

A Genetically Informed Study of the Association Between Childhood Separation Anxiety, Sensitivity to CO₂, Panic Disorder, and the Effect of Childhood Parental Loss

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Context: Childhood separation anxiety disorder can pre-date panic disorder, which usually begins in early adulthood. Both disorders are associated with heightened sensitivity to inhaled CO₂ and can be influenced by childhood parental loss.

Objectives: To find the sources of covariation between childhood separation anxiety disorder, hypersensitivity to CO₂, and panic disorder in adulthood and to measure the effect of childhood parental loss on such covariation.

Design: Multivariate twin study.

Participants: Seven hundred twelve young adults from the Norwegian Institute of Public Health Twin Panel, a general population cohort.

Main Outcome Measures: Personal direct assessment of lifetime panic disorder through structured psychiatric interviews, history of childhood parental loss, and separation anxiety disorder symptoms. Subjective anxiety response to a 35% CO₂/65% O₂ inhaled mixture compared with compressed air (placebo).

Results: Our best-fitting solution yielded a common pathway model, implying that covariation between separation anxiety in childhood, hypersensitivity to CO₂, and panic disorder in adulthood can be explained by a single latent intervening variable influencing all phenotypes. The latent variable governing the 3 phenotypes' covariation was in turn largely (89%) influenced by genetic factors and childhood parental loss (treated as an identified element of risk acting at a family-wide level), which accounted for the remaining 11% of covariance. Residual variance was explained by 1 specific genetic variance component for separation anxiety disorder and variable-specific unique environmental variance components.

Conclusions: Shared genetic determinants appear to be the major underlying cause of the developmental continuity of childhood separation anxiety disorder into adult panic disorder and the association of both disorders with heightened sensitivity to CO₂. Inasmuch as childhood parental loss is a truly environmental risk factor, it can account for a significant additional proportion of the covariation of these 3 developmentally related phenotypes.

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THE TYPICAL ONSET OF PANIC disorder (PD) occurs in early adulthood,¹ but a closer investigation of one's developmental years may increase our understanding of this illness. Relatively distinct behavioral and psychophysiological antecedents have been described for PD.^{2,3} Some adverse events occurring early in life appear to increase the risk of later manifesting the disorder in addition to—or in interaction with—familial genetic causal factors.^{4,5}

An operationally defined abnormal childhood behavior that may represent an antecedent to adult-onset PD is separation anxiety disorder (SAD).^{6,7} Separation anxiety disorder has been found to be specifically associated with heightened individual risk to develop PD in con-

trolled, long-term follow-up studies of clinical and nonclinical pediatric samples^{8,9} and in retrospective studies of adults.¹⁰ Yet, some authors have not found an association between SAD and panic attacks.¹¹ Moreover, epidemiological data alone are not powerful enough to clarify the nature of continuity¹² between SAD and PD, which could reflect ongoing genetic or environmental influences or a combination of the two.

Several studies of CO₂ sensitivity in children and adults offer an opportunity to assess a neural substrate common to SAD and PD. Controlled studies of children and adolescents with anxiety show a consistent association of SAD with CO₂ hypersensitivity, both defined by symptom reports,^{13,14} and with abnormal respiratory measures during exposure to hypercap-

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nia.¹⁴ The responses to CO₂ stimulation in children with SAD thus appear similar to those seen in adults with PD.^{13,15,16}

At least 3 potential limitations apply, however. First, although longitudinal designs would best address the question of the extent to which childhood SAD predicts CO₂ hypersensitivity in adulthood, our study uses retrospective assessments of SAD symptoms in adults who underwent CO₂ stimulation. Second, the possible role of other diagnoses, anxiety disorders most prominently, in influencing the subjective anxiety response to CO₂ stimulation should be considered, given the rates of comorbid disorders described for both PD¹ and SAD.¹⁴ Third and foremost, the underlying causes of covariation between reported symptoms of SAD in the developmental years, CO₂ reactivity, and PD in adulthood remain unaddressed.

Turning to the role of early life experiences in bringing about continuities and discontinuities in psychopathology, actual separation events during childhood (encompassing, eg, parental death, separation or divorce, relocation, sometimes cumulatively referred to as childhood parental loss [CPL]⁴) have been recognized as risk factors to predict PD in adulthood by general population and clinical studies.^{4,17} On the other hand, studies of adults with PD¹⁸ that found that both SAD and separation events were associated with PD reported a near-zero correlation between actual separation experiences and childhood SAD. This may mean that at least partially independent developmental pathways lead from parental loss and/or excessive worry about separation in childhood to PD in adulthood. However, heterogeneity may act as a confounder: if both PD and SAD were heterogeneous, failure to distinguish between subgroups would reduce the correlations.

