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Generalized Anxiety and C-Reactive Protein Levels: A Prospective, Longitudinal Analysis

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Abstract

Background—Generalized Anxiety Disorder (GAD) is highly comorbid with depression. Depression is associated with elevated levels of the inflammation marker C-reactive protein (CRP), cross-sectionally and over time. To date, no studies have looked at the association of CRP with GAD.

Methods—Ten waves of data from the prospective population-based Great Smoky Mountains Study ($N=1,420$) were used, covering children in the community aged 9–16, 19, and 21 years old. Structured interviews were used at each assessment to assess GAD symptoms, diagnosis, and cumulative episodes. Bloodspots were collected and assayed for CRP levels.

Results—GAD was associated with increased levels of CRP in bivariate cross-sectional analyses. These bivariate associations, however, were attenuated after accounting for demographic, substance use, and health-related covariates. In longitudinal models, there was little evidence that CRP predicted later GAD. Associations from GAD to later CRP were attenuated in models adjusted for health-related covariates and there was evidence that the GAD-CRP association was mediated by BMI and medication use.

Conclusions—Similar to depression, GAD was associated with elevated levels of CRP, but the effect of GAD on CRP levels was explained by the effect of GAD on health-related behaviors such as BMI and medication use. This study suggests differences in the association between inflammation and depression and GAD.

Keywords

Inflammation; CRP; Generalized Anxiety Disorder; Epidemiology; Childhood; Adolescence

Introduction

Generalized anxiety disorder (GAD) is characterized by excessive or uncontrollable anxiety and worry, is associated with a range of physical symptoms (American Psychiatric Association, 1994), and is highly comorbid with depression at every stage of life (Angold et al., 1999a, Kessler et al., 2005) (Moffitt et al., 2007b). Several lines of empirical research

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have established that GAD and depression share many, although not all psychosocial and biological risk factors and correlates (American Psychiatric Association, 1994). Depression is associated with elevated levels of pro-inflammatory cytokines and the proteins released in response to increasing cytokine levels. The acute phase protein C-reactive protein (CRP) has been the focus of extensive epidemiologic investigation because of the association of elevated plasma CRP levels (>3 mg/L) with cardiovascular risk (The Emerging Risk Factors Collaboration, 2010, Shah et al., 2009) and aspects of metabolic syndrome (Tamakoshi et al., 2003, Pradhan et al., 2002, Ridker et al., 2003). Recent work on depression has identified robust cross-sectional and longitudinal associations with CRP (Howren et al., 2009, Copeland et al., 2012). Are such associations also observable between GAD and CRP?

Several previous findings suggest that such an association should be identified. First, family and twin studies have documented co-transmission of depression and GAD (Kendler et al., 1995, Eley and Stevenson, 1999, Thapar and McGuffin, 1997, Middeldorp et al., 2005) as well as strong bivariate genetic correlations between depression and GAD (Kendler et al., 2007, Kendler, 1996). Second, hypothalamic-pituitary adrenal axis and autonomic nervous system activity have both been implicated in depressive symptoms and CRP production (for reviews (Raison et al., 2006, Dantzer et al., 2008)); these systems are generally also activated in the context of anxiety (O'Donovan et al., 2010, Toker et al., 2005, Pitsavos et al., 2006), although few studies of physiological correlates have specifically focused on GAD. Third, depression and GAD tend to be responsive to the same active ingredients in drugs (Kuzma and Black, 2004). Finally, a number of studies comparing psychosocial correlates of depression and GAD have identified at least some similarities (Moffitt et al., 2007a).

Recent work has, however, also identified differences between depression and GAD, suggesting at least partially different etiological pathways. For example, divergence in correlates was found for a number of psychosocial adversities (Moffitt, 2007, Shanahan, 2008) and temperamental traits (Moffitt, 2007). Furthermore, a family history of depression was specifically associated with depression, but not with GAD (Moffitt, 2007; Shanahan, 2008), suggesting potential differences in at least some of the biological bases of these disorders.

The goal of this study was to test whether GAD would be associated with increased levels of the systemic inflammation marker CRP, both cross-sectionally and over time. It is also plausible, as was observed in part with depression (Copeland et al., 2012), that a portion of any identified association is accounted for by other health-related variables such as body-mass index, medication use, and smoking. Such an association would provide additional evidence for biological overlap between depression and GAD and provide some support for the combination of these disorders in DSM-5. A lack of association between GAD and CRP would implicate distinct biological processes in these otherwise closely-linked disorders.

Methods and Materials

Participants

The Great Smoky Mountains Study is a longitudinal study of the development of psychiatric disorder and need for mental health services in rural and urban youth (Costello et al., 1996, Costello et al., 2003). A representative sample of three cohorts of children, age 9, 11, and 13 at intake, was recruited from 11 counties in western North Carolina using a multi-stage selection process. All children scoring above a predetermined cut-point (the top 25% of the total scores) on a screening questionnaire, plus a 1-in-10 random sample of the rest, were

recruited for detailed interviews. Of all subjects recruited, 80% (N=1420) agreed to participate.

Subjects were assessed annually to age 16 then again at ages 19 and 21. Participants were interviewed as closely as possible to their birthday each year. Across all waves, participation rates averaged 84% (range: 74-94%). About 8% of the area residents and the sample are African American, less than 1% are Hispanic, and 3% are American Indian.

Procedures

The parent (biological mother for 83% of interviews) and subject were interviewed by trained interviewers separately until the subject was 16, and subjects only thereafter. Before the interviews began, parent and child signed informed consent forms approved by the Duke University Medical Center Institutional Review Board. Each parent and child received an honorarium for their participation.

