Online Supplement

Objects list:

eMethods – Recruitment details

eTable 1– Multivariate model fit statistics in adolescence and early adulthood: excluding siblings

eTable 2 – Multivariate genetic analyses at 15 and 17 years, inclusive of physical injury variable

eTable 3 – Univariate results

eTable 4 – Longitudinal phenotypic continuity of anxiety subscales, within and across anxiety measures.

eTable 5 – Multivariate model fit statistics in adolescence and early adulthood. Submodel comparisons to drop C and A.

eMethods – Recruitment details

ECHO

Emotions, Cognitions, Heredity and Outcome (ECHO) study is a spin-off from a larger longitudinal sample of twins born in England and Wales during 1994-1996 (TEDS)[1]. In order to maximize power and include children with high emotional symptoms, the majority of twins (N=247 pairs) were recruited due to one or both of them scoring within top 15% on child anxiety at age 7, as reported by parents. A smaller group of 'control' pairs were chosen, out of which none of the twins scored high on anxiety symptoms (N=53 pairs). This selection ensured that the data represented a full range of scores on test measures. A total of 11 twin pairs (4%) were excluded because at least one of the twins had co-morbid diagnosis of neurological impairments, autistic spectrum disorders, severe receptive language impairments or persistent attentional difficulties. Zygosity was established using parent-report questionnaires. This method is estimated to be over 95% accurate[2, 3]. Where zygosity was ambiguous, DNA was collected from cheek swabs in order to assign zygosity. The social-economic status (SES) of ECHO participants was somewhat higher than a population based sample, where for example 32% of parents were in education until 18 years or more[4]. The sample characteristics at both waves are presented in the Table 1.

For both waves, parents/guardians provided written informed consent through the post prior to data collection. Data collection was conducted at the Institute of Psychiatry (King's College London, United Kingdom), apart from a small number of children who were visited in their homes. The study was granted ethical approval by the Maudsley Hospital Ethics Committee (London, United Kingdom).

In order to be able to generalize the results from this selected sample to the whole population, a weight was incorporated into all analyses. The weight controls for biases due to ascertainment - oversampling symptomatic children. The weight used the ratio of the selection probability of high symptom families to that of nonsymptomatic families to control for bias associated with ascertainment across waves, and the inverse of the predicted probability of families remaining at Wave 2 to control for bias associated with attrition. In short, lower weights were assigned to individuals from categories over-represented in the sample, and higher weights to individuals from categories under-represented in the sample relative to the population distribution. The weight did not change the results in a way that would alter the interpretation.

G1219

The G1219 study is a longitudinal study of 3,640 adolescent twins and siblings. The sample was recruited from two sources. First, adolescent offspring of adults from a large-scale population-based study (GENESIS[5]) were invited to participate in this or another study[6]. Of the 3,600 responses, 1,818 adolescents (51%) from 1,294 families agreed to participate in G1219. Second, a random selection of live twin births born between 1985 and 1988 identified by the UK Office of National Statistics were recruited by Heath Authorities and General Practitioners on behalf of G1219 team. Of the 2,947 families contacted, 1,381 (47%) participated. Only respondents aged 12 to 19 were included within the final sample. The present analyses focus on waves 2-4 of the data collection, when the participants were on average 15, 17 and 20 years old. Zygosity was established using parent-report questionnaires assessing the physical similarity between pairs. This method is estimated to be over 95% accurate[2, 3]. When there was disagreement between zygosity ratings between wave one and two, DNA was obtained (N=26 pairs) before final classifications were made. The sample characteristics at three waves are presented in the Table 1. Weight was not included in the analyses due to the non-selected nature of the sample. The social-economic status (SES) of G1219 participants was somewhat higher than a population based sample, with 39% educated to A-level or above compared to 32% in the nationally representative sample.[4] Parents from the G1219 sample were also more likely to own their own homes (82% compared to 68%).

For all waves, informed consent was obtained from parents/guardians of all participating adolescents under 16 and from participants themselves when over 16. The study was granted ethical approval by the Research Ethics Committees of the Institute of Psychiatry, South London and Maudsley NHS Trust for all waves, and Goldsmiths, University of London at wave 4.

eTable 1 – Multivariate model fit statistics in adolescence and early adulthood: excluding siblings.

