CAUSES OF COMORBIDITY AMONG INTERNALIZING DISORDERS OF CHILDHOOD AND ADOLESCENCE: THE ROLES OF NEUROTICISM, GENES AND ENVIRONMENT

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CAUSES OF COMORBIDITY AMONG INTERNALIZING DISORDERS
OF CHILDHOOD AND ADOLESCENCE:
THE ROLES OF NEUROTICISM, GENES AND ENVIRONMENT

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ABSTRACT

Sources of variance and covariance among mother-reported separation anxiety disorder (SAD) and self-reported depression (DEP) and generalized anxiety disorder (GAD) were studied in a population-based sample of 4,320 female twins. The relationship of self-reported neuroticism to each of the three internalizing disorders was also examined. Correlations among the four phenotypes were computed and logistic regression was used to predict SAD, DEP and GAD from neuroticism. Structural equation modeling (SEM) was conducted to parse the causes of variation in neuroticism, SAD, DEP and GAD into additive genetic (A), shared environmental (C) and nonshared environmental (E) effects. SEM was also conducted to parse sources of covariation among the four phenotypes into A, C, and E effects.

All four phenotypes were positively and significantly associated with one other. Genetic and nonshared environmental factors were important in the etiology of all four phenotypes, whereas shared environmental influences were only significant for SAD. Covariation among the phenotypes was due primarily to additive genetic and nonshared environmental effects. These results suggest a common underlying genetic liability may explain comorbidity among internalizing disorders and their association with neuroticism.

INTRODUCTION

Internalizing disorders, the central feature of which is disorder of emotion or mood, incorporate various anxiety disorders and mood disorders, including depression (Kovacs & Devlin, 1998). Anxiety and depressive disorders are common and debilitating problems in the United States today. Large epidemiological studies have revealed that the prevalence rates of anxiety disorders such as generalized anxiety disorder as well as depression are quite high. Results from the National Comorbidity Survey (NCS) revealed lifetime prevalence rates of 15.8% for major depressive episodes and 5.1% for generalized anxiety disorder (Kessler, McGonagle, Zhao, Nelson, Hughes, Eshleman et al., 1994). Not only do these conditions affect millions of Americans, but they are also debilitating to the affected individuals' families, as well as to society as a whole. According to the National Institute of Mental Health, "major depressive disorder is the leading cause of disability in the U.S." (National Institute of Mental Health, 2002). The result is that individuals who suffer from these conditions experience decreased productivity as well as a reduction in the quality of their lives, making investigations into the etiology of anxiety and depression of critical importance.

One stable finding in the literature has been the greater prevalence of anxiety and depression in women than in men. Women are significantly more likely to develop a depressive or anxiety disorder and to have comorbid conditions than men (Kessler et al., 1994). Despite increased attention paid to their etiology and treatment in the scientific community, the prevalence of these disorders has not decreased and the proportion of disease burden they account for is expected to rise (National Institute of Mental Health,

2002). Therefore, it is imperative that investigation continues into the etiological pathways responsible for these disorders.

Anxiety and depression are not problems specific to adults. Epidemiological studies have shown that depression and anxiety are all too common in child and adolescent populations. For example, in the Great Smoky Mountains Study, a large population-based study of children 9-13 years old, 3-month prevalence estimates of separation anxiety disorder and generalized anxiety disorder and major depression in the sample were 3.5%, 1.7%, and 0.03%, respectively (Costello, Angold, Burns, Stangl, Tweed, Erkanli et al., 1996). In the Dunedin Multidisciplinary Health and Development study, 12-month prevalence estimates of separation anxiety disorder, overanxious disorder (currently subsumed under generalized anxiety disorder in DSM-IV) and major depressive episodes among 15 year-olds were 2.0%, 5.9%, and 1.9%, respectively (McGee, Feehan, Williams, Partridge, Silva, & Kelly, 1990). These prevalence estimates demonstrate the importance of understanding the causes and correlates of anxiety and depression in children and adolescents. This paper will focus specifically on two forms of anxiety, separation anxiety disorder and generalized anxiety disorder, as well as depression. These forms of anxiety were chosen due to their prevalence and availability within a large population-based sample of twins. It is important to understand the etiology of each of these disorders, as well as the basis of their comorbidity with one another.

Separation Anxiety Disorder

Separation anxiety disorder (SAD), a condition specific to children and adolescents, is a significant problem, with prevalence estimates often ranging from 2% to

3% (e.g. Bowen, Offord, & Boyle, 1990; Verhulst, van der Ende, Ferdinand, & Kasius, 1997) in community samples with some estimates reaching as high as 13% to 15% (Cohen, Cohen, Kasen, Velez, Hartmark, Johnson et al., 1993; Simonoff, Pickles, Meyer, Silberg, Maes, Loeber et al., 1997). This disorder is characterized by excessive and developmentally inappropriate worry about separation or threat of separation from an attachment figure. Children and adolescents with this disorder often refuse to be separated from their caregivers or their homes, including refusing to go to school or to sleep alone, often reporting nightmares and complaining of physical symptoms in anticipation of separation (e.g. stomachaches). The nature of this disorder makes it debilitating not only for the affected children, but for parents and families as well. Despite the prevalence of and disruption in functioning caused by this disorder, the etiological mechanisms that lead to the development of SAD and its relationship to other anxiety disorders and depression are not well understood.

Six studies using samples ranging in age from 3 to 66 years have examined the genetic and environmental influences in the etiology of SAD (Cronk, Slutske, Madden, Bucholz, Reich, & Heath, 2002; Cronk, Slutske, Madden, Bucholz, & Heath, 2004; Eaves, Silberg, Meyer, Maes, Simonoff, Pickles et al., 1997; Feigon, Waldman, Levy, & Hay, 2001, Silove, Manicavasagar, O'Connell, & Morris-Yates, 1995; Topolski, Hewitt, Eaves, Silberg, Meyer, Rutter et al., 1997). These studies have demonstrated support for a significant role for genetic influences, but findings are mixed regarding the role of family environmental factors. The behavior genetic models in these studies propose three latent factors to account for variance in liability to disorder: an additive genetic factor, a shared (family) environmental factor and an individual-specific (nonshared)

environmental factor. These models are fit to determine the proportion of variance in the trait of interest attributable to each of the three latent sources. The proportion of variance attributable to genetic factors is referred to as the heritability of a given trait. In studies using parental reports of SAD symptoms in twins, estimates of heritability range from .28 to .74, reaching significance in all cases (Cronk et al., 2004; Cronk et al., 2002; Eaves et al., 1997; Feigon et al., 2001). In two of these four studies, based on large samples of female twins and their parents, estimates of family environmental influences were significant and ranged from .20 to .52 (Cronk et al., 2004; Feigon et al., 2001). The two studies that did not find a significant role for family environmental influences were based on symptom counts (Cronk et al., 2002; Eaves et al., 1997). These results indicate that whereas genetic influences are consistently found to be important in parent-reported SAD, the significance of family environmental influences has been more difficult to establish.