Using a previously described procedure (Worthman and Stallings, 1997), blood samples were obtained at the beginning of each in-person assessment, as follows: two finger-prick samples (yielding 10 spots total per visit) were collected at a 20-minute intervals, applied to standardized collection paper, immediately refrigerated upon drying, and express shipped (without refrigeration) to the laboratory within two weeks of collection. Samples were then stored at -28°C until they were assayed. This protocol is consistent with the rigorous quality control program developed for newborn screening programs (Mei et al., 2001) and has been used in a number of epidemiologic studies involving blood spot CRP measures (McDade et al., 2006, Williams and McDade, 2009).

Assessment

Generalized Anxiety is characterized by excessive anxiety and worry that a person finds difficult to control, and is associated with a range of physical symptoms (restlessness, easily fatigued, difficulty concentrating, irritability, muscle tension, and sleep disturbance) (American Psychiatric Association, 1994). Generalized anxiety disorder variables were assessed using the *Child and Adolescent Psychiatric Assessment* until age 16, and its upward extension, the *Young Adult Psychiatric Assessment* thereafter (Angold and Costello, 2000, Angold et al., 1999b). These structured interviews were coded by a trained interviewer and each interview was then checked by a supervisor. The time frame for determining the presence of most behaviors was the past three months to minimize forgetting and recall biases, although onset dates were also collected for all symptoms. A symptom was counted as present if reported by either parent or child or both, as is standard in clinical practice. Scoring programs written in SAS by the senior authors combined information about the date of onset, duration, and intensity of each symptom to create GAD diagnoses according to the fourth edition of the Diagnostic and Statistical Manual of the American Psychiatric Association (American Psychiatric Association, 1994). Two-week test-retest reliability of diagnoses is comparable to that of other highly-structured child psychiatric interviews (Angold and Costello, 1995). Construct validity as judged by 10 different criteria including comparison to other interviews and ability to predict mental health service use is good to excellent (Angold and Costello, 2000). Generalized anxiety disorder status was measured by two variables: Current generalized anxiety disorder (binary) and the number of total generalized anxiety symptoms (range: 0-6). Cumulative generalized anxiety exposure was assessed by looking at the total number of GAD episodes reported up until the current assessment (range: 0-3). This variable was included because of the evidence that cumulative depressive episodes predicted later CRP levels and other studies linking perturbations in inflammation levels to repeated psychological distress (Danese et al., 2008, Copeland et al., 2012).

C-Reactive Protein—Our assay for CRP in whole-blood spots was a biotin-Streptavidin based immunofluorometric system improving on a previously published method (McDade et al., 2004). One assay was completed for each subject at each observation. Streptavidin A-coated microtiter plates bind a biotinylated capture antibody to CRP, clone C2. A second Europium-labeled antibody then binds to the Streptavidin A Biotin-C2-CRP complex; fluorescence of the resultant complex is directly proportional to the CRP concentration in each well. Minimum detectable dose for the assay is .010 mg/L. For low, medium, high, and very high controls (.022, .259, 1.208, and 3.271 mg/L, respectively), within-assay coefficient of variation (CV)(precision) is 2.0%, 1.2%, 1.6%, and 1.4%, respectively, while between-assay CV (reliability) runs 14.4%, 13.9%, 12.3% and 10.9%, respectively. CRP remains stable in dried blood spots for at least 5 days at room temperature or 14 days at 4 °C, and stable for years at –26 °C.

A validation study was performed with matched serum and blood spot samples assayed for CRP (n=38). As has been reported for many other analytes including CRP (Worthman and Stallings, 1997, McDade et al., 1997, McDade et al., 2004), a close linear correlation was identified between serum and blood spot CRP values (n=29; $R^2 = .98$, $p < .0001$). Serum equivalents therefore were calculated using the following algorithm based on the serum-blood spot regression: serum (hsCRP) = $1.38 * (\text{Blood Spot CRP Value}) - 0.97$. Blood spot CRP measures have been used in a number of epidemiologic studies (Williams and McDade, 2009, McDade et al., 2006, McDade et al., 2005). Observations with values above 10 mg/L indicate frank infection and were removed from statistical analysis (N=190 from a total of 6000 observations), whereas values below 10 mg/L the extent of chronic low grade systemic inflammation associated with cardiovascular and metabolic risk (Pearson et al., 2003).

Covariates included variables typically associated with variation in CRP levels (Woodward et al., 2003, Elovainio et al., 2006) or used as covariates in studies of CRP and depression (Gimeno et al., 2009, Stewart et al., 2009, Janicki Deverts et al., 2010, Matthews et al., 2010). These included age, sex, race, BMI, medication use, substance use, low SES, and recent physical ailments. BMI was calculated from weight and height measurements completed at each assessment. For all assessments completed before subjects were 20, BMI values were corrected for age and sex (Ogden et al., 2002). The substance use assessment of the CAPA and YAPA interviews assesses current nicotine, alcohol, and illicit drug use. Dichotomous variables were included to indicate recent use of these substances. A physical health problems survey adapted from Form HIS-1A (1998), US Department of Commerce for the U.S. Public Health Service was administered to assess 39 common ailments (e.g., diabetes, anemia, mononucleosis). A binary variable indicating any health ailments within the last 12 months was used for all analyses. Analyses were also tested using the following separate health categories: atopic (e.g., food/digestive allergy, asthma, and, respiratory allergy), injuries, infections (tonsillitis, ear infection, frequent diarrhea or colitis, and urinary tract infections) and chronic diseases (e.g., diabetes, epilepsy, cancer and chronic heart disease). Medication use within the prior year was also assessed from the Child and Adolescent Services Assessment (Ascher et al., 1996), which focuses on psychotropic medications, but also assessed prescribed medications not related to psychiatric problems. All analyses were tested using a broad-based medication use variables as well as categories for individual medication groups (e.g., antidepressant, stimulant, and other prescribed medications). Low SES coded whether the subject's family displayed any 2 of the following 3 indicators: income below the federal poverty line, low parental education attainment, and low parental occupation status. Additional physiological covariates evaluated alongside CRP in older samples at risk for cardiovascular problems (e.g., blood pressure, lipids, or insulin) were not assessed.