The present study sought to address some of these issues within a genetically informed design. In a sample of young adult twins from the Norwegian Institute of Public Health Twin Panel, we examined 3 principal questions. First, we wanted to clarify the sources of covariation between retrospectively assessed SAD symptoms, sensitivity to CO₂, and the emergence of PD in adulthood. Second, we wanted to understand whether the genetic and/or environmental causes of such covariation are best conceived as acting directly and independently on phenotypes or through the mediation of an additional common higher-order factor. Third, we wanted to assess whether, and to what extent, adding CPL as an identified element of risk to a causal model of covariation between SAD, sensitivity to CO₂, and PD would improve our ability to explain the nature of these associations.

METHODS

RECRUITMENT AND ASSESSMENT

As outlined in detail elsewhere (A.O. et al, unpublished data, 2008),^{19,20} from 2002 to 2004 we consecutively recruited a sample of 712 subjects (346 complete pairs plus 20 single twins) from the Norwegian Twin Study on the Genetics of Personality and Mental Health (a cohort sequential design program based on a general population cohort of twins). From these twins, we gath-

ered extensive information on CO₂ reactivity, occurrence of life events, antecedents of PD, and direct psychiatric evaluations. Although the large majority of participants were randomly ascertained to guarantee a sufficient number of individuals who would be informative at the CO₂ challenge, 12% of pairs were selectively ascertained on the endorsement of anxiety-related items in a mailed questionnaire they had answered in 1998.¹⁹ Despite the partially nonrandom ascertainment, factors such as age, frequency of contact with co-twin, and self-rated symptoms of generalized anxiety disorder or depression had no or marginal ability to predict participation in the CO₂ study.¹⁹ To assess individual sensitivity to CO₂, we used the 35% CO₂ single-breath test,^{19,21} which is a safe procedure with a good ability to distinguish patients with PD from controls and patients with other mental disorders.^{16,22,23}

After complete description and screening of exclusion criteria,^{19,21} signed informed consent was obtained as approved by the Regional Committee for Research Ethics and the Norwegian government. Two gas mixtures were used: compressed air (placebo) and a mixture of 35% CO₂ and 65% O₂. Participants inhaled the gasses through a self-administration mask connected to a Mark 20 Wright respirometer (Ferraris Medical, Hertford, England) to measure vital capacity and the gas volume delivered at each inhalation. Participants were informed before the challenge that they would inhale 2 harmless gas mixtures that might elicit some discomfort, from few physical symptoms to clear anxiety. After vital capacity was measured, the participants inhaled 1 vital capacity of compressed air. Then, after an interval of 30 minutes, they inhaled 35% CO₂/65% O₂. At the end of each inhalation, participants held their breath for 4 seconds. According to this standardized procedure,^{21,23} the test is valid if at least 80% of vital capacity is inhaled.

In the interval between the 2 inhalations, 2 interviewers (trained and supervised by M.B. and K.T.) who were masked to participants' diagnoses investigated, using a semistructured interview, the participants' lifetime history of stressful events, SAD, and CPL (A.O. et al, unpublished data, 2008). The occurrence of symptoms of SAD was retrospectively assessed by the 12-item questionnaire by van der Molen et al²⁴ adapted for DSM-IV criteria.⁷ As in previous studies of adults,⁴ CPL was defined as a period of at least 1 year of unexpected or unscheduled separation from 1 or both biological parents that occurred prior to the participants' 17th birthday. As part of the Norwegian Twin Study on the Genetics of Personality and Mental Health,²⁵ all twins were also interviewed for lifetime Axis I and II disorders with the Composite International Diagnostic Interview (CIDI)²⁶ and the Structured Clinical Interview for DSM-IV Personality,²⁷ which were administered by experienced psychology students or psychiatric nurses who had adequate training.²⁸

OUTCOME VARIABLES

The response evoked by the 35% CO₂/65% O₂ test was measured by the Panic Symptom List III-Revised²⁹ and the Visual Analog Scale for Anxiety (VASA).³⁰ Because post-CO₂ VASA scores have better reliability³¹ and discriminative power,²² we based our study on these scores (0, no anxiety at all; 100, the worst anxiety imaginable) obtained immediately after inhalation.