Two twin studies based on self-reports of symptoms have yielded significant heritability estimates of .31 to .41 and estimates of family environmental influences of .00 to .02, suggesting that genetic and individual-specific environmental influences adequately explain variation in self-reported SAD (Eaves et al., 1997; Silove et al., 1995). In a third twin study based on self-reports, Topolski et al. (1997) reported a non-significant role for genetic influences (a^2=.04) and found that family environmental (c^2=.40) and individual-specific environmental influences (e^2=.56) best accounted for variation in SAD. One likely reason for the difference in findings may be the definition of SAD employed in these studies. Eaves et al. (1997) and Silove et al. (1995) employed symptom counts, whereas Topolski et al. (1997) employed a trichotomous outcome (i.e.

no symptoms, some symptoms but not enough for diagnosis, sufficient symptoms for DSM-III-R diagnosis). Taken together, these studies provide evidence for a moderate role of genetic influences in the development of SAD and, less consistently, suggest the potential importance of the shared family environment.

Generalized Anxiety Disorder

Generalized anxiety disorder (GAD), as the name implies, is a condition characterized by excessive anxiety that is generalized across different activities or events (American Psychiatric Association, 1994). The pervasive nature of the worry in GAD distinguishes it from other anxiety disorders, such as phobias, which are characterized by anxiety or worry about specific activities, objects or situations. Individuals who suffer from GAD have difficulty controlling their worry, which is associated with feelings of restlessness, muscle tension, fatigue, difficulty concentrating, irritability and difficulties sleeping. GAD, as in its current form, emerged in the revised third edition of the DSM (DSM-III-R) (American Psychiatric Association, 1987). Several genetically informed investigations into the etiology of GAD have been conducted since its formulation.

DSM-IV diagnostic criteria require that symptoms of GAD persist for at least 6 months. This duration requirement is consistent with the DSM-III-R duration criterion and represents a change from the DSM-III which required only one month duration of symptoms to meet criteria for GAD. However, several large epidemiological studies have demonstrated no significant differences in comorbidity or outcome for cases of GAD lasting 6 months versus those lasting less than 6 months (Bienvenu, Nestadt, & Eaton, 1998; Carter, Wittchen, Pfister, & Kessler, 2001; Kessler, Brandenburg, et al., 2005) Kendler, and colleagues (1992a) investigated the genetic and environmental

sources of liability to GAD, using both one-month and six-month minimum duration of symptoms requirements. This study was conducted with data from the Virginia Twin Registry (VTR), a large population-based sample of female twin pairs age 17 to 55. The authors found that for both definitions of GAD (i.e. one-month and six-month duration of symptoms), an AE model, including only genetic (A) and individual-specific (E) environmental effects, provided the most parsimonious fit to the data. Heritability for GAD in this study was estimated at approximately 30%. Similarly, Skre, Onstad, Torgersen, Lygren and Kringlen (1993) found evidence for genetic influence in the development of GAD in a sample of 81 twin pairs. In this study, co-twins of GAD probands were more likely to meet criteria for GAD (33%) than cotwins of comparison twins (10%). Additionally, MZ cotwins of GAD probands were four times more likely to meet criteria for GAD than DZ cotwins of GAD probands, suggesting a substantial role for genetic factors. Finally, Hettema, Neale and Kendler (2001) conducted a meta-analysis of three twin studies of GAD and found that nearly 32% of variance in liability to GAD was accounted for by genetic factors, with a small proportion of variance attributable to shared family environmental factors, and the remaining variance attributable to individual-specific environmental factors for women. The results of these twin studies of GAD in adult samples to date have been consistent in finding approximately 30% of variance in liability for this disorder is due to genetic factors and a small proportion of variance due to shared family environmental factors.

Prior to the fourth edition of the DSM, children and adolescents who experienced excessive generalized anxiety that was not limited to specific situations or triggers, were diagnosed with overanxious disorder (OAD), rather than GAD. Many of the diagnostic

criteria for OAD overlapped with criteria for GAD, therefore OAD was subsumed under the GAD diagnosis in the DSM-IV. Two twin studies of overanxious disorder have been conducted using data from the Virginia Twin Study of Adolescent Behavioral Development (VTSABD) (Eaves et al., 1997; Topolski et al., 1997). The VTSABD is a large population-based sample of 1,412 male and female twins age 8-16, which assessed symptoms of psychopathology occurring during the 3-month period prior to assessment. Both studies based on these data found that for child self-reports, genetic and individual-specific environmental influences accounted for risk for overanxious disorder and that shared family environmental influences were not significant. Topolski et al. (1997) reported a heritability estimate of .37 for a trichotomous definition of overanxious disorder and Eaves et al. (1997) reported an estimate of .46 for symptom counts. Eaves et al. (1997) also found heritability estimates of .66 and .59 for maternal and paternal ratings of overanxious disorder, respectively, and found that shared family environmental influences were not significant. Taken together, these studies are consistent with findings in the adult literature for GAD, which suggest that genetic and individual-specific environmental influences are important in the development of generalized anxiety, but that shared family environmental influences may not be significant. There has consistently been little agreement between parent- and child-reported internalizing disorders (e.g. Choudhury, Pimental, & Kendall, 2003; Foley et al., 2004; Foley et al., 2005). The findings reported above for GAD and OAD demonstrate higher additive genetic effects for parent-reported symptoms relative to child-reported symptoms. This finding was consistent across multiple forms of anxiety as well as depression in the VTSABD (Eaves et al., 1997).