Analytic framework

All subjects were assigned a weight inversely proportional to their probability of selection, so that the results are representative of the population from which the sample was drawn and not biased from the oversampling procedure. CRP values were positively skewed and were log₁₀-transformed after scaling for non-negative values by adding 1. Models predicting GAD symptoms or cumulative GAD episodes employed Poisson regression, models predicting diagnostic status employed logistic regression and those predicting CRP employed linear regression. All associations were tested using weighted regression models in a generalized estimating equations framework implemented by SAS PROC GENMOD. For all analyses, multiple observations were used for each subject and robust variance (sandwich type) estimates adjusted the standard errors of the parameter estimates for the repeated observations. The covariance matrix was specified as autoregressive in all analyses.

All *longitudinal* models used values from the prior assessment to predict current levels of the outcome variable. As such, only subjects with multiple assessments were included and subjects with more than two assessments contributed multiple observations. For example, if a subject had 5 assessments, then they contributed 4 observations to the current analyses (wave 1 predicting wave 2, wave 2 predicting wave 3, wave 3 predicting wave 4, and wave 4 predicting wave 5).

Missing data

By age 21, 8806 total assessments had been completed. Of these, bloodspots were obtained for 6087 (69.1%). Bloodspots were not available either because the subject refused or because the interviews were completed by phone. For in-person interviews, 79.4% of subjects agreed to provide bloodspots. Comparisons of observations in which blood spots were collected to those in which the subjects refused indicated no significant differences on any of the GAD outcomes. Of the 6087 bloodspots collected, 6000 (98.6%) were successfully assayed for CRP (190 were removed due to elevated CRP levels indicating infection). Of the 1420 study participants, 1,334 (93.9%) provided blood spot samples assayed for CRP in one year or more. The median number of CRP samples provided was 5 (mean =4.77 (SD=2.24); range =1-9).

Results

Sample characteristics

Table 1 shows the characteristics of the full analytic sample in person-observations (5810 observations of 1334 subjects). GAD-related outcomes were significantly associated with sex, age, BMI, tobacco use, alcohol use, illicit drug use, medication use, and current health ailments. CRP was associated with age, BMI, tobacco use, alcohol use, other drug use, medication use, current health ailments and low SES. This pattern of findings suggests that all covariates merit inclusion.

The overlap between GAD and depression variables was high. Thirty-nine-point-nine percent of those meeting criteria for GAD also met criteria for a depressive disorder (OR=40.9; 95%CI =18.0-92.9, $p < 0.0001$). There was similarly high overlap between symptoms ($r = 0.72$, $p < 0.0001$) and cumulative episodes to date ($r = 0.55$, $p < 0.0001$). If CRP is associated with GAD, it is reasonable to ask if such associations are independent of depression.

Cross-sectional Associations

A series of regression models tested the cross-sectional associations between CRP and all GAD-related outcomes (table 2). For each outcome, a simple bivariate model was tested as

well as a model adjusting for covariates. There was strong evidence of association between CRP and GAD-related outcomes in bivariate models. All associations were attenuated to nonsignificance by the inclusion of health-related covariates. BMI and medication use were significant predictors in all models predicting CRP.

Longitudinal Associations

Longitudinal bi-directional associations were tested in a series of similar models similar to those for the cross-sectional associations (table 3). The simple longitudinal model predicted the outcome using the predictor and prior status on the outcome variable (e.g., prior CRP and GAD symptoms predicting current GAD symptoms). As such, this model predicts change in the outcome variable across assessments. There was little evidence of either CRP predicting later GAD-related outcomes or vice versa in the simple models. The lone exception was GAD symptoms predicting later CRP levels adjusting for prior CRP. This association, like other marginal associations, was attenuated after accounting for current health-related covariates. The models testing effects of GAD-related variables on later CRP levels implicated a common set of covariates which included race, time since the last interview, BMI, nicotine use, and current medication use.

These concurrent covariates may mediate or account for the association between past GAD symptoms and current CRP as has been observed, in part, for depression (Copeland et al., 2012). Each significant covariate was tested as a potential mediator using a multistage regression approach (Baron and Kenny, 1986, MacKinnon et al., 2002). Identification of potential mediators required the following: 1) GAD symptoms were associated with the covariate (table 4, column 1); 2) In models controlling for the GAD symptoms, the covariate was associated with CRP (column 2); 3) In models controlling for the covariate, the association between the GAD symptoms and CRP was either no longer statistically significant or was attenuated (column 3); and 4) A statistically significant indirect path existed between the GAD symptoms and CRP through the covariate, as measured by the Sobel test (Sobel, 1982) (column 4). Neither race nor time since last interview were associated with the GAD symptoms and, thus, were not included in table 3. Both BMI and medication mediated the association from prior GAD symptoms to current CRP.