			Comparison to Saturated Model			Comparison to Correlated Factors Solution			Comparison to 2 Factors Independent Pathway Model				
	-2LL	df	χ^2	Δ df	p-value	χ^2	Δ df	p-value	χ^2	Δ df	p-value	AIC	BIC (size-adjusted)
Adolescence (15 years)													
Saturated Model	26715.40	9491										7733.40	28198.25
Correlated Factors Solution	**27125.78**	**9777**	**410.38**	**286**	**<.01**							**7571.78**	**27303.72**
2 Factors Independent Pathway Model	27162.21	9784	446.81	293	<.01	36.43	7	<.01				7594.22	27308.22
1 Factor Independent Pathway Model	27210.27	9787	494.87	296	<.01	84.49	10	<.01	48.06	3	<.01	7636.27	27342.59
Adolescence (17 years)													
Saturated Model	15521.20	5914										3693.20	17004.05
Correlated Factors Solution	**15928.29**	**6200**	**407.09**	**286**	**<.01**							**3528.29**	**16106.23**
2 Factors Independent Pathway Model	15992.47	6207	471.27	293	<.01	64.18	7	<.01				3578.46	16138.47
1 Factor Independent Pathway Model	16010.00	6210	488.80	296	<.01	81.71	10	<.01	17.53	3	<.01	3589.99	16142.31
Young Adulthood (20 years)													
Saturated Model	18182.31	5616										6950.31	19665.16
Correlated Factors Solution	18524.03	5902	341.73	286	.01							6720.03	18701.98
2 Factors Independent Pathway Model	**18537.01**	**5909**	**354.70**	**293**	**.01**	**12.98**	**7**	**0.07**				**6719.01**	**18683.02**
1 Factor Independent Pathway Model	18555.80	5912	373.49	296	<.01	31.77	10	<.01	18.79	3	<.01	6731.80	18688.11

eTable 1– Multivariate model fit statistics in adolescence and early adulthood: excluding siblings. (Continued)

Note:

The adolescence sample comes from waves 2-3 and the young adult sample comes from wave 4 from G1219 study. Mean ages provided in the headings.

-2LL – minus twice the log likelihood; *df*- degrees of freedom; *Δ df* – degrees of freedom difference; *p* – probability; *AIC* – Akaike's information criterion; *BIC* – Bayesian's information criterion.

The best fitting model (shown in bold) was selected based on the principle of parsimony and lowest AIC and BIC value.

The analyses were repeated excluding siblings in order to establish whether the results hold for narrower age ranges. The exclusion of siblings has not altered the results in a meaningful way, supporting the conclusion that they are applicable to the developmental periods investigated.

eTable 2 – Multivariate genetic analyses at 15 and 17 years, inclusive of physical injury variable

		Descriptive Statistics				Univariate results			Phenotypic correlations with depression		Correlated Factors Solution results			
Wave	N	Mean (SD)	Skew	Kurtosis	α	A	C	E	Full (r_{ph})	Partial	r_A with depression	r_E with depression	Proportion of r_{ph} due to A	Proportion of r_{ph} due to E
Adolescence (15 years)	2628	3.18 (2.47)	.87	3.75	.50	.37 (.25-.44)	.00 (.00-.08)	.63 (.56-.71)	.31 (.28-.39)	-.02 (-.06-.02)	.46 (.35-.58)	.16 (.08-.24)	.69 (.52-.85)	.31 (.15-.48)
Adolescence (17 years)	1590	3.02 (2.51)	.96	3.96	.50	.31 (.07-.40)	.00 (.00-.15)	.69 (.60-.81)	.30 (.26-.34)	.06 (.01-.11)	.23 (.04-.40)	.25 (.15-.35)	.36 (.06-.62)	.64 (.38-.94)

Note

The adolescence sample comes from waves 2-3 from G1219 study. Mean ages provided in the headings.

A – additive genetic influences, *C* – shared environmental influences, *E* – non-shared environmental influences, r_{ph} – phenotypic correlation, r_{ph} – genetic correlation, r_{ph} – non-shared environmental correlation

Descriptive and phenotypic results presented on untransformed variables for comparison with other published samples.

95% Confidence Intervals (CIs) are presented in brackets. CIs not inclusive of zeros indicate significant correlations. Non-overlapping CIs mean significant difference between the values.

Partial correlations controlled for all other anxiety variables within time.