Depression

As discussed above, major depressive disorder is a common and debilitating form of internalizing psychopathology. Results from the National Comorbidity Survey Replication indicate lifetime prevalence estimates of 6.7%, with 30% of these cases characterized as serious in severity (i.e. accompanied by a suicide attempt, disability, or impairment in functioning) (Kessler, Chiu, Demler, & Walters, 2005). Additionally, depression has consistently been demonstrated to be much more prevalent in women than men (Kessler, Berglund, Demler, Jin, & Walters, 2005). Gender differences in depression begin to emerge during early adolescence and remain constant throughout adolescence and into adulthood (Nolen-Hoeksema & Girgus, 1994). Depression is characterized by one or more major depressive episodes (DEP) that consist of at least five symptoms that occur together for a period of two-weeks or longer. These symptoms include depressed mood, reduced interest in activities, appetite disturbance, sleep disturbance, fatigue, psychomotor agitation or retardation, feelings of excessive guilt or worthlessness, difficulty concentrating and recurrent thoughts of death (American Psychiatric Association, 1994). In order to meet criteria for DEP, these symptoms cannot be attributable to bereavement unless they persist for more than two months, indicating major depression, beyond normal bereavement.

Depression has long been known to be familial, with family members of depressed individuals at greater risk for developing depression than individuals who are not related to depressed individuals (McGuffin & Katz, 1986). Behavior genetic studies have sought to parse the source of the familiality of depression into genetic and environmental sources. Twin studies of depression have been conducted with both

clinical and community samples in a number of different countries. One such study of depressed individuals in a clinical sample was carried out in England. McGuffin, Katz, Watkins, and Rutherford (1996) conducted a twin study of depression using data from the Maudsley Hospital Twin Register in London. This study of 181 probands with depressive disorder and their cotwins yielded significant heritability estimates of over 70%.

Numerous twin studies of depression based on community samples have also consistently demonstrated significant estimates of the heritability of depression. In an attempt to examine the effects on etiology of using different definitions of depression, Kendler, Neale, Kessler, Heath, and Eaves (1992c) used data from the VTR to investigate the genetic and environmental influences on nine different definitions of depression. Definitions of depression in this study included DSM-III and DSM-III-R criteria as well as Research Diagnostic Criteria (Spitzer, Endicott, & Robins, 1975), among others. They found that regardless of the definition of depression employed, variance in liability of risk for depression was best explained by genetic and individual-specific environmental influences, with heritability estimates ranging from .21 to .45. Similar results have been found in twin studies in other countries including Australia (Bierut, Heath, Bucholz, Dinwiddie, Madden, Statham et al., 1999) and Sweden (Kendler, Pedersen, Neale, & Mathé, 1995). Sullivan, Neale and Kendler (2000) conducted a meta-analysis of six twin studies of depression and found that the variance in liability to depression was attributable to genetic effects (a^2=.37; 95% confidence interval .31 - .42) and individual-specific environmental effects (e^2=.63; 95% confidence interval .58 - .67). Shared environmental effects were not significant (c^2=.00; 95% confidence interval .00 - .05).

Behavior genetic studies of depression in adult women (i.e. age 18 and older) have been consistent in finding significant genetic effects and nonsignificant shared family environmental effects. Eaves et al. (1997) analyzed interview and questionnaire data from parent- and twin self-reports of depression in the VTSABD. They found that for girls age 8 to 16, parent interview and questionnaire as well as twin interview data, genetic influences (a^2=.19-.66) and individual-specific environmental influences (e^2=.34-.81) were significant and shared family environmental influences were not. The only exception was for twin questionnaire data where genetic (a^2=.15), shared family environmental (c^2=.59) and individual-specific environmental influences (e^2=.26) were all significant. Also using data from the VTSABD, Silberg and colleagues (1999), investigated the effects of pubertal status on the etiology of depression. They found that genetic effects were important for pubertal females (a^2=.28), but not for prepubertal females. This finding is consistent with previous findings indicating an increased role for genetic effects on depression with increasing age. For example, Thapar and MGuffin (1994) found that in their sample of 411 twin pairs, the most parsimonious model explaining variance in depression for children (age 8-11) included shared family environmental factors (c^2=.77) but not genetic factors. Alternatively, for adolescents in this sample (age 11-16), the most parsimonious model included genetic factors (a^2=.82) but not shared family environmental factors.

The genetically informed studies of DEP and GAD are consistent in attributing the variance in liability for these disorders to genetic influences and aspects of the environment not shared between cotwins, with no significant role for shared family environmental effects. This finding appears to be consistent for adult and adolescent

populations. There has also been consistent support for the significant role of genetic effects in the etiology of SAD. However, unlike the findings for DEP and GAD, some, but not all twin studies of SAD, have demonstrated a significant role of shared family environmental influences in risk for this disorder.

Comorbidity of Anxiety and Depression

To further complicate the clinical picture, anxiety disorders are frequently comorbid with other anxiety disorders as well as with depression. Data from the National Comorbidity Survey suggest that among individuals who meet criteria for lifetime depression, over 26% have comorbid social phobia (Kessler, Stang, Wittchen, Stein, & Walters, 1999). Additionally, in the National Comorbidity Survey Replication, significant correlations existed among 12-month diagnoses of depression, panic disorder, social phobia, generalized anxiety disorder, separation anxiety disorder and specific and social phobias (Kessler, Chiu, et al., 2005). The frequency of comorbid anxiety and depressive disorders has been well documented and has led some to suggest that pure anxiety and pure depressive conditions are the exception while comorbid cases may be the rule (Wadsworth, Hudziak, Heath, & Achenbach, 2001). The phenomenon of comorbidity among anxiety and depressive disorders has been investigated from a behavioral genetic framework in several twin studies, which have attempted to parse the sources of covariation among these disorders into genetic and shared environmental factors.