Followup analyses

CRP levels greater than > 3 mg/L indicate increased risk for cardiovascular disease in adults (Pearson et al., 2003). There was no evidence that changes to the definition of CRP, either by dichotomizing CRP levels at $CRP > 3$ mg/L or by including cases with $CRP > 10$ mg/L (which is indicative of acute infection) affected the results of either the cross-sectional or longitudinal analysis. It is plausible that the association of CRP with GAD differs is found in some subgroups but not in others. To test for this pattern, interaction terms were entered into each of the simple/bivariate between the predictor and each demographic variable (sex and race) or significant covariate. Significant interactions occurred at the rate expected by chance and were no more likely to include one demographic variable or covariate than another. Finally, it is possible - albeit unlikely - that it is the portion of GAD that is independent of depression that is associated with CRP levels. All analyses were rerun including depression variables as covariates. As expected, this did not change the pattern of associations.

Discussion

GAD is highly comorbid with depression (Moffitt et al., 2007b), and depression is both directly and indirectly associated with elevated levels of CRP (Howren et al., 2009). On this basis, we predicted that GAD would also be associated with elevated levels of CRP. There

was evidence supporting this hypothesis in bivariate associations both cross-sectionally and over time. These associations attenuated, however, with the inclusion of health-related covariates and both BMI and medication use which mediated the longitudinal associations between GAD variables and later CRP levels. Thus, GAD does affect levels of CRP, but this effect is accounted for by the effect GAD on health-related behaviors. The direct association with elevated low-grade inflammation is an area of divergence between GAD and depression for the age-groups examined here.

Despite the extensive literature on the depression-CRP link, only few studies have looked at the association between CRP and any measure of anxiety. The ATTICA study examined concurrent associations between State-Trait Anxiety Inventory scores and a number of markers of immunity/inflammation including hsCRP in a sample of 853 adults (Pitsavos et al., 2006). They found a small, but significant, association ($\rho=0.18$) that persisted after controlling for relevant covariates. A case-control study of 27 anxious patients and 29 nonanxious controls found no association between CRP levels and anxiety as measured by a self-report questionnaire (O'Donovan et al., 2010). Finally, a test of the CRP-anxiety association in a large sample of employees completing a routine health exam at an Israeli medical center found no association (Toker et al., 2005). These studies suggest a consensus for little evidence of association between CRP with anxiety. In our study which employed structured interviews to assess GAD, the aspect of anxiety most closely tied to depression, there was no independent association.

If indeed depression, but not GAD, is associated with CRP directly, what does this specificity suggest? Although the areas of overlap between GAD and depression are legion (Moffitt et al., 2007b, Watson, 2005), divergence in biological correlates may reinforce the notion that these disorders have at least partially distinct neurobiological profiles. There is also evidence that GAD and depression are preceded by somewhat different profiles of psychosocial risk factors (Moffitt et al., 2007a). The study of biological correlates has generally been limited to markers of HPA axis function. Here again, results have been mixed with some studies showing increased levels of basal and peak salivary cortisol levels (Mantella et al., 2008) and other studies failing to find any association of GAD with diurnal cortisol levels (Hoehn-Saric et al., 1991). Similar mixed results have been reported regarding nonsuppression of cortisol in response to the dexamethasone suppression test (Schweizer et al., 1986, Tiller et al., 1988). Studies of HPA-related indicators and depression, however, have been plagued by similar inconsistencies (Kaufman et al., 2001). One general conclusion from the literature is that disorder-biological correlate associations still tend to be small, affected by a range of health-related behaviors, and often limited to specific subgroups (by age, number of disorder episodes). Thus, while our findings are consistent with a conceptualization of GAD and depression as closely-linked entities with distinct biological correlates, more work needs to be done on the conditions under which such associations are detectable for all psychiatric disorders.

Bivariate associations between GAD and CRP seem to be attenuated by a broad set of health-related variables including substance use, body-mass index, medication use. A similar set of variables attenuated the associations of depression-related variables on later CRP levels (albeit not to the point of nonsignificance for cumulative depressive episodes). Analyses testing bivariate associations ignore the normative clustering of these multiple health-risk behaviors and the elevated levels of CRP. Although the influence of any single risk factor may be important in terms of understanding the pathophysiology of mild inflammation, these risk factors are much difficult to tease apart in the real world. Risk reduction strategies may opt to use this normative clustering to identify subjects at highest risk for elevated inflammation patterns rather than focusing on individual risk factors.

Limitations

The Great Smoky Mountains study has several strengths besides its longitudinal, prospective design: A population-based design that minimized selection biases; anxiety variables assessed repeatedly with structured interviews that allowed us to look at various aspects of anxiety; repeated collection of bloodspots that allowed for subjects to provide up to 9 values of CRP across 12 years; and assessment of a wide range of domains that allowed us to control for covariates of anxiety and CRP. But the sample is not representative of the U.S. population (Native Americans overrepresented and African Americans and Latinos underrepresented).

The criteria for GAD may change in DSM-5. The primary proposed changes for DSM-5 GAD include removing criterion B (difficulty controlling worry), reducing the threshold for criterion C (associated symptoms), and adding a behavioral avoidance criterion (DSM-5 Anxiety Disorders Work Group, 2010). The first change is only meant to remove a redundancy and the reduced threshold for criterion C already applies in children (Andrews et al., 2010). The primary change then, as it relates to the current study, is the additional of the behavioral avoidance criterion. One goal of this addition is indeed to increase differentiation with other anxiety and mood disorders (Andrews et al., 2010). While this new criterion is only propositional, this change, if adopted, may affect the association of GAD with CRP.

Conclusion

Our study provides little support for a robust association between GAD and CRP. Instead, significant cross-sectional or longitudinal associations were entirely attenuated by a cluster of health-related covariates known to predict both CRP and anxiety. In the case of depression, there is evidence for an independent effect of recurrent depressive episodes on later CRP levels (Copeland et al., 2012), but this was not the case for GAD. This suggests caution in interpretation of the recent evidence of overlap between GAD and depression in developmental course and lifetime comorbidity. Despite general similarities, it has not been demonstrated that depression and GAD share biological correlates related either to HPA-axis function or CRP levels. These differences may prove instructive in understanding the specificity of associations between negative emotions and inflammation.

