grandchild | 3774 |
| Avuncular | 2815 |
| Half-siblings | 322 |
| Grand avuncular | 2928 |
| Half avuncular | 918 |
| 1st Cousins | 2610 |
| 1st Cousins, 1 removed | 932 |
| Half 1st cousins, 1 removed | 441 |
| 2nd cousins | 4363 |
| Other relationships | 390 |
| Total | 23253 |

**Supplementary Table 2:** Age group distribution of males and females at rest.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Age groups**  **(in years)** | **Males** | | | **Females** | | |
| **Frequency** | **Percentage** | **Cumulative** | **Frequency** | **Percentage** | **Cumulative** |
| <10 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10-19 | 136 | 23.33 | 23.33 | 120 | 16.15 | 16.15 |
| 20-29 | 197 | 33.79 | 57.12 | 251 | 33.78 | 49.93 |
| 30-39 | 87 | 14.92 | 72.04 | 116 | 15.61 | 65.55 |
| 40-49 | 63 | 10.81 | 82.85 | 120 | 16.15 | 81.7 |
| 50-59 | 42 | 7.20 | 90.05 | 74 | 9.96 | 91.66 |
| 60-69 | 31 | 5.32 | 95.37 | 42 | 5.65 | 97.31 |
| 70-79 | 14 | 2.40 | 97.77 | 12 | 1.62 | 98.92 |
| 80-89 | 11 | 1.89 | 99.66 | 7 | 0.94 | 99.87 |
| ≥90 | 2 | 0.34 | 100 | 1 | 0.13 | 100 |
| Total | 583 | 100 |  | 743 | 100 |  |

**Supplementary Table 3:** Univariate estimates for log-transformed heart rate variability measurements and heart rate during rest and physical stress test (cold pressor test) limited to the participants of the word-conflict test. Covariates bmi, sex, age, and age2 were included in the analyses.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **HRV measurements and HR** | **REST** | | | **WCT** | | | **CPT** | | |
| N | h2 (SE) | Proportion of variance due to covariates | N | h2 (SE) | Proportion of variance due to covariates | N | h2 (SE) | Proportion of variance due to covariates |
| lnSDNN | 658 | **0.16** (0.08) | 0.10 | 666 | **0.28** (0.09) | 0.09 | 648 | **0.24** (0.09) | 0.08 |
| lnRMSSD | 658 | **0.24** (0.09) | 0.06 | 664 | **0.36** (0.10) | 0.03 | 648 | **0.33** (0.10) | 0.08 |
| lnHF | 632 | **0.17** (0.08) | 0.04 | 640 | **0.17** (0.08) | 0.05 | 609 | **0.27** (0.09) | 0.07 |
| lnLF | 632 | **0.17** (0.07) | 0.09 | 640 | **0.21** (0.09) | 0.10 | 609 | **0.33** (0.09) | 0.12 |
| lnVLF | 632 | **0.20** (0.08) | 0.09 | 640 | 0.09 (0.07) | 0.14 | 609 | 0.09 (0.07) | 0.12 |
| lnTP | 632 | **0.14** (0.07) | 0.07 | 640 | **0.13** (0.09) | 0.12 | 609 | **0.18** (0.08) | 0.12 |
| Ln(LF/HF) | 637 | **0.41** (0.11) | 0.03 | 639 | **0.38** (0.09) | 0.09 | 613 | **0.33** (0.09) | 0.06 |
| lnHR | 689 | **0.31** (0.09) | 0.11 | 700 | **0.43** (0.10) | 0.08 | 685 | **0.31** (0.10) | 0.18 |

**Bold** numbers are statistically significant: p-value < 0.05

WCT: word conflict test; CPT: cold pressor test. SE: Standard Error; SDNN: standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive differences; HF: high frequency; LF: low frequency; VLF: very low frequency; TP: total power; LF/HF: low frequency/high frequency ratio; HR: heart rate