The majority of information regarding the genetic and environmental sources of covariation among anxiety and depressive disorders has been conducted with multivariate genetic models based on data from adult female twins in the VTR. Like univariate

genetic models, multivariate models propose three latent factors: additive genetic, shared environmental and nonshared environmental factors. Whereas univariate models seek to explain variation in liability to one phenotype (i.e. diagnosis), multivariate models seek to explain covariation among two or more phenotypes. The results from multivariate genetic analyses conducted with the VTR suggest that anxiety disorders including phobias, panic disorder, and generalized anxiety disorder as well as major depression share common sources of genetic variation (Kendler, Heath, Martin & Eaves, 1987; Kendler, Neale, Kessler, Heath, & Eaves, 1992b, 1993b, 1995; Kendler, Prescott, Myers, & Neale, 2003; Kendler, Walters et al., 1995). These multivariate analyses reveal that the common sources of shared family environmental variation appear to be non-significant, indicating that aspects of the shared environment of twins are not shared in common among anxiety and depressive disorders. However, individual-specific environmental factors may be shared in common between depression and generalized anxiety disorder (Kendler et al., 1992b), depression and agoraphobia, and between social phobia and situational phobia (Kendler et al., 1993b).

Comorbidity of anxiety and depressive disorders in childhood and adolescence has also been well established (e.g. Axelson, D. A. & Birmaher, B., 2001; Brady & Kendall, 1992; Eley & Stevenson, 1999a; Last, Strauss, & Francis, 1987). Two twin studies to date have examined the genetic and environmental sources of covariation among these disorders. Thapar and McGuffin (1997) found that covariation between maternal reports of symptoms of anxiety and depression was mostly due to common genetic factors. Similarly, Eley and Stevenson (1999b) found that common genetic factors accounted for almost all of the covariation (80%) in self-reported anxiety and

depression scale scores.

The results of the adult studies of comorbidity and the two twin studies of child and adolescent anxiety and depression comorbidity are consistent in suggesting that common genetic factors may be contributing to the co-occurrence of these disorders. However, there has been no investigation into the mechanisms of comorbidity between SAD and depression and between SAD and other anxiety disorders. It remains to be seen how much genetic and environmental factors may contribute to the comorbidity of SAD with DEP or SAD with GAD in a sample of children.

Krueger (1999) proposed a model to explain the relationships among internalizing disorders (i.e. anxiety and depressive disorders), using data from the National Comorbidity Survey. This model proposes a latent internalizing factor that is indicated by two lower order factors labeled "Distress" and "Fear." Depression, dysthymia, and GAD load on the Distress factor, whereas agoraphobia, social phobia, specific phobia and panic disorder load on the Fear factor. The Fear and Distress factors are highly correlated with one another (r =.74), reflecting the high rates of comorbidity among the measured disorders. This model of comorbidity has been replicated in other studies (e.g. Vollebergh, Iedema, Bijl, de Graaf, Smit, & Ormel, 2001) including a meta-analysis of existing comorbidity studies (Krueger & Markon, 2006). Kendler and colleagues examined this model from within a behavior genetic framework with data from the Virginia Twin Registry (Kendler et al., 2003). The results of this study showed that major depression and generalized anxiety disorder loaded strongly on one genetic factor, whereas animal phobia and situational phobia loaded on a separate but correlated genetic factor. These two groupings were termed "Anxious-Misery" and "Fear" and correspond

to Krueger's Distress and Fear factors and demonstrated significant genetic influences.

Neuroticism and the Comorbidity of Anxiety and Depression

Behavior genetic studies suggest that there is strong evidence for common genetic sources of comorbidity among anxiety and depressive disorders, which makes the search for measured characteristics that might explain some of the latent genetic sources of comorbidity a vital next step. Potential candidates to explain this common genetic risk are variables that are both strongly influenced by genetic factors themselves and are also strongly associated with anxiety and depressive disorders. Given these criteria, personality traits, specifically neuroticism, are prime candidates for this area of investigation.

The behavioral genetic basis of personality has been investigated and genetic factors have reliably been found to account for a significant proportion of variability in personality traits (e.g. Loehlin, 1992). Specifically, neuroticism has been shown to be moderately to highly heritable in a number of large twin studies (e.g. Lake, Eaves, Maes, Heath, & Martin, 2000; Rose, Koskenvuo, Kaprio, Sarna, & Lainginvainio, 1988; Tambs, Sundet, Eaves, Solaas, & Berg, 1991). The use of personality traits to explain the genetic basis of comorbidity among psychological disorders has been conducted in the study of alcohol dependence, where the personality traits of positive and negative emotionality, and especially behavioral undercontrol, explained much of the common genetic overlap between alcohol dependence and conduct disorder (Slutske, Heath, Madden, Bucholz, Statham, & Martin, 2002).

The personality trait of neuroticism has been consistently and strongly linked to depression (e.g. Boyce, Parker, Barnett, Cooney, & Smith, 1991) and anxiety (e.g. Clark

et al., 1994). Neuroticism is characterized by a vulnerability to stress or negative emotional reaction under stress and has also been termed 'negative affectivity' or 'negative emotionality'. The strong and consistent relationship between neuroticism and the anxiety and depressive disorders has led some to suggest that neuroticism may be the link among these different disorders. Clark and Watson (1991) proposed a tripartite model of depression and anxiety in which they described three factors that underlie the different disorders. Clark, Watson and Mineka (1994) described physiological hyperarousal as specific to anxiety, low levels of positive emotionality specific to depression and high levels of negative affectivity (i.e. neuroticism) common to both anxiety and depression. This model focuses on neuroticism as a means of explaining the high rates of comorbidity among anxiety and depressive disorders and has been supported in population-based samples (e.g. Weinstock & Whisman, 2006).

Kendler, Neale, Kessler, Heath and Eaves (1993a) noted that the relationship between neuroticism and depression could be explained in two ways. First, high levels of neuroticism could predispose individuals to experience depressive episodes. Alternatively, the experience of depression could result in higher levels of neuroticism ("scar effect"). Using a genetically-informed longitudinal design, they found a strong association between neuroticism and depression and that 70% of the correlation between depression and neuroticism was due to shared genetic influences. In a follow-up study with an overlapping sample, it was found that this pattern was similar for both men and women (Fanous, Gardner, Prescott, Cancro and Kendler (2002). Kendler et al., (1993a) also found strong support for the role of neuroticism as a potential causal factor in the development of depression and modest support for a "scar effect" of depression on

neuroticism. Clinical studies have failed to find evidence of such a "scar effect" (Duggan, Sham, Lee, & Murray, 1987; Zeiss & Lewinsohn, 1988).

Whereas the literature is consistent in demonstrating a strong association between the personality trait neuroticism and the anxiety and depressive disorders, these findings are limited predominantly to adult populations. To date, there has been scant research examining the genetic epidemiology of the personality trait of neuroticism and anxiety and depressive disorders in child and adolescent populations.