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References

- AMERICAN PSYCHIATRIC ASSOCIATION. Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV). American Psychiatric Press, Inc; Washington, DC: 1994.
- ANDREWS G, HOBBS MJ, BORKOVEC TD, BEESDO K, CRASKE MG, HEIMBERG RG, RAPEE RM, RUSCIO AM, STANLEY MA. Generalized worry disorder: a review of DSM-IV generalized anxiety disorder and options for DSM-V. *Depression and Anxiety*. 2010; 27:134–147. [PubMed: 20058241]
- ANGOLD A, COSTELLO E. The Child and Adolescent Psychiatric Assessment (CAPA). *Journal of the American Academy of Child and Adolescent Psychiatry*. 2000; 39:39–48. [PubMed: 10638066]

- ANGOLD A, COSTELLO EJ. A test-retest reliability study of child-reported psychiatric symptoms and diagnoses using the Child and Adolescent Psychiatric Assessment (CAPA-C). *Psychological Medicine*. 1995; 25:755–762. [PubMed: 7480452]
- ANGOLD A, COSTELLO EJ, ERKANLI A. Comorbidity. *Journal of Child Psychology and Psychiatry*. 1999a; 40:57–87. [PubMed: 10102726]
- ANGOLD, A.; COX, A.; PRENDERGAST, M.; RUTTER, M.; SIMONOFF, E.; COSTELLO, EJ.; ASCHER, BH. The Young Adult Psychiatric Assessment (YAPA). Duke University Medical Center; Durham, NC: 1999b.
- ASCHER BH, FARMER EMZ, BURNS BJ, ANGOLD A. The Child and Adolescent Services Assessment (CASA): Description and psychometrics. *Journal of Emotional and Behavioral Disorders*. 1996; 4:12–20.
- BARON RM, KENNY DA. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *Journal of Personality and Social Psychology*. 1986; 51:1173–1182. [PubMed: 3806354]
- COPELAND WE, SHANAHAN L, WORTHMAN C, ANGOLD A, COSTELLO EJ. Cumulative Depression Episodes Predict Later C-Reactive Protein Levels: A Prospective Analysis. *Biological Psychiatry*. 2012; 71:15–21. [PubMed: 22047718]
- COSTELLO EJ, ANGOLD A, BURNS B, STANGL D, TWEED D, ERKANLI A, WORTHMAN C. The Great Smoky Mountains Study of Youth: Goals, designs, methods, and the prevalence of DSM-III-R disorders. *Archives of General Psychiatry*. 1996; 53:1129–1136. [PubMed: 8956679]
- COSTELLO EJ, MUSTILLO S, ERKANLI A, KEELER G, ANGOLD A. Prevalence and development of psychiatric disorders in childhood and adolescence. *Archives of General Psychiatry*. 2003; 60:837–844. [PubMed: 12912767]
- DANESE A, MOFFITT TE, PARIANTE CM, AMBLER A, POULTON R, CASPI A. Elevated Inflammation Levels in Depressed Adults With a History of Childhood Maltreatment. *Arch Gen Psychiatry*. 2008; 65:409–415. [PubMed: 18391129]
- DANTZER R, O'CONNOR JC, FREUND GG, JOHNSON RW, KELLEY KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008; 9:46–56. [PubMed: 18073775]
- DSM-5 ANXIETY DISORDERS WORK GROUP. Proposed revisions for Generalized Anxiety Disorder. American Psychiatric Association; Washington, DC: 2010. Online
- ELEY T, STEVENSON J. Exploring the covariation between anxiety and depression symptoms: A genetic analysis of the effects of the age and sex. *Journal of Child Psychiatry*. 1999; 40:1273–1282.
- ELOVAINIO M, KELTIKANGAS-JÄRVINEN L, PULKKI-RÅBACK L, KIVIMÄKI M, PUTTONEN S, VIKARI L, RÄSÄNEN L, MANSIKKANIEMI K, VIKARI J, RAITAKARI OT. Depressive symptoms and C-reactive protein: The Cardiovascular Risk in Young Finns Study. *Psychological Medicine: A Journal of Research in Psychiatry and the Allied Sciences*. 2006; 36:797–805.
- GIMENO D, KIVIMÄKI M, BRUNNER EJ, ELOVAINIO M, DE VOGLI R, STEPTOE A, KUMARI M, LOWE GDO, RUMLEY A, MARMOT MG, FERRIE JE. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychological Medicine*. 2009; 39:413–423. [PubMed: 18533059]
- HOEHN-SARIC R, MCLEOD DR, LEE YB, ZIMMERLI WD. Cortisol levels in generalized anxiety disorder. *Psychiatry Research*. 1991; 38:313–315. [PubMed: 1754641]
- HOWREN MB, LAMKIN DM, SULS J. Associations of Depression With C-Reactive Protein, IL-1, and IL-6: A Meta-Analysis. *Psychosomatic Medicine*. 2009; 71:171–186. [PubMed: 19188531]
- JANICKI DEVERTS D, COHEN S, DILILLO VG, LEWIS CE, KIEFE C, WHOOLEY M, MATTHEWS KA. Depressive Symptoms, Race, and Circulating C-Reactive Protein: The Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Psychosomatic Medicine*. 2010; 72:734–741. [PubMed: 20668285]
- KAUFMAN J, MARTIN A, KING R, CHARNEY D. Are child-, adolescent-, and adult-onset depression one and the same disorder? *Biological Psychiatry*. 2001; 49:980–1001. [PubMed: 11430841]