After the 35% CO₂/65% O₂ test, self-rated anxiety scores typically yield skewed distributions without an a priori ideal threshold to define a positive response.^{16,19,22} To deal with these issues, we adopted 2 thresholds—the 75th and 90th VASA score percentiles of the sample—whereby each participant could be classified as a responder or nonresponder. The 90th percentile corresponds here to the 26% increment of anxiety, which has been shown²² to be the ideal threshold to distinguish people

with PD from controls, while the 75th percentile threshold was set to identify a more lenient level of sensitivity to hypercapnia, suitable for participants from the general population.¹⁹

Likewise, because categorically defined (present or absent) PD is relatively rare in the population,^{1,32,33} we organized CIDI diagnostic information into 3 categories: 0, unaffected (participant had never experienced a spontaneous panic attack); 1, broad PD (participant had experienced ≥ 1 panic attacks, but failed to meet the full lifetime diagnosis or to have a current diagnosis of *DSM-IV* PD); and 2, narrow PD (participant satisfied lifetime or current criteria for *DSM-IV* PD). As we did with VASA scores and PD, we imposed 2 thresholds (the 75th and 90th percentiles of the sample) on the semicontinuously distributed SAD scores, whereby each participant could be classified as 0, unaffected; 1, having mild separation anxiety; or 2, having substantial separation anxiety/SAD. Owing to limited power, CPL was sorted dichotomously (0, no CPL; 1, CPL) without differentiating between parental death, divorce, continued separation, or maternal vs paternal CPL.

By specifying 2 thresholds on all traits, we adopted a multiple threshold model³⁴ approach, which assumes different degrees of severity on the same normally distributed underlying continuum of risk. Controls for the appropriateness of this assumption made by multiple threshold tests with PRELIS³⁵ for monozygotic (MZ) and dizygotic (DZ) twins for each of the 3 phenotypes provided good fits (range, MZ twin relative to post-CO₂ VASA score, $P = .12$; MZ twin relative to SAD, $P = .89$), suggesting that, within the limits of these data and procedures, the milder and stronger responses to CO₂ and broad and narrow SAD and PD are on the same continua of liability, as has been consistently shown in previous reports.^{19,20,32,33}

CLINICAL AND SOCIODEMOGRAPHIC CHARACTERISTICS OF THE SAMPLE

The characteristics of the sample have been detailed elsewhere (A.O. et al, unpublished data, 2008).^{19,20} Briefly, of 712 participants, 64% were women, 49% were MZ twins, and the mean age was 30.95 years (standard deviation, 3.6 years). No participant had an anxious response to inhalation of compressed air (placebo). A lifetime diagnosis of *DSM-IV* PD was present in 6.6% of participants, and a retrospective diagnosis of SAD was attributed to 10.4% of participants for having endorsed 3 or more SAD symptoms for at least 1 month and having significant interference during childhood to adolescence. Both values are consistent with those reported in people in the general population of comparable age who were interviewed for lifetime PD³⁶ and retrospectively for SAD.³⁷

Causes of CPL included parental death (4.1% of participants) and separation events (divorce, job relocation, military service, etc, endorsed by 11.1% of participants). These were reported concordantly by the large majority of twin pairs. The few cases in which only 1 twin in a pair had reported CPL were, after inspection, attributable to minor discrepancies (usually a few months) in dating the occurrence of a separation event shortly before or after the twins' 17th birthday. Therefore, to increase sensitivity, all pairs in which at least 1 twin had reported CPL were considered concordant-positive (17.7%), while all other pairs were concordant for not having experienced CPL (82.3%). Consequently, herein CPL is considered a fully concordant family-wide environmental element of risk.

STATISTICAL ANALYSIS

Preliminary analyses with logistic regression (model $\chi^2 = 30.621$, $P < .001$) showed that positive responses to CO₂ were predicted by SAD (Wald $\chi^2 = 22.1$; odds ratio, 2.75; 95% confi-

dence interval, 1.8-4.2; $P < .001$) and PD (Wald $\chi^2 = 4.3$; odds ratio, 2.09; 95% confidence interval, 1.1-4.2; $P = .04$) but not by lifetime diagnoses of social phobia, obsessive-compulsive disorder, generalized anxiety disorder, depression, or blood or injury phobias on the CIDI. Amongst the nonsignificant predictors mentioned, depression had the largest effect ($P = .26$). Likewise, when the correlations between post-CO₂ VASA score and PD and between post-CO₂ VASA score and SAD were controlled for each of the aforementioned lifetime diagnoses on the CIDI, we observed only modest variations of correlation coefficients (mean variation in correlation coefficients, 6.5%; minimum variation, 0.1% between PD and VASA score after controlling for generalized anxiety disorder; maximum variation, 15% decrease of correlation between PD and VASA score after controlling for depression), all of which remained significant. Therefore, our multivariate analyses encompassed PD and SAD but no other lifetime diagnoses on the CIDI.