In conclusion, a great deal of important work has already been done investigating the comorbidity of anxiety and depressive disorders and the associations of personality variables with anxiety and depression. However, these areas of investigation have remained largely separate from one another. There is a need to incorporate the knowledge of correlates of psychopathology with the investigation into the co-occurrence of anxiety and depression in order to achieve a more complete developmental model of their associations. This research integrates the work that has been done investigating the behavior genetic sources of comorbidity among anxiety and depression and the work investigating known risk factors for anxiety and depression. This research extends this work to a child and adolescent population to not only investigate the comorbidity of depression and anxiety disorders in childhood and adolescence, but to explain the mechanisms by which common genes and environment result in comorbid internalizing disorders.

Goals of the Present Study

The goals of this study are threefold. 1) To investigate the lifetime co-occurrence of SAD with DEP and GAD. It is hypothesized that the presence of SAD will be

positively associated with both DEP and GAD and that DEP and GAD will be positively associated with one another. 2) To examine the genetic, shared environmental and nonshared environmental sources of variation in SAD, DEP and GAD as well as the genetic, shared and nonshared environmental causes of covariation among SAD, DEP and GAD using univariate and multivariate genetic analyses, respectively. It is hypothesized that significant genetic influences will be found for all three disorders but that shared environmental influences will be more modest. It is also hypothesized that SAD, DEP and GAD will share common genetic influences, whereas common sources of shared environmental effects among the three disorders will be modest. 3) To examine the relationship of the personality trait of neuroticism (negative affectivity) to each of the three disorders. It is hypothesized that neuroticism will be strongly and positively associated with each of the disorders and that genetic contributions to neuroticism will be strong. It is also hypothesized that neuroticism will share common genetic influences with each of the three disorders.

METHOD

Participants

The participants for the present research project include female twins and their biological mothers from the Missouri Adolescent Female Twin Study (MOAFTS), which is a prospective twin study of the genetic and environmental risks for alcoholism (PI: Andrew Heath, D.Phil. at Washington University School of Medicine). In addition to assessment of alcohol-related variables, the MOAFTS includes a rich collection of information regarding child and adolescent psychopathology as well as characteristics of the home and family environments of twin pairs. Drawn using a cohort-sequential design, this population-based sample consists of female twins born in Missouri to Missouri residents between 1975 and 1987. These twin pairs were identified through birth records and located using computerized tracing methods. Once located, telephone contact was attempted in order to gain cooperation of these families to participate in the study. In 1995 and 1996, the first wave of telephone interviews was conducted with parents and/or guardians of the twin pairs as well as with each twin separately. Interviews were successfully completed with biological mothers and at least one twin for 76% and 66%, respectively, of the families that had been successfully located (Heath, Madden, Grant, McLaughlin, Todorov, & Bucholz, 1999). This sample reflects the racial composition of the region from which it was drawn. Table 1 summarizes the measures used and their sequence in time.

In addition to biological mothers of twins, other parental figures including adoptive mothers, biological and adoptive fathers, among others, were interviewed when

possible. For the analyses involving parental reports in this project, only the reports of biological mothers were used. The decision to use the reports of biological mothers was made for the sake of consistency in reporting. One-third of the twins in this sample experienced the loss of a father at some point prior to the age of 13, making fathers an unreliable source of information. By far the most common source of information regarding the early development and symptomatology of twins in this sample based on the parent interview was biological mothers (96% of cases).

Only twins with zygosity information were retained in the final sample, which consists of 4,320 twins from 2,370 families. This includes 1,950 twin pairs (90% of total) with at least some information from both twins and 420 pairs (10% of total) from which all information is missing from one twin. Zygosity determinations were based on the responses of mothers and twins to a set of established questions designed to assess zygosity in twin samples which have been shown to be at least 95% accurate in assigning zygosity compared to the use of genotyping (see Eaves, Eysenck, & Martin, 1989). These questions pertained to the similarity of eye, skin and hair coloring of the twins as well as their heights and weights. Further questions assessed how often the twins were mistaken for one another by parents, teachers and strangers and whether they look as alike one another as "two peas in a pod." In the sample used for the analyses reported here, zygosity was based on the report of biological mothers when present; otherwise on the reports of twins themselves. In the vast majority of cases (93.7%) zygosity was based on biological mothers' reports. In cases where information was available from both sources, there was good agreement between mothers and twins (kappa=.73).

Measures

Mother Report. Though the interviews included in the MOAFTS assessed a wide range of individual and family characteristics, only the items relevant to the current research project will be discussed at length here. In the initial wave of telephone interviews, mothers were asked to report about lifetime symptoms of separation anxiety disorder (SAD) in each of their twins. SAD symptoms were assessed by nine items adapted from the Diagnostic Interview for Children and Adolescents - Parent version for use in telephone interviews (DICA-P) (Reich, 2000). Mothers were asked to report separately for each twin about the lifetime occurrence of symptoms, clustering of symptoms within a month, as well as impairment and treatment-seeking when the twins were six years of age and older. Based on these reports, 12% of twins exhibited at least three SAD symptoms (DSM-IV Criterion A), 6% exhibited clustering of these symptoms at the same time for at least one month (DSM-IV Criterion B), 7% demonstrated impairment in functioning or treatment-seeking for symptoms (DSM-IV Criterion D) and 4% met full DSM-IV criteria for SAD diagnosis (see Table 2).

Twin Self-Report. In the initial wave of telephone interviews, twins were asked to report retrospectively about their early environments as well as their lifetime experience of symptoms of several psychological disorders including depression and generalized anxiety disorder. The child version of the DICA, modified for use in telephone interviews, was used to assess lifetime experience of depression and generalized anxiety disorder symptoms, as well as the clustering of symptoms, impairment and treatment-seeking, in accordance with DSM-IV diagnostic criteria. The DICA has good test-retest reliability for depression and anxiety among adolescents age 13-18 (kappa = .71-.80) and among children age 6-12 (kappa = .55-.65) (Reich, 2000). Based on these interviews,

approximately 15% of twins reported experiencing at least 5 symptoms of DEP (DSM-IV Criterion A), 12% reported that these symptoms lasted for a period of at least two weeks (DSM-IV Criterion A) and 10% experienced impairment in functioning and/or treatment-seeking for their symptoms (DSM-IV Criterion C). Nearly 7% met full DSM-IV criteria for depression (see Table 2). Similarly, 14% of twins reported experiencing at least 3 symptoms of excessive worry (DSM-IV Criterion A) combined with at least three physical symptoms such as restlessness and muscle tension (DSM-IV Criterion C) of GAD. Only 4% reported that these symptoms lasted for at least 6 months (DSM-IV Criterion A) and less than 2% reported impairment in functioning and/or treatment-seeking (DSM-IV Criterion E). Less than 2% of twins met full DSM-IV criteria for GAD (see Table 2).