- KENDLER KS. Major depression and generalised anxiety disorder. Same genes, (partly)different environments--revisited. *Br J Psychiatry Suppl.* 1996;68–75. [PubMed: 8864151]
- KENDLER KS, GARDNER CO, GATZ M, PEDERSEN NL. The sources of co-morbidity between major depression and generalized anxiety disorder in a Swedish national twin sample. *Psychol Med.* 2007; 37:453–62. [PubMed: 17121688]
- KENDLER KS, WALTERS EE, NEALE MC, KESSLER RC, HEATH AC, EAVES LJ. The structure of the genetic and environmental risk factors for six major psychiatric disorders in women. Phobia, generalized anxiety disorder, panic disorder, bulimia, major depression, and alcoholism. *Archives of General Psychiatry.* 1995; 52:374–383. [PubMed: 7726718]
- KESSLER RC, CHIU WT, DEMLER O, WALTERS EE. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the national comorbidity survey replication. *Archive of General Psychiatry.* 2005; 62:617–627.
- KUZMA JM, BLACK DW. Integrating pharmacotherapy and psychotherapy in the management of anxiety disorders. *Current Psychiatry Reports.* 2004; 6:268–273. [PubMed: 15260942]
- MACKINNON D, LOCKWOOD C, HOFFMAN J, WEST S, SHEETS V. A comparison of methods to test mediation and other intervening variable effects. *Psychological Methods.* 2002; 7:83–104. [PubMed: 11928892]
- MANTELLA RC, BUTTERS MA, AMICO JA, MAZUMDAR S, ROLLMAN BL, BEGLEY AE, REYNOLDS CF, LENZE EJ. Salivary cortisol is associated with diagnosis and severity of late-life generalized anxiety disorder. *Psychoneuroendocrinology.* 2008; 33:773–781. [PubMed: 18407426]
- MATTHEWS KA, SCHOTT LL, BROMBERGER JT, CYRANOWSKI JM, EVERSON-ROSE SA, SOWERS M. Are there bi-directional associations between depressive symptoms and C-reactive protein in mid-life women? *Brain, Behavior, and Immunity.* 2010; 24:96–101.
- MCDADE TW, BURHOP J, DOHNAL J. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clinical Chemistry.* 2004; 50:652–654. [PubMed: 14981035]
- MCDADE TW, HAWKLEY LC, CACIOPPO JT. Psychosocial and Behavioral Predictors of Inflammation in Middle-Aged and Older Adults: The Chicago Health, Aging, and Social Relations Study. *Psychosomatic Medicine.* 2006; 68:376–381. [PubMed: 16738067]
- MCDADE TW, LEONARD WR, BURHOP J, REYES-GARCÍA V, VADEZ V, HUANCA T, GODOY RA. Predictors of C-reactive protein in Tsimane' 2 to 15 year-olds in lowland Bolivia. *American Journal of Physical Anthropology.* 2005; 128:906–913. [PubMed: 16118783]
- MCDADE W, STALLINGS JF, WORTHMAN CM. Psychosocial stress and cell-mediated immune function: Validation of a blood spot method for Epstein-Barr virus antibodies. *American Journal of Physical Anthropology.* 1997; 24(Suppl):164–65.
- MEI JV, ALEXANDER JR, ADAM BW, HANNON WH. Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens. *The Journal of Nutrition.* 2001; 131:1631S–1636S. [PubMed: 11340130]
- MIDDELDORP C, BIRLEY A, CATH D, GILLESPIE N, WILLEMSSEN G, STATHAM D, DE GEUS E, ANDREWS J, VAN DYCK R, BEEM A, SULLIVAN P, MARTIN N, BOOMSMA D. Familial clustering of major depression and anxiety disorders in Australian and Dutch twins and siblings. *Twin Research & Human Genetics.* 2005; 8:609–15. [PubMed: 16354503]
- MOFFITT TE, CASPI A, HARRINGTON H, MILNE BJ, MELCHIOR M, GOLDBERG D, POULTON R. Generalized anxiety disorder and depression: childhood risk factors in a birth cohort followed to age 32. *Psychological Medicine.* 2007a; 37:441–452. [PubMed: 17201999]
- MOFFITT TE, HARRINGTON H, CASPI A, KIM-COHEN J, GOLDBERG D, GREGORY AM, POULTON R. Depression and Generalized Anxiety Disorder: Cumulative and Sequential Comorbidity in a Birth Cohort Followed Prospectively to Age 32 Years. *Arch Gen Psychiatry.* 2007b; 64:651–660. [PubMed: 17548747]
- O'DONOVAN A, HUGHES BM, SLAVICH GM, LYNCH L, CRONIN M-T, O'FARRELLY C, MALONE KM. Clinical anxiety, cortisol and interleukin-6: Evidence for specificity in emotion-biology relationships. *Brain, Behavior, and Immunity.* 2010; 24:1074–1077.
- OGDEN CL, KUCZMARSKI RJ, FLEGAL KM, MEI Z, GUO S, WEI R, GRUMMER-STRAWN LM, CURTIN LR, ROCHE AF, JOHNSON CL. Centers for Disease Control and Prevention 2000