All structural equation modeling analyses were run with the Mx program³⁸ using raw data, including the twin pairs with incomplete information by using the method of maximum likelihood. The within-twin and cross-trait (between 2 traits in the same twin), the cross-twin and within-trait (between twin 1 and twin 2 of the same pair for the same trait), and the cross-twin and cross-trait (between 1 trait in 1 twin and the other trait in the co-twin) polychoric correlations (based on the assumption of an underlying continuous bivariate normal liability distribution) were obtained by running the script *ordSATmt2.mx*, available in the Mx library.³⁹ Owing to limited power, we did not explore possible sex-specific variance effects. The polychoric correlations were then calculated by assuming no sex differences and by only using 2 zygosity categories: MZ and DZ. We began estimations of the polychoric correlations by calculating the relative fit of a model without constraints (ie, a saturated model, which contains as many parameters as there are unknowns), against which we compared the fit of simpler models with progressively more elaborated constraints. We successively tested the likelihood of imposing no difference in thresholds between the first and second twins in a pair and MZ and DZ pairs for SAD, positive post-CO₂ response measured with the VASA, and PD (all 3 traits under categories 0, 1, 2, as explained in the "Outcome Variables" section).

We then applied a multivariate twin design to the data. A multivariate twin design models the causal sources of covariation between 3 or more conditions and allows for the separation of the total phenotypic variance and covariance of traits into proportions owing to (1) additive genetic, (2) shared environmental (eg, experiences associated with socioeconomic and/or religious background), and (3) unique (individual-specific) environmental (like most illness, interpersonal relationships, etc) factors. The models compare MZ and DZ twins' phenotypic resemblance, assuming correlations of 1.0 for MZ pairs and 0.5 for DZ pairs between their additive genetic influences (DZ twins share half of their segregating genes on average) and a correlation of 1.0 for both MZ and DZ pairs between their shared environmental influences; unique environmental influences are uncorrelated for all twin pairs (with the equal environment assumption). While the purpose of the univariate twin design is to explain the causes of individual differences for a single phenotype, multivariate models involve twin correlations for different traits taken into account simultaneously and thus can be viewed as a simultaneous factor analysis on the genetic and environmental variances and covariances.⁴⁰ By comparing the cross-trait twin correlations in MZ and DZ twins, the sources of covariance between the traits are quantified so that greater MZ than DZ cross-twin cross-trait correlations suggest genetic influences on covariance.

We considered 3 alternative multivariate models with progressively more elaborate constraints to be compared with a saturated model: the Cholesky, the independent pathway, and

Table 1. Polychoric Correlation Estimates Calculated Using Best-Fit Results in 712 Twins

Twin Type	Within-Trait Polychoric Correlations			Cross-Trait Polychoric Correlations					
	SAD ^a	VASA Score ^b	PD ^c	Within-Twin			Cross-Twin		
				VASA Score With SAD	VASA Score With PD	SAD With PD	VASA Score With SAD	VASA Score With PD	SAD With PD
Monozygotic (n=349)	0.77	0.52	0.40	0.39	0.40	0.46	0.38	0.36	0.38
Dizygotic (n=363)	0.27	0.19	0.19	0.39	0.40	0.46	0.25	0.17	0.33

Abbreviations: PD, panic disorder; SAD, separation anxiety disorder; VASA, Visual Analog Scale for Anxiety.

^aRetrospective assessment of *DSM-IV* SAD through direct interview.

^bAfter 35% CO₂/65% O₂ inhalation test.

^cLifetime occurrence of *DSM-IV* PD.

the common pathway models. For *n* variables, a Cholesky decomposition includes *n* independent genetic and environmental factors. The first factor loads on all traits, the second loads on all traits except the first, the third loads on all traits except the first 2, and so on. The independent pathway model^{41,42} predicts that 1 or more common latent genetic and/or environmental factors influence covariation of the observed variables directly (ie, without the mediation of any higher-order factor) and allows the influence of the overlapping factors to differ quantitatively so that the common genetic and environmental factors do not necessarily cause similar groupings of variables. In a common pathway model, covariation is accounted for by genetic and environmental factors through a shared pathway⁴²; in this model, a latent intervening variable determined by higher-order latent genetic and environmental factors influences all phenotypes. In terms of life science concepts, such a latent intervening variable could be thought of as 1 or more unifying (patho)physiological mechanisms or systems common to SAD, CO₂ hypersensitivity, and PD, such as a suffocation detector system, as Klein hypothesized.⁶

Although these 3 multivariate models make different assumptions, they all distinguish between common factors that influence all phenotypes and factors specific to each phenotype. While common genetic and environmental factors contribute to explaining the phenotypic covariance, the specific factors explain residual variance not shared by the different phenotypes.