One year after the initial interview, twins were each mailed a self-report questionnaire, which included assessments of various aspects of their personality (see Table 1). Twelve items assessing neuroticism were adapted from the Junior Eysenck Personality Questionnaire (JEPQ) (Eysenck & Eysenck, 1975). The JEPQ is a well-established and reliable instrument, with an average reliability estimate of .80 (Caruso & Edwards, 2001). An example of an item assessing neuroticism is "Do you ever feel 'just miserable' for no good reason?" and respondents were instructed to circle "Yes" or "No" for each item. The maximum score an individual in this sample could have for neuroticism would be 12, indicating endorsement of all twelve neuroticism items. Neuroticism scores in this sample ranged from 0 to 12 with a mean of 5.4 (s.d.=3.3). Coefficient alpha for the neuroticism scale in this sample was .81. This variable was later collapsed for model-fitting purposes into a seven level variable ranging from 0 to 6.

(Individuals with scores of zero remained zero, scores of 1 and 2 became 1, 3 and 4 became 2, 5 and 6 became 3, 7 and 8 became 4, 9 and 10 became 5 and 11 and 12 became 6.) This transformation resulted in no significant differences in phenotypic correlations, twin correlations, or univariate model-fitting results. Therefore, only results based on the collapsed variable will be discussed. The median and mode values for the collapsed variable in the sample as a whole and stratified by age group are presented in Figure 1.

Internalizing Disorder Variables. SAD, DEP and GAD were operationalized for these analyses as dichotomous variables, based on symptom cut-offs. Dichotomous variables (versus symptom counts) were used in this present research for purposes of model-fitting. For SAD, the cutoff for the dichotomous variable was the presence of 3 or more symptoms of SAD out of nine, corresponding to DSM-IV Criterion A. For GAD, the presence of three domains of reported worry, corresponding to DSM-IV Criterion A, and three physical symptoms of GAD, corresponding to DSM-IV Criterion C was required. For DEP, the cutoff for the dichotomous variable was the presence of 5 or more symptoms of DEP, out of nine, corresponding to DSM-IV Criterion A. These definitions differ from DSM-IV diagnostic criteria in that they do not require significant interference with functioning, and do not exclude the presence of other forms of psychopathology. The effects of using more or less stringent diagnostic criteria on estimates of genetic and environmental influences on SAD have been investigated previously in this sample (Cronk et al., 2004). Specifically, it was found that although the prevalence of SAD as defined by the presence of three or more symptoms (12.6%) was higher than the prevalence of full DSM-IV SAD diagnosis (4.7%), the sources of variance in these diagnostic definitions did not differ. Estimates of additive genetic, shared and nonshared

environmental effects for the presence of three or more symptoms (a^2=.55; c^2=.37; e^2=.08) were not substantially different from the effects for full diagnosis (a^2=.61; c^2=.23; e^2=.16).

Data Analyses

Aim 1 – Comorbidity. In order to assess the comorbidity of internalizing disorders, correlations among mother-reported SAD and self-reported DEP and GAD were computed. Tetrachoric correlations were computed for the dichotomous phenotypes and the proportion of individuals meeting criteria for one disorder who also met criteria for either of the other disorders (e.g. proportion of individuals meeting criteria for SAD who also met criteria for DEP) were computed using SAS.

Aim 2 - Causes of Comorbidity. Before the environmental and genetic sources of comorbidity among the internalizing disorders were investigated, the relative contributions of genetic, shared and nonshared environmental factors were established for each disorder, in turn. First, MZ and DZ twin correlations were computed in SAS and univariate genetic analyses were conducted for SAD, DEP and GAD using the Mx program (Mx: Statistical Modeling, 4th ed., 1999, available from M.C. Neale, Box 126 MCV, Richmond, VA 23298). An example of the univariate model is depicted in Figure 2 for the case of SAD. This model proposes three latent factors (additive genetic, shared environmental and nonshared environmental) that contribute to twin similarity for a given phenotype (e.g. SAD). The effect of the additive genetic factor (A) is also known as the heritability of the phenotype. The shared environmental factor (C) represents elements of the environment that contribute to twin similarity, also known as the environment common to both twins. The nonshared environmental factor (E) represents elements of

the environment that are unique to each twin and also contains measurement error. The analyses conducted with dichotomous outcome measures (SAD, DEP, and GAD) assume a normal underlying distribution of liability to disorder. It should also be noted that the estimation of genetic and environmental sources of variance in liability to disorder using the twin study method is based on a number of assumptions including the assumption of equal environments. This assumption states that monozygotic (MZ) twins do not experience more similar etiologically relevant environments than do dizygotic (DZ) twins. This assumption has been tested and its validity supported in this sample (Cronk et al., 2002). The fit of these models is estimated using the maximum likelihood method, which yields the "-2 times the log likelihood" (-2lnL) fit statistic.

Once the full model was fitted, parameters were dropped in an attempt to discover the model that provided the most parsimonious fit to the data. The difference between two -2lnL statistics is distributed as a chi-square statistic and nested models were evaluated by means of the chi-square difference test. A reduced model that did not result in a significant worsening of fit was more parsimonious than, and thus preferred to, the full model.

In order to evaluate the nature of the relationships between SAD, GAD and DEP, multivariate genetic analyses were conducted using the Mx program. A Cholesky model was fitted in an attempt to examine the nature of the comorbidity among the three disorders within a behavioral genetic framework (see Fig. 3). This model proposes three latent additive genetic factors (A_1 to A_3), three latent shared environmental factors (C_1 to C_3) and three latent nonshared environmental factors (E_1 to E_3) to explain covariation among phenotypes. In Figure 3, the paths from A_1 represent additive genetic effects

common to all three disorders. Paths from A_2 represent additive genetic effects common to DEP and GAD, not shared with SAD, and so on.