**Supplementary Table 4:** Bivariate quantitative analyses of log-transformed heart rate variability measurements and heart rate examining the genetic (rG), environmental (rE) and phenotypic (rP) correlations between rest and the word conflict test (WCT) and cold pressor test (CPT), limited to the WCT participants. Covariates bmi, sex, age, and age2 were included in the analyses.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **HRV measurements and HR** | **N** | **WCT** | | | | | | **CPT** | | | | | |
| **h2**  **(SE)** | | **Genetic**  **correlation** | **Environmental**  **correlation** | **Phenotypic**  **correlation** | **Proportions of rP** | **h2**  **(SE)** | | **Genetic**  **correlation** | **Environmental**  **correlation** | **Phenotypic**  **correlation** | **Proportions of rP** |
| **REST** | **WCT** | **rG**  **(SE)** | **rE**  **(SE)** | **rP** | **A/E#** | **REST** | **CPT** | **rG** | **rE** | **rP** | **A/E#** |
| lnSDNN | 658 | 0.17  (0.08) | 0.27  (0.09) | **0.63**\*  (0.16) | 0.66  (0.04) | 0.65 | 0.20/0.77 | 0.17  (0.08) | 0.23  (0.09) | **0.64\***  (0.18) | 0.56  (0.05) | 0.58 | 0.22/0.72 |
| lnRMSSD | 658 | 0.26  (0.09) | 0.36  (0.10) | **0.83**\*  (0.07) | 0.80  (0.03) | 0.80 | 0.31/0.69 | 0.24  (0.09) | 0.33  (0.10) | **0.63**\*  (0.14) | 0.76  (0.04) | 0.72 | 0.25/0.75 |
| lnHF | 633 | 0.18  (0.08) | 0.18  (0.09) | 0.84\*  (0.09) | 0.85  (0.02) | 0.85 | 0.18/0.81 | 0.18  (0.08) | 0.27  (0.09) | 0.69\*  (0.15) | 0.76  (0.04) | 0.74 | 0.20/0.80 |
| lnLF | 633 | 0.19  (0.08) | 0.22  (0.09) | **0.85**\*  (0.09) | 0.74  (0.03) | 0.76 | 0.22/0.76 | 0.18  (0.07) | 0.33  (0.09) | **0.91**  (0.09) | 0.57  (0.05) | 0.65 | 0.35/0.65 |
| lnVLF | 633 | 0.20  (0.08) | 0.08  (0.07) | 0.70  (0.21) | 0.53  (0.05) | 0.55 | 0.16/0.79 | 0.20  (0.08) | 0.09  (0.07) | **0.82**  (0.30) | 0.26  (0.07) | 0.33 | 0.34/0.66 |
| lnTP | 633 | 0.14  (0.08) | 0.12  (0.08) | 0.75\*  (0.17) | 0.75  (0.03) | 0.75 | 0.13/0.85 | 0.14  (0.07) | 0.18  (0.08) | **0.83**  (0.20) | 0.47  (0.05) | 0.52 | 0.25/0.75 |
| Ln(LF/HF) | 639 | 0.40  (0.10) | 0.39  (0.09) | **0.78\***  (0.10) | 0.43  (0.08) | 0.57 | 0.16/0.82 | 0.40  (0.10) | 0.35  (0.09) | **0.79\***  (0.11) | 0.34  (0.09) | 0.51 | 0.57/0.43 |
| lnHR | 689 | 0.34  (0.09) | 0.48  (0.10) | 0.89  (0.04) | 0.77  (0.04) | 0.81 | 0.44/0.56 | 0.31  (0.09) | 0.30  (0.10) | **0.76**\*  (0.11) | 0.65  (0.05) | 0.68 | 0.40/0.39 |

Covariates: bmi, age, sex, age2; SE: Standard Error; SDNN: standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive differences; HF: high frequency; LF: low frequency; VLF: very low frequency; TP: total power; LF/HF: low frequency/high frequency ratio; HR: heart rate

#A/E is the percentage of the phenotypic correlation that is caused by genes (A) or environment (E), based on the following equation [4]:

**Bold** numbers are genetic correlation significantly different from zero; \*genetic correlations are significantly different from 1

**Supplementary Table 5:** Bivariate quantitative analyses of log-transformed heart rate variability measurements and heart rate examining the genetic (rG), environmental (rE) and phenotypic (rP) correlations between cold pressor test (CPT) and word conflict test (WCT), of the overlapping WCT participants. Covariates bmi, sex, age, and age2 were included in the analyses.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **HRV measurements and HR** | **N** | **h2**  **(SE)** | | **Genetic**  **correlation** | **Environmental**  **correlation** | **Phenotypic**  **correlation** | **Proportions of rP** |
| **WCT** | **CPT** | **rG**  **(SE)** | **rE**  **(SE)** | **rP** | **A/E\*** |
| lnSDNN | 658 | 0.28  (0.09) | 0.23  (0.09) | **0.82**\*  (0.12) | 0.52  (0.06) | 0.59 | 0.36/0.64 |
| lnRMSSD | 658 | 0.37  (0.10) | 0.32  (0.10) | **0.71**\*  (0.11) | 0.73  (0.05) | 0.73 | 0.34/0.66 |
| lnHF | 633 | 0.17  (0.08) | 0.25  (0.09) | **1.00**  (nc) | 0.75  (0.03) | 0.80 | 0.26/0.74 |
| lnLF | 633 | 0.20  (0.08) | 0.32  (0.09) | **1.00**  (nc) | 0.62  (0.05) | 0.7 | 0.36/0.64 |
| lnVLF | 633 | 0.12  (0.07) | 0.10  (0.07) | **1.00**  (nc) | 0.24  (0.06) | 0.32 | 0.35/0.65 |
| lnTP | 633 | 0.14  (0.07) | 0.19  (0.08) | **1.00**  (nc) | 0.43  (0.05) | 0.53 | 0.32/0.68 |
| Ln(LF/HF) | 639 | 0.36  (0.09) | 0.31  (0.09) | **0.96**  (0.10) | 0.48  (0.07) | 0.64 | 0.51/0.49 |
| lnHR | 689 | 0.47  (0.10) | 0.31  (0.10) | **0.74\***  (0.09) | 0.69  (0.06) | 0.70 | 0.41/0.59 |

WCT: word conflict test; CPT: cold pressor test; SE: Standard Error; SDNN: standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive differences; HF: high frequency; LF: low frequency; VLF: very low frequency; TP: total power; LF/HF: low frequency/high frequency ratio; HR: heart rate; nc: not computable

**Bold** numbers are genetic correlation significantly different from zero

\*genetic correlations are significantly different from 1.