After having identified a best-fitting model to explain phenotypic covariation, we proceeded to test whether the addition of a specified (ie, measured) family-wide environmental agent such as CPL could improve the model's ability to explain covariance. We chose CPL for 2 reasons. First, the classic twin study approach often fails to detect a substantial role for shared environmental agents,⁴³ but the introduction of specified agents into the models sometimes reveals a contribution of such elements, explaining a proportion of familial aggregation of traits.^{32,44} Second, CPL as a specified form of familial environment has been reported to account for 4.9% of total variance in liability to PD.⁴

Preliminarily, we assessed whether the presence or absence of CPL was associated with different parameter estimates, ie, whether its action could to some extent be described in terms of interaction. We calculated the relative fit of a common pathway model (ie, the best-fitting model according to multivariate analyses, see the "Results" section), which allowed the estimates for SAD, post-CO₂ VASA score, and PD to differ across pairs concordant-positive and concordant-negative for CPL against the fit of a simpler model that imposed the same parameter estimates for pairs who had and had not experienced CPL. Then we used our best-fitting model (to which CPL was added as a specified family-shared factor with

variance fixed to unity) as a starting point to assess whether (1) early parental loss could further characterize the model by explaining an additional, substantial proportion of variation or covariation, (2) the role of early parental loss could best be described as impinging directly on 1 or more phenotypes (via a residual model whereby CPL directly and differentially influenced the phenotypes), or (3) the role of early parental loss could best be described as impinging on a latent common factor (via a factor model whereby CPL influenced phenotypic covariation via a common, underlying liability or factor shared by all 3 phenotypes).

We based the selection of the model best fitted to the raw data and parameter estimations on a maximum-likelihood approach. The significance of factors was tested by stepwise deletion of variance components in progressively more parsimonious models. Submodels were compared using hierarchical χ^2 tests, as the difference between twice the negative log likelihood (-2LL) for the reduced and the full models have a χ^2 distribution, with *df* given by the difference between the *df* for the 2 models.⁴⁵ Models were also compared on the basis of the Akaike information criterion (AIC=-2LL-2 *df*), with the lowest AIC value reflecting a balance between goodness of fit and parsimony.

RESULTS

A full model with the thresholds of all variables (SAD, post-CO₂ VASA score, and PD) allowed to differ across zygosity groups and within twin pairs (first and second twins in a pair) yielded the following: -2LL=2275.15, *df*=2037, and AIC=-1798.85. By applying progressively more elaborate constraints, we observed improvements in parsimony without significant worsening of the fit indices: a model in which the thresholds were constrained to be equal for the first and second twins within a pair and across MZ and DZ pairs yielded the following: -2LL=2303.31, *df*=2055, and AIC=-1806.69. We were able to further reduce the number of parameters via a submodel that estimated 1 cross-twin cross-trait polychoric correlation for each zygosity group and we constrained the within-twin cross-trait correlation to be the same for MZ and DZ pairs⁴⁶ and for the first and second twins in a pair (-2LL=2318.36, *df*=2070, and AIC=-1821.64). **Table 1** presents the polychoric correlations for MZ and DZ twins, calculated on the basis of the latter, best-fitting phenotypic model. The size of within-twin phenotypic correlations confirm that the 3 traits covary moderately in our sample. The differences