The inclusion of the fear of physical injury variable has not altered the fit statistics of the multivariate models in a way that would change the interpretation of results – the correlated factors solution remained the best fitting model at both ages. The fear of physical injury has been modelled as an additional 'fear' variable in the two factors independent pathway model.

C influences were dropped from the multivariate models without a significant deterioration of the fit.

eTable 3 – Univariate results

Trait	Parameter	Childhood (8 years)	Childhood (10 years)	Adolescence (15 years)	Adolescence (17 years)	Young adult (20 years)
Depression	A	.31 (.00-.50)	.00 (.00-.40)	.45 (.26-.57)	.45 (.20-.53)	.40 (.24-.50)
	C	.05 (.00-.33)	.37 (.05-.48)	.06 (.00-.19)	.00 (.00-.19)	.00 (.00-.11)
	E	.65 (.50-.83)	.63 (.48-.75)	.49 (.43-.57)	.55 (.47-.64)	.60 (.50-.70)
Generalized Anxiety	A	.31 (.06-.44)	.27 (.00-.43)	.45 (.25-.52)	.40 (.24-.49)	.36 (.06-.49)
	C	.00 (.00-.17)	.00 (.00-.26)	.00 (.00-.14)	.00 (.00-.10)	.03 (.00-.23)
	E	.69 (.56-.84)	.73 (.57-.91)	.55 (.48-.63)	.60 (.51-.70)	.61 (.51-.74)
Panic	A	.19 (.00-.34)	.14 (.00-.44)	.27 (.06-.45)	.29 (.09-.39)	.32 (.10-.41)
	C	.00 (.00-.20)	.13 (.00-.35)	.10 (.00-.25)	.00 (.00-.13)	.00 (.00-.15)
	E	.81 (.66-.97)	.73 (.56-.90)	.63 (.55-.71)	.71 (.61-.82)	.68 (.59-.79)
Separation Anxiety	A	.28 (.11-.43)	.35 (.02-.50)	.34 (.13-.49)	.41 (.28-.50)	.36 (.21-.45)
	C	.00 (.00-.09)	.00 (.00-.25)	.08 (.00-23)	.00 (.00-.08)	.00 (.00-.10)
	E	.72 (.57-.87)	.65 (.50-.81)	.58 (.51-.66)	.60 (.51-.69)	.64 (.55-.75)
Social Anxiety	A	.05 (.00-.22)	.39 (.00-.53)	.43 (.30-.50)	.28 (.00-.45)	.44 (.16-.54)
	C	.00 (.00-.11)	.00 (.00-.29)	.00 (.00-.08)	.08 (.00-.28)	.01 (.00-.21)
	E	.95 (.83-1.00)	.61 (.47-.78)	.57 (.50-.65)	.65 (.55-.76)	.55 (.46-.66)

eTable 3 – Univariate results (continued)

Note:

The childhood sample comes from ECHO study, the adolescence sample comes from waves 2-3 and the young adult sample comes from wave 4 from G1219 study. Mean ages provided in the headings.

A – additive genetic influences, *C* – shared environmental influences, *E* – non-shared environmental influences

95% Confidence Intervals are presented in brackets. CIs not inclusive of zeros indicate significant correlations. Non-overlapping CIs mean significant difference between the values. The difference in CIs width between the ECHO and G1219 time points reflects larger sample size of G1219 which results in greater power to estimate the parameters precisely.

The ECHO sample was too small to examine sex differences so these were only examined in G1219. Quantitative sex differences imply that genetic and environmental influences differ in magnitude across sex whilst scalar sex differences indicate variance differences between males and females. Scalar sex differences were evident for all variables apart from social concerns at times 3-5, suggesting that males and females showed different variance on most measures. To account for these differences, a scalar was fitted in all twin modeling analyses at these time points.

Depression at time 2 in child sample (ECHO) showed different pattern of parameter estimates than other variables, being influenced by moderate shared environmental factors with no genetic influence. This is due to a low power to distinguish between A and C in the ECHO sample.

eTable 4 – Longitudinal phenotypic continuity of anxiety subscales, within and across anxiety measures.