- Growth Charts for the United States: Improvements to the 1977 National Center for Health Statistics Version. *Pediatrics*. 2002; 109:45–60. [PubMed: 11773541]
- PEARSON TA, MENSAH GA, ALEXANDER RW, ANDERSON JL, CANNON RO III, CRIQUI M, FADL YY, FORTMANN SP, HONG Y, MYERS GL, RIFAI N, SMITH SC JR, TAUBERT K, TRACY RP, VINICOR F. Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003; 107:499–511. [PubMed: 12551878]
- PITSAVOS C, PANAGIOTAKOS DB, PAPAGEORGIOU C, TSETSEKOU E, SOLDATOS C, STEFANADIS C. Anxiety in relation to inflammation and coagulation markers, among healthy adults: The ATTICA Study. *Atherosclerosis*. 2006; 185:320–326. [PubMed: 16005881]
- PRADHAN AD, MANSON JE, RIFAI M, BURING JE, RIDKER PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association*. 2002; 286:327–334. [PubMed: 11466099]
- RAISON CL, CAPURON L, MILLER AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol*. 2006; 27:24–31. [PubMed: 16316783]
- RIDKER PM, BURING JE, COOK NR, RIFAI N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: An 8-year follow-up of 14,719 initially healthy american women. *Circulation*. 2003; 107:391–397. [PubMed: 12551861]
- SCHWEIZER EE, SWENSON CM, WINOKUR A, RICKELS K, MAISLIN G. The dexamethasone suppression test in generalised anxiety disorder. *The British Journal of Psychiatry*. 1986; 149:320–2. [PubMed: 3779298]
- SHAH T, CASAS JP, COOPER JA, TZOULAKI I, SOFAT R, MCCORMACK V, SMEETH L, DEANFIELD JE, LOWE GD, RUMLEY A, FOWKES FGR, HUMPHRIES SE, HINGORANI AD. Critical appraisal of CRP measurement for the prediction of coronary heart disease events: new data and systematic review of 31 prospective cohorts. *International Journal of Epidemiology*. 2009; 38:217–231. [PubMed: 18930961]
- SOBEL ME. Asymptotic confidence intervals for indirect effects in structural equation models. *Sociological Methodology*. 1982:290–312.
- STEWART JC, RAND KL, MULDOON MF, KAMARCK TW. A prospective evaluation of the directionality of the depression-inflammation relationship. *Brain, Behavior, and Immunity*. 2009; 23:936–944.
- TAMAKOSHI K, YATSYA H, KONDO T, ET AL. The metabolic syndrome is associated with elevated circulating c-reactive protein in healthy reference range, a systemic low-grade inflammatory state. *International Journal of Obesity and Related Metabolic Disorders*. 2003; 27:443–449. [PubMed: 12664077]
- THAPAR A, MCGUFFIN P. Anxiety and depressive symptoms in childhood: A genetic study of comorbidity. *Journal of Child Psychology and Psychiatry*. 1997; 38:651–656. [PubMed: 9315975]
- THE EMERGING RISK FACTORS COLLABORATION. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *The Lancet*. 2010; 375:132–140.
- TILLER JWG, BIDDLE N, MAGUIRE KP, DAVIES BM. The dexamethasone suppression test and plasma dexamethasone in generalized anxiety disorder. *Biological Psychiatry*. 1988; 23:261–270. [PubMed: 3337861]
- TOKER S, SHIROM A, SHAPIRA I, BERLINER S, MELAMED S. The Association Between Burnout, Depression, Anxiety, and Inflammation Biomarkers: C-Reactive Protein and Fibrinogen in Men and Women. *Journal of Occupational Health Psychology*. 2005; 10:344–362. [PubMed: 16248685]
- WATSON D. Rethinking the Mood and Anxiety Disorders: A Quantitative Hierarchical Model for DSM-V. *Journal of Abnormal Psychology*. 2005; 114:522–536. [PubMed: 16351375]
- WILLIAMS SR, MCDADE TW. The Use of Dried Blood Spot Sampling in the National Social Life, Health, and Aging Project. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences*. 2009; 64B:i131–i136.

- WOODWARD M, RUMLEY A, LOWE GDO, TUNSTALL-PEDOE H. C-reactive protein: associations with haematological variables, cardiovascular risk factors and prevalent cardiovascular disease. *British Journal of Haematology*. 2003; 122:135–141. [PubMed: 12823355]
- WORTHMAN CM, STALLINGS JF. Hormone measures in finger-prick blood spot samples: New field methods for reproductive endocrinology. *American Journal of Physical Anthropology*. 1997; 103:1–21. [PubMed: 9185948]

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Table 1
Descriptive information and concurrent associations between primary variables and covariates

	Total sample (N=5810)	GAD sx. ^a		GAD dx. ^c		Cumulative episodes ^b		CRP ^a	
		Odds or means ratio		Odds or means ratio		Odds or means ratio		Odds or means ratio	
Primary									
Mean GAD sx.	0.38 (0.76)	--	--	--	--	--	--	--	--
GAD dx.	2.2% (146)	7.73 (5.48-10.91)	--	--	--	--	--	--	--
Mean cumulative episodes	0.08 (0.31)	2.45 (2.03-2.97)	51.36 (18.19-145.0)	--	--	--	--	--	--
Mean CRP levels (mg/L)	1.04 (2.02)	1.50 (1.11-2.02)	2.53 (1.00-6.39)	3.18 (1.87-5.41)	--	--	--	--	--
Covariates									
Sex (% female)	48.7% (2766)	1.26 (1.00-1.59)	2.02 (1.04-3.92)	1.63 (0.88-3.01)	1.02 (0.99-1.05)				
Mean age	14.21 (3.19)	1.03 (1.01-1.06)	1.12 (1.03-1.22)	1.18 (1.13-1.25)	1.02 (1.01-1.02)				
Race (% White)	89.7% (3905)	0.86 (0.71-1.05)	0.69 (0.39-1.21)	0.75 (0.45-1.28)	1.14 (1.12-1.17)				
Mean BMI	22.37 (5.62)	1.01 (1.00-1.03)	1.03 (1.00-1.06)	1.08 (1.05-1.11)	1.02 (1.02-1.02)				
Alcohol use	12.8% (850)	1.40 (1.13-1.75)	2.44 (1.16-5.12)	2.24 (1.57-3.19)	1.08 (1.05-1.11)				
Tobacco use	13.5% (1143)	1.41 (1.11-1.78)	2.86 (1.43-5.74)	2.41 (1.61-3.60)	1.11 (1.08-1.15)				
Other drug use	8.1% (571)	1.50 (1.11-2.03)	3.17 (1.32-7.60)	1.97 (1.27-3.05)	1.07 (1.03-1.11)				
Medication use	30.2% (1842)	1.75 (1.46-2.09)	2.00 (1.13-3.54)	1.48 (0.93-2.37)	1.06 (1.04-1.09)				
Recent health ailments	34.7% (2013)	1.61 (1.37-1.91)	1.97 (1.16-3.34)	1.55 (1.23-1.95)	1.05 (1.03-1.08)				
Low SES	20.1% (1607)	1.18 (0.98-1.42)	1.22 (0.65-2.28)	1.44 (0.96-2.16)	1.05 (1.02-1.07)				