Table 2. Multivariate Models' Statistics and Comparisons

Model ^a	-2LL	df	AIC	χ^2	df	P Value	Change in χ^2	Change in df	P Value
Model 1, saturated	2275.15	2037	-1798.85						
Model 2, Cholesky	2324.54	2070	-1815.46	49.39	33	.03			
Model 3, independent	2324.64	2070	-1815.36	49.49	33	.03			
Model 4, common	2325.57	2074	-1822.43	50.42	37	.07			
Model 5, common + A _C = 0	2332.51	2075	-1817.49	57.36	38	.02	6.94 ^b	1	.01 ^b
Model 6, common + C _C = 0	2325.57	2075	-1824.43	50.42	38	.09	0.00 ^b	1	>.99 ^b
Model 7, model 6 + E _C = 0	2325.65	2076	-1826.35	50.49	39	.10	0.07 ^c	1	.79 ^c
Model 8, model 7 + C _S VASA, C _S PD, C _S SAD = 0	2325.65	2079	-1832.35	50.49	42	.17	0.00 ^d	3	>.99 ^d
Model 9, model 8 + A _S VASA = 0	2327.36	2080	-1832.64	52.21	43	.16	1.71 ^e	1	.19 ^e
Model 10, model 9 + A _S SAD = 0	2333.54	2081	-1828.46	58.39	44	.07	6.18 ^f	1	.01 ^f
Model 11, model 9 + A _S PD = 0 ^g	2327.36	2081	-1834.64	52.21	44	.19	0.00 ^f	1	>.99 ^f

Abbreviations: A, genetic factor; AIC, Akaike information criterion; C, shared environmental factor; E, unique environmental factor; PD, panic disorder; SAD, separation anxiety disorder; VASA, Visual Analog Scale for Anxiety; -2LL, twice the negative log likelihood.

^aThe subscript C indicates that the influence of the factor is common to the phenotypes, while the subscript S indicates that the influence of the factor is specific, or uncorrelated between phenotypes.

^bCompared with model 4.

^cCompared with model 6.

^dCompared with model 7.

^eCompared with model 8.

^fCompared with model 9.

^gBest-fitting model.

Table 3. Comparison Between Models Implying Different Modes of Influence for CPL

Model	-2LL	df	AIC	χ^2	P Value
Common pathway model ^a + CPL as residual model ^b (model 1)	2645.33	2439	-2232.67		
Common pathway model ^a + CPL as factor model ^c (model 2)	2648.59	2441	-2333.41		
Model 2 + effect of CPL = 0	2660.86	2442	-2223.14	12.27 ^d	.001 ^d

Abbreviations: AIC, Akaike information criterion; CPL, childhood parental loss; -2LL, twice the negative log likelihood.

^aBest-fitting model (Table 2).

^bChildhood parental loss independently influencing the variance of observed phenotypes.

^cChildhood parental loss influencing the common intervening variable L.

^dCompared with model 2.

between MZ and DZ cross-twin within-trait correlations are consistent with data showing different degrees of heritability for PD,^{32,33} SAD,^{5,47} and the acute anxious response to CO₂ stimulation.¹⁹ The greater cross-twin cross-trait correlations in MZ twins compared with DZ twins in turn suggest the importance of genetic factors in explaining phenotypic covariation.

Table 2 presents the results of fitting 3 alternative models compared with a saturated model to explain phenotypic covariation. The common pathway model (model 4) provided a more parsimonious fit than the Cholesky (model 2) and the independent pathway (model 3) models and therefore was selected as the starting point for further refinement. Dropping the common genetic factor from the common pathway model provided a clear and significant deterioration of the fit (model 5), whereas the common shared environmental and the common unique environmental factors could be dropped from the model without significant fit deterioration and with improvement of the AIC (models 6 and 7). Further modeling showed that dropping all specific shared environmental factors (SAD, VASA score, and PD [model 8]), the specific genetic factor for VASA score (model 9), and the specific genetic factor for PD (model 11) did not

worsen the model's fit, while a significant deterioration of the model was attained when attempting to drop the specific genetic factor for SAD (model 10). Overall, these analyses show that the best-fitting model is a common pathway model with 1 common genetic higher-order factor, 1 specific genetic variance component for SAD, and specific unique environmental variance components for all 3 phenotypes to explain residual variance.

A common pathway model in which all parameter estimates were allowed to differ across twin pairs who had or had not experienced CPL for all phenotypes yielded -2LL = 2286.53 and AIC = -1843.47, whereas a model that equated parameters' estimates across pairs who had or had not experienced CPL yielded -2LL = 2288.26 and AIC = -1863.74. We interpret this finding as evidence against interactive (ie, gene by environment) effects of CPL.