	Age 15 – Age 17 (Within SCAS)	Age 15- Age 20 (SCAS to RCADS)	Age 17- Age 20 (SCAS to RCADS)
Generalized Anxiety	.47 (.43-.51)	.36 (.32-.40)	.53 (.49-.56)
Panic	.43 (.39-.47)	.39 (.35-.43)	.48 (.44-.52)
Separation Anxiety	.36 (.32-.40)	.39 (.35-.43)	.35 (.31-.39)
Social Phobia	.53 (.49-.56)	.46 (.42-.50)	.58 (.54-.62)

Note:

The adolescence sample comes from waves 2-3 and the young adult sample comes from wave 4 from G1219 study. Mean ages provided in the headings.

SCAS - Spence Children's Anxiety Scale[7]; *RCADS* - Revised Child Anxiety and Depression Scale[8].

95% Confidence Intervals (CIs) are presented in brackets. CIs not inclusive of zeros indicate significant correlations. Non-overlapping CIs mean significant difference between the values.

The analyses (Pearson's correlations) were conducted in order to check for measurement effects, and the results suggest a comparable continuity of the scores within and across anxiety measures.

eTable 5 – Multivariate model fit statistics in adolescence and early adulthood. Submodel comparisons to drop C and A.

			Comparison to Saturated Model			AIC	BIC (size-adjusted)
	-2LL	df	X^2	Δ df	p-value		
Adolescence (15 years)							
Correlated Factors Solution – ACE model	35203.32	12649				9905.32	35470.98
Correlated Factors Solution – AE model	**35207.38**	**12664**	**4.06**	**15**	**1.00**	**9879.38**	**35400.69**
Correlated Factors Solution – CE model	35262.08	12664	58.76	15	<.01	9934.08	35455.39
Adolescence (17 years)							
Correlated Factors Solution – ACE model	19754.74	7668				4418.74	20022.40
Correlated Factors Solution – AE model	**19758.02**	**7683**	**3.28**	**15**	**1.00**	**4392.02**	**19951.33**
Correlated Factors Solution – CE model	19813.65	7683	58.91	15	<.01	4447.65	20006.96
Young Adulthood (20 years)							
2 Factor Independent Pathway Model – ACE model	23559.32	7543				8473.32	23767.51
2 Factors Independent Pathway Model – AE model	**23566.13**	**7553**	**6.81**	**10**	**.74**	**8460.13**	**23724.74**
2 Factor Independent Pathway Model –CE model	23619.75	7556	60.43	13	<.01	8507.74	23763.49

Note:

The adolescence sample comes from waves 2-3 and the young adult sample comes from wave 4 from the G1219 study. Mean ages provided in the headings.

-2LL – minus twice the log likelihood; *df*- degrees of freedom; *Δ df* – degrees of freedom difference; *p* – probability; *AIC* – Akaike's information criterion.

The best fitting model (shown in bold) was selected based on the principle of parsimony and lowest AIC and BIC value. Shared-environmental, but not genetic influences can be dropped from the models without significant deterioration of the fit. The AIC values suggest that dropping C lead to improvement of the model fit at these three waves.

Supplementary references

1. Trouton A, Spinath FM, Plomin R. Twins Early Development Study (TEDS): A multivariate, longitudinal genetic investigation of language, cognition and behaviour problems in childhood. *Twin Res.* 2002;5(5):444-448.

2. Goldsmith HH. A zygosity questionnaire for young twins: A research note. *Behavior Genetics.* 1991;21(3):257-269.

3. Price TS, Freeman B, Craig IW, Petrill SA, Ebersole L, Plomin R. Infant zygosity can be assigned by parental report questionnaire data. *Twin Res.* 2000;3:129-133.

4. Meltzer H, Gatward R, Goodman R, Ford T. *Mental health of children and adolescents in Great Britain*. London: The Stationary Office; 2000.

5. Sham PC, Sterne A, Purcell S, et al. GENESiS: Creating a composite index of the vulnerability to anxiety and depression in a community-based sample of siblings. *Twin Res.* 2000;3(4):316-322.

6. Curran S, Mill J, Sham P, et al. CHIP: Defining a dimension of the vulnerability to Attention Deficit Hyperactivity Disorder (ADHD) using sibling and individual data of children in a community-based sample. *Behavior Genetics.* in press 2003;119(1):86-97.

7. Spence SH. A measure of anxiety symptoms among children. *Behav Res Ther.* 1998;36(5):545-566.

8. Chorpita BF, Yim L, Moffitt C, Umemoto LA, Francis SE. Assessment of symptoms of DSM-IV anxiety and depression in children: A revised child anxiety and depression scale. *Behav Res Ther.* 2000;38(8):835-855.