Means and standard deviations are reported for continuous variables and percentages and sample sizes are included for dichotomous variables. Reported CRP mean and standard deviation values are from untransformed variables. All associations between CRP and other variables use log [0-transformed values. Associations of primary variable with other variables were tested with either linear^a, Poisson^b, or logistic^c regression. CRP=C-Reactive Protein; BMI = Body-mass index; SES= socioeconomic status.

Table 2

Cross-sectional associations between CRP and generalized anxiety

Predictor	Outcome	Effect of predictor				Significant Covariates
		Bivariate		Adjusted for covariates		
		B (SE)	p	B (SE)	p	
CRP	GAD sx. ^b	0.38 (0.16)	0.01	0.17 (0.18)	0.33	2, 9, 10
CRP	GAD dx. ^c	0.90 (0.48)	0.06	0.50 (0.59)	0.40	
CRP	Cumulative episodes ^b	0.97 (0.31)	0.002	0.23 (0.21)	0.28	1, 2, 3
GAD sx.	CRP ^a	0.02 (0.007)	0.02	0.007 (0.007)	0.33	2, 5, 9
GAD dx.	CRP ^a	0.06 (0.04)	0.14	0.03 (0.04)	0.43	2, 5, 6, 9
Cumulative episodes	CRP ^a	0.07 (0.03)	0.02	0.004 (0.02)	0.85	2, 5, 6, 9

Models were tested with either linear^a, Poisson^b, or logistic^c regression. Covariates include the following: 1= sex; 2= race; 3=age; 5=BMI; 6=current nicotine use; 7 = current alcohol use; 8= current illicit drug use; 9 = current medication use; 10=recent health ailments; 11= current low SES. CRP=C-Reactive Protein; BMI = Body-mass index; SES= socioeconomic status.

Table 3

Bidirectional longitudinal associations between CRP and generalized anxiety

Predictor status at preceding assessment	Outcome	Effect of prior predictor				Significant Covariates
		Simple		Adjusted for covariates		
		B (SE)	p	B (SE)	p	
CRP	GAD sx. ^b	0.17 (0.25)	0.49	0.17 (0.24)	0.48	4, 9, 10
CRP	GAD dx. ^c	0.35 (0.61)	0.56	0.06 (0.61)	0.92	4, 10
CRP	Cumulative episodes ^b	0.64 (0.34)	0.06	0.12 (0.25)	0.64	2, 3
GAD sx.	CRP ^a	0.03 (0.009)	0.007	0.02 (0.01)	0.09	2, 4, 5, 6, 9
GAD dx.	CRP ^a	0.06 (0.04)	0.12	0.03 (0.03)	0.33	2, 4, 5, 6, 9
Cumulative episodes	CRP ^a	0.05 (0.03)	0.09	0.02 (0.03)	0.37	2, 4, 5, 6, 9

Models were tested with either linear^a, Poisson^b, or logistic^c regression. Simple models include prior status on the outcome variable. Covariates include the following: 1 = sex; 2 = race; 3 = age; 4 = time since last interview; 5 = BMI; 6 = current nicotine use; 7 = current alcohol use; 8 = current illicit drug use; 9 = current medication use; 10 = recent health ailments; 11 = current low SES. CRP = C-Reactive Protein; BMI = Body-mass index; SES = socioeconomic status.

Protein; BMI = Body-mass index; SES = socioeconomic status. Sx = symptoms; Dx = diagnoses.

Table 4

Tests of potential mediators of the association between GAD symptoms and later CRP

	1. GAD symptoms to mediator	2. Mediator to CRP	3. GAD symptoms to CRP	4. Sobel test
	B (SE)	B (SE)	B (SE)	<i>p</i>
BMI	0.54 (0.18), <i>p</i> <0.01	0.01 (0.001), <i>p</i> <0.001	0.02 (0.009), <i>p</i> =0.07	<0.01
Nicotine Use	0.26 (0.07), <i>p</i> <0.001	0.04 (0.02), <i>p</i> <0.05	0.02 (0.009), <i>p</i> <0.05	0.08
Medication Use	0.25 (0.06), <i>p</i> <0.001	0.04 (0.01), <i>p</i> <0.001	0.02 (0.009), <i>p</i> <0.05	<0.01

Models were tested with either Poisson^a or logistic^b regression. Columns numbered 2 and 3 provide results from models in which both GAD symptoms variable and potential mediator predicted current CRP status. Models adjusted for demographic covariates and prior CRP status. Sobel test assess significance of indirect pathway. CRP=C-Reactive Protein; BMI = Body-mass index. Sx = symptoms.