Twin Research and Human Genetics

Article Title: Higher Anxiety Is Associated With Lower Cardiovascular Autonomic Function In Female Twins

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Supplementary materials

Supplementary material 1. Experiment details & quality control

Twin pairs typically arrived at 9:00 and the protocol started with blood samples taken as well as weight/height/circumference assessments. Cardiovascular autonomic functions (BRS, IBI, HRV) were assessed in an experimental laboratory task with four standardised conditions in the following order: Rest (R1), Stress with visual feedback (S1), Stress with auditory feedback (S2) and Rest (R2). Stress conditions were employed to measure the shift in sympathetic/parasympathetic balance. The stress tasks exposedparticipants to a modified version of the 'emotion face dot probe task' (Mogg & Bradley, 1999; Riese et al., 2006a), involving a series of trials whereby a pair of faces was presented for 19 ms, followed by a mask for 50 ms. Following this, dots appeared in the location previously occupied by the two masked faces: 11 dots on one side, and three or four dots on the other side. Participants had to indicate whether three or four dots appeared as quickly as possible using a button response.

The task is modified in that it involves the use of dots for the response frame rather than horizontal or vertical semi-colons. Visual feedback was given by presenting the correct number of dots for 1000 ms in the centre of the screen; "3 stippen! (3 dots!)" or "4 stippen! (4 dots!)", in the Courier New font with 18 font size. Auditory feedback involved exposing participants to 100dB white noise for 500 ms when a wrong response was given. The auditory feedback was presented to participants twice before this second session started.

Cardiovascular measurements began after the participants relaxed in a sitting position for a minimum of 10 minutes. Each experimental condition lasted approximately 5 minutes.

Quality control

Measurements were excluded if signal recording failed. For continuous blood pressure (BP) and heart rate, artefacts, outliers and missing values were corrected for using linear interpolation of four data points surrounding the artefact. Visual inspection led to 976 measurements suitable for BRS calculation in the CARSPAN spectral analysis program (Mulder, 1988; Robbe et al., 1987). The method has also been the basis of calculating BRS in various other studies (Althaus et al., 2004; Dietrich et al., 2006; Lefrandt et al., 1999; Van Roon, Mulder, Althaus, & Mulder, 2004). The program enables discrete Fourier transformation of non-equidistant systolic BP and IBI series. These time series were corrected for artefacts and checked for stationarity. BRS was defined as the mean between spectral IBI variability and BP variability values in the 0.07-0.14 frequency band, expressed in ms/mmHg. The gain in the 0.07–0.14 Hz frequency band is influenced by both branches of the autonomic nervous system (Akselrod et al., 1985) and it has been demonstrated that the narrow band (around 0.10 Hz) is valid for determining changes in short-term blood pressure regulation (Robbe et al., 1987). For respiration, spectral power values were calculated, which were used in the BRS quality control procedure (Jorna, 1992).

The quality of the dataset was assured by excluding:
(1) 20 BRS values obtained based on less than three frequency points (i.e. less than 3 out of the 8 points in the 0.07-0.14 frequency band);

(2) 13 BRS values that were based on measurements that had more than 10% of the BP signal corrected by CARSPAN and/or contained too many artefacts (that is, time-series with supraventricular extra systoles, showing signal gaps of more than 5s of IBIs and/or more than 10s in systolic BP signals);
(3) 9 BRS values obtained from measurements lasting less than 100s; and
(4) 19 BRS values based on unreliable IBI spectral power values due to power influences from the respiration signal in the 0.07–0.14 Hz band, caused by slow breathing (during normal breathing the respiration peak can be expected around 0.25 Hz).

Participants with no reliable BRS values were excluded in analyses of IBI and HRV. Two participants' HRV measurements deviated more than 3 S.D. from the mean and were also excluded. Two participants were excluded because of supraventricular extrasystoles (8 BRS values), and 31 BRS values were excluded because of other reasons such as talking, coughing during the measurement, or IBI power in the 0.15– 0.50 Hz band instead of the 0.07–0.14 Hz band (Riese et al. 2006).

<u>Supplementary material 2.</u> Twin model fitting analysis using structural equation modelling (SEM)

The parameter estimates of the full 'ACE' model, those of subsequently fitted reduced models and 95% confidence intervals were estimated using maximum likelihood methods in the OpenMx package in R (Neale et al., 2016; Neale & Miller, 1997). Goodness of fit of models were determined using Akaike's information criterion (AIC; Akaike, 1987) and the χ^2 statistic. The AIC judges the fit of the model (χ^2) relative to the number of parameters; a lower AIC shows goodness of fit and parsimony, indicating whether to accept or reject further sub-models.

Phenotypic factor model

The phenotypic pathway model estimated MZ and DZ twin correlations between the latent factors (**Figure 1**). We applied constraints to this model to obtain: (a) one set of within-twin (within individual), cross-trait correlations between ANX, BRS, HRV and IBI(e.g. ANX Twin 1 –BRS Twin 1). This was regardless of twin order or zygosity group. Additionally, (b) one set of cross-twin cross-trait correlations for MZ and DZ pairs separately (e.g. MZ; ANX Twin1 – BRS Twin 2). This was independent of twin order (e.g. ANX Twin1 – BRS Twin2 = ANX Twin2 – BRS Twin1). Finally, (c) the cross-twin within-trait correlations (e.g. ANX Twin1 – ANX Twin2) were free to vary across zygosity groups.

Genetic factor model

Classical twin models estimate the effects of latent (unobserved) genetic and environmental influences on the variance of an observed trait. The power to estimate these variance components is through the differences in covariance (or correlation) of the trait among MZ and DZ twin pairs. The cross twin within-trait correlations allow variances of each latent factor to

be decomposed into additive genetic (A or a^2), common environmental (C or c^2) and unique environmental effects (E or e^2). The power to distinguish between different sources of covariance comes through the cross-twin cross-trait correlations (e.g. ANX Twin1 –BRS Twin 2). If the phenotypic relationships between latent anxiety and autonomic factors are significant, this would imply common aetiology and significant cross-twin cross-trait correlations suggest that this aetiology is familial. The ratio of the MZ/DZ cross-twin cross-trait correlations indicate to what extent the common aetiology is genetic or environmental in origin; a 2:1 ratio suggests the effects of A, a 1:1 ratio suggests the effects of C and nonsignificant cross- trait cross-twin correlations suggest that the common aetiology is due to E (Neale & Cardon, 2013).

Correlations between latent factors were modelled as a function of their latent A, C and E influences. The model is expressed in correlated factors: six each for the A, C and E factors (**Figure 2**; only genetic correlations, $r_{g,}$ are shown). The degree to which latent A factors contribute to the phenotypic correlation between any two latent factors is gained by multiplying square roots of the standardized estimates (a^2) of the latent phenotypes with their matching genetic correlation (r_g). The same procedure is done to obtain the contributions of C and E to the phenotypic correlation. The total phenotypic correlation is therefore a sum of the A, C and E correlations.

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