Table 3 presents the results of model fitting when CPL is added to the best-fitting common pathway model (model 11) (Table 2), with parameter estimates equated across pairs with or without a history of CPL. The factor common pathway model (model 2) had a better balance between goodness of fit and parsimony than the residual common pathway model (model 1). The added value of CPL to computations is shown by worsening of

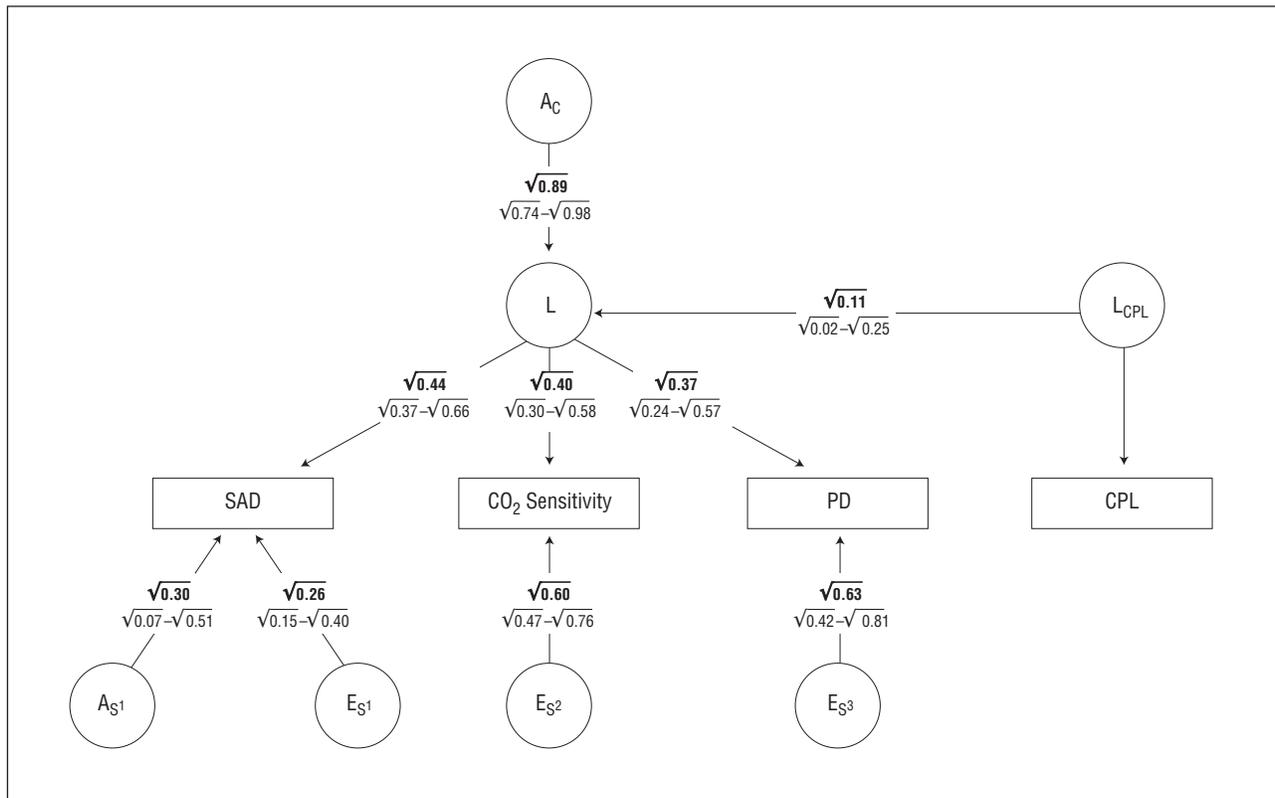


Figure. Best-fitting factor common pathway model with childhood parental loss (CPL) fitted as a shared environmental risk factor and parameters estimates (95% confidence intervals). Values in bold are path values and those beneath are 95% confidence intervals. L indicates common latent intervening variable determined by higher-order latent genetic and environmental factors that influence all phenotypes; PD, panic disorder; and SAD, separation anxiety disorder.

the AIC once this measured risk factor is constrained to 0 (model 3). The **Figure** shows the best-fitting factor common pathway model with CPL fitted as shared environmental risk factor and the parameters estimates.

COMMENT

Our results show that the covariation between separation anxiety in childhood, hypersensitivity to CO₂ (as indexed by the anxiety response to a 35% CO₂/65% O₂ mixture), and PD in adulthood can be explained by a single, shared underlying latent variable influencing the 3 phenotypes. These findings appear to concur with a body of evidence that was collected during almost 5 decades and stemmed from the findings of shared response of PD and SAD to imipramine.⁴⁸ Successively, the symptoms of air hunger and the psychobiological trait of sensitivity to lactate and CO₂⁶ were pivotal in demonstrating the distinctiveness of PD from other anxiety syndromes and led to the recent formulation of falsifiable models⁴⁹ of the neurobiological commonalities linking SAD to PD.