Aim 3 - Examining the Role of Neuroticism. In order to assess the relationship between neuroticism and each of the three disorders, polychoric correlations were computed using SAS. Logistic regression analyses were also conducted regressing each of the three disorders on neuroticism. Multivariate models were fitted examining the genetic, shared, and nonshared environmental sources of covariation among neuroticism and the three disorders. First, a Cholesky model was fitted in an attempt to examine the nature of the role of neuroticism in the comorbidity among the three disorders within a behavioral genetic framework (see Fig. 4). This is an extension of the Cholesky model presented in Figure 3 and allows for the examination of the genetic and environmental effects on each of the three disorders "left over" after the effects in common with neuroticism are accounted for. Second, an independent pathway model was fitted to the data (see Fig. 5). The independent pathway model is a subset of the full Cholesky model and proposes that covariation among the measured phenotypes (neuroticism, SAD, DEP, and GAD) is accounted for by one common additive genetic factor, one common shared environmental factor, and one common nonshared environmental factor. Any unique variance in liability to each phenotype (i.e. not shared with the other phenotypes) is accounted for by specific A, C, and E factors represented in the bottom segment of Figure 5.

Third, a common pathway model was fitted to the data (see Fig. 6). The common pathway model proposes that covariation among the measured phenotypes is accounted for by the effect of a single latent phenotype, which is effected by latent additive genetic,

shared and nonshared environmental factors. The appropriateness of the independent pathway and common pathway models may be ascertained by comparing the resulting Akaike's Information Criterion (AIC) with that of the full Cholesky model, with lower values indicating the more appropriate model.

Role of Age in Analyses. The assessments in this study span a broad and critical range of ages. With increased age, the length of recall period for SAD increases. Additionally, twins report on their DEP and GAD symptoms at varying stages of the risk period for onset of DEP and GAD. Finally, neuroticism scores are known to vary throughout adolescence into early adulthood (Roberts & DelVecchio, 2000). Therefore, it was important to investigate the role of age on each of the phenotypes of interest in this sample as well as their relationships with one another. Ages in this sample ranged from 12 to 23 (mean = 15.5; s.d.=2.42) and were collapsed into four age groups for model-fitting purposes. There were only 43 individuals between the ages of 21 and 23, therefore this age group was dropped from many analyses, due to insufficient power. The four age groups, 12-14, 15-17, 18-20, 21-23, consist of 43%, 33%, 23% and 1% of the sample. Logistic regressions predicting each of the four phenotypes from age were conducted. Prevalence rates, twin correlations and twin concordances were computed separately for each age group. Univariate models were fit allowing thresholds and parameter estimates to vary by age in order to determine whether genetic and environmental effects differed significantly across age groups.

RESULTS

Role of Age

Two-thirds (67%) of twins meeting criteria for DEP and 95% of twins meeting criteria for GAD in Age Group 3 (18-20) had onset of symptoms prior to age 18. In Age Group 4, 8 of the 9 cases (89%) of GAD and 7 of the 13 cases (54%) of DEP reported onset of symptoms prior to age 18. Overall, the vast majority of twins meeting criteria for GAD (98%) and DEP (80%) reported onset of symptoms in childhood or adolescence.

Logistic regressions revealed no significant relationship between age and SAD or between age and neuroticism. Significant linear effects of age on GAD and DEP were found and a quadratic effect on DEP was also significant (See Table 3). For example, the odds of meeting criteria for DEP increase 1.26 times given a one year increase in age. As age increases, the odds of meeting criteria for DEP and GAD increase significantly. Differences in MZ and DZ twin correlations for SAD, DEP GAD and neuroticism across age groups were tested using Fisher's r to z transformation. Twenty-four pairs of correlations (three age groups X four phenotypes X two zygosities) were compared and of these only five were significantly different. Specifically, the MZ twin correlation for SAD among 12-14 year olds was significantly lower than the MZ twin correlations among 15-17 year olds and 18-20 year olds. The DZ twin correlation for DEP among 18-20 year olds was significantly lower than the DZ twin correlations among 12-14 year olds and 15-17 year olds and the DZ twin correlation for GAD among 18-20 year olds was significantly lower than the DZ twin correlation among 15-17 year olds. These differences were significant at $p < .05$, which is a conservative estimate, given the large

number of comparisons, indicating minimal age differences in twin correlations. Given the limited differences in twin correlations, subsequent analyses of covariation were not stratified by age.

Comorbidity

The three internalizing disorders were significantly ($p < .05$) correlated with one another with tetrachoric correlations ranging from .19 to .52 (see Table 4). The strongest association was between self-reported depression and generalized anxiety. Neuroticism was significantly ($p < .05$) associated with each of the three disorders with polychoric correlations ranging from .20 to .34. Neuroticism was most strongly associated with generalized anxiety.

Causes of Variation in Phenotypes

MZ and DZ twin correlations for each of the three internalizing disorders are presented in Table 5. In cases where genetic influences are strong, we would expect MZ twin correlations to be close to unity, because MZ twins share 100% of their genes in common. To the extent that additive genetic influences are present and shared environmental influences are not, we would expect an MZ twin correlation twice as large as the DZ correlation for a particular phenotype. This is because MZ twins share twice as much of their genetic material in common than DZ twins (100% vs. 50%). To the extent that shared environmental influences are affecting a given phenotype, we would expect the DZ correlation to be greater than ½ the MZ twin correlation for that phenotype. This is because the shared environment is likely to affect the phenotype similarly for both MZ and DZ twins. To the extent that twin correlations are higher than zero, the presence of familiality of a phenotype is established. If twin correlations don't differ from zero, it

can be concluded that the phenotype is not familial (i.e. determined by unique environmental and not genetic or shared environmental influences).

Inspection of the twin correlations for the total sample in Table 5 reveals that all twin correlations in the total sample are greater than zero, reaching significance for SAD and neuroticism, indicating familiality of each of the phenotypes. In the case of SAD, MZ twin correlations are close to 1, indicating strong genetic influences. DZ twin correlations are greater than ½ of the MZ correlations indicating a possible role for shared environmental influences. The pattern of correlations for DEP and GAD are similar to one another and different from SAD. For DEP and GAD, MZ twin correlations are not significantly different from zero, indicating that familial effects may not be strong. Alternatively, MZ twin correlations are nearly twice as large as DZ twin correlations, indicating that familiality of these disorders may be accounted for by additive genetic influences. Twin correlations for neuroticism are similar, indicating limited familiality in this sample, attributed primarily to additive genetic influences.