Inasmuch as the hypersensitivity to CO₂ can be considered a relatively specific biological marker,^{16,22} our data favor a developmental continuity between SAD in childhood and PD in adulthood, whereby the same underlying neural substrate of excessive sensitivity to a suffocative stimulus⁶ appears to act as a bridging element between these 2 anxiety disorders. In turn, genetic effects appear to be the most important underlying cause of such continuity, because the latent variable governing the 3 phe-

notypes' covariation is largely (89%) influenced by additive genetic determinants, according to our best-fitting model solution.

Like many other studies based on the classic twin study approach, we found that common and specific shared environmental effects could be dropped from stepwise modeling without significant loss of fit. However, as in several other studies, adding CPL (a shared familial factor) to our best-fitting model explained a significant proportion (11%) of the covariation between variables. By multiplying the standardized coefficient paths, one can easily obtain the amount of variance in liability for each phenotype attributable to the additive action of CPL. For instance, in the case of PD, this yields 4.1% (0.11 × 0.37), a value close to that obtained by a previous study on the effect of CPL on liability for PD in adult women (4.9%).⁴ Therefore, inasmuch as CPL can be fully considered to be an aspect of family-wide environment, it helps to explain the familial aggregation of the phenotypes being studied here as well as their covariation within the same individuals.

These results should be interpreted with regard to 7 potential limitations. First, while this is probably the largest sample ever probed for CO₂ reactivity, it is relatively small for structural equation modeling analyses of twin data. Reduced participation rates and relatively small samples, however, remain inevitable constraints of studies that use moderately stressful procedures. Moreover, the use of categorical data and the low prevalence of positive responses to a challenge applied to participants in

the general population somewhat reduces the power of this study. The consequences include the modest precision of several parameter estimates, the relatively wide confidence intervals, and reduced power to effectively choose between alternative multivariate, nested models solutions. Based on the AIC, we can quite safely conclude that CPL is important in explaining the covariation of PD, CO₂ sensitivity, and SAD. There is, however, only a small margin to support the conclusion that CPL exerts a similar proportional effect on all 3 phenotypes through the mediation of the latent intervening variable (ie, the factor model), rather than acting directly and possibly differently on SAD, CO₂ sensitivity, and PD (ie, the residual model). Second, individual response to the 35% CO₂/65% O₂ test appears to be reasonably reliable³¹ and stable^{50,51} for some but not all PD symptoms. Moreover, while we found that depression and several anxiety disorders did not predict heightened sensitivity to the 35% CO₂/65% O₂ test, we did not measure and could not control for the possible effect of neuroticism, which some⁵² found to partially mediate the response to CO₂ stimulation. Third, we did not control for sex effects. Because both PD and heightened reactivity to CO₂ are more common in women than men, the twin correlations for both traits in opposite-sex pairs could be lower than those of same-sex pairs, resulting in artificially increased differences in MZ-DZ correlations. Liability-threshold model approaches to large samples of twins in the general population, however, have not found sex effects on the genetic risk factors for different definitions of PD syndromes.³³ Fourth, the findings are based on the method's assumptions, including the independence and additivity of the latent variables, random mating, and the equal environment assumption. However, regression analyses of the questionnaire items that assessed the degree of environmental closeness between sibs and the possible influence of shared experiences on MZ-DZ twin concordance revealed that these measures of closeness could not predict concordance for either response to the CO₂ test and PD ($P = .13-.97$)²⁰ or SAD ($P = .21$ for DZ twins, $P = .87$ for MZ twins), suggesting that shared environmental experiences are unlikely to have biased the estimation of genetic covariation between the traits being analyzed. Fifth, this is a partially nonrandomly ascertained sample, but previous controls of the effect of this possible bias on parameters' estimates showed relatively modest effects in our data set.²⁰ Sixth, each phenotype was assessed at 1 time, which potentially confounds the effects of individual-specific environmental and measurement error, possibly including a recollection bias specific to SAD, which is generally seen in retrospective assessments.^{53,54} Seventh, by finding that genetic causes are the main reason for covariation between the studied phenotypes, we partially disagree with 1 study that failed to confirm CO₂ hypersensitivity as a familial risk marker for PD in children and adolescents¹⁴ who were exposed to 5% CO₂ mixtures. However, we are in broad agreement with 5 studies (reviewed by Pine et al¹⁴) that found greater response to a single breath of 35% CO₂ in adult offspring of patients with PD than in controls. Such inconsistencies may relate to several methodological factors, including the anxiogenic properties of different CO₂

and O₂ concentrations (eg, a 35% CO₂/65% O₂ mixture is simultaneously hypercarbic and hyperoxic) and statistical power issues.¹⁴

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