An alternative way of examining genetic and environmental risk is to compute probandwise concordance rates for each of the internalizing disorders. Probandwise concordance rates are estimates of risk and must be interpreted in relation to the overall risk in the sample. For instance, the overall risk of meeting criteria for SAD in this sample is 12% (prevalence rate). Probandwise concordance rates higher than .12 would indicate greater risk for individuals with an affected co-twin. Inspection of Table 6 reveals that the MZ and DZ probandwise concordance rates for SAD are .72 and .49, respectively, indicating substantially greater risk for meeting criteria for SAD, given an affected co-twin. Therefore, a twin's risk of meeting criteria for SAD given that her MZ

co-twin has met criteria is 72%. The same risk, given that her DZ co-twin has met criteria is 49%. These risk estimates are substantially higher than the risk in the total sample of 12%, indicating substantial familiality. Furthermore, the risk for MZ twins relative to DZ twins, given an affected co-twin, is substantially higher, indicating strong genetic influences.

Inspection of probandwise concordance rates for DEP and GAD indicate that MZ twins are at higher risk of meeting criteria than DZ twins, given an affected co-twin. However, this risk is not substantially higher, indicating a more modest role of additive genetic influences relative to SAD. Additionally the MZ and DZ concordance rates for DEP and GAD are not as much higher than the risk in the total sample as the MZ and DZ concordance rates for SAD are, indicating less familiality for DEP and GAD relative to SAD.

Univariate model-fitting results are depicted in Table 7 and correspond to values suggested by inspection of the twin correlations and probandwise concordance rates. These parameter estimates indicate significant additive genetic influences for each of the four phenotypes. The additive genetic effect on SAD is particularly strong, accounting for over half of the variation in liability for this disorder. Shared environmental influences contribute significantly to variation in liability for SAD, but not for DEP, GAD, or neuroticism. As indicated by the twin correlations, non familial influences are substantial for DEP, GAD and neuroticism, accounting for 54% to 61% of variation in these phenotypes, but not for SAD (accounting for only 8% of variation).

Age was incorporated into the univariate model-fitting analyses in order to assess whether sources of variation in each of the four phenotypes differed significantly across

the three age groups. The univariate models were therefore fit allowing thresholds and parameter estimates to vary across age groups. Results indicated that thresholds and parameter estimates could be constrained to be equal across age groups for SAD and neuroticism without significantly worsening model fit. Parameter estimates could be equated across all age groups and thresholds for age groups 2 and 3 could be equated for models of DEP. Thresholds could be equated across all age groups and parameter estimates could be equated for 15-17 year olds and 18-20 year olds for models of GAD. Given the minimal influence of age on univariate results, age differences were not incorporated into multivariate model fitting analyses.

Sources of Covariation

Multivariate Cholesky model-fitting results are depicted in Table 8. The parameter estimates listed in this table are substantially influenced by the order of the phenotypes in the model. For example, all of the genetic and environmental sources of variation in liability to neuroticism are constrained to be unique to neuroticism. However, the proportion of each bivariate correlation attributable to genetic, shared and nonshared environmental sources remains consistent regardless of the order of phenotypes in the model. The proportions of each correlation attributable to each of the three latent sources are depicted in Table 9. Examination of the proportions in this table reveals several important findings. First, over half of the relationship of SAD with each of the other phenotypes is accounted for by common shared environmental influences. The remainder of these associations is split nearly evenly between common additive genetic and nonshared environmental influences. Second, the associations of neuroticism with DEP and neuroticism with GAD are accounted for primarily by shared additive

genetic influences (70% and 63%, respectively). The remainder of these associations is attributable to common nonshared environmental influences. Finally, nearly half of the association between DEP and GAD appears to be attributable to common genetic influences, with the rest of the association split nearly evenly between common shared and nonshared environmental influences.

Next, the Independent Pathway model was fit and results from this model are depicted in Table 10. The results of this model are similar to those of the Cholesky model. These estimates reveal that the majority of the risk for SAD is unique to this disorder and not shared with any of the other phenotypes, with 91% of the additive genetic effect, 91% of the shared environmental effect and 81% of the nonshared environmental effect unique to SAD. Specifically, the total estimate of risk for SAD attributed to genetic influences is .56, or 56% of the total risk. From Table 10, it can be seen that the parameter estimate corresponding to the effect of the common additive genetic factor (A_c) is .23, whereas the corresponding estimate for the additive genetic factor specific to SAD (A_s) is .71. Using tracing rules, it follows that the estimate of the effect of A_c on SAD is .05 (.23x1x.23) which accounts for only 9% (.05/.56) of the additive genetic effect on SAD and the estimate of the effect of A_s is .50 (.71x1x.71), accounting for the remaining 91% (.50/.56) of the additive genetic effect on SAD. Similarly, none of the shared environmental effect and little of the nonshared environmental effect (15%) on neuroticism are shared in common with the three internalizing disorders. On the other hand, virtually all of the genetic risk for neuroticism (95%) is common to all of the phenotypes.

Similar to the Cholesky model-fitting results, most of the nonshared

environmental effects on DEP (71%) and GAD (70%) are unique to each of these disorders and not shared in common with the other phenotypes. Alternatively, most of the familial influences on each of these disorders are not unique, but shared in common with all of the phenotypes. Specifically, 75% and 81% of the genetic effects on DEP and GAD, respectively, are shared in common with all of the phenotypes. Additionally, all of the shared environmental effects on DEP and GAD are attributable to the shared environmental factor common to all phenotypes.

Common Pathway model-fitting results are depicted in Table 11. This model proposes one latent factor that accounts for all the covariation among the measured phenotypes and which is itself influenced by genetic and environmental factors. The results in Table 11 reveal that most (59%) of the variance in this factor is attributable to latent additive genetic effects with the remainder due to shared (12%) and nonshared environmental (29%) effects. These results also indicate that SAD loads least strongly on this latent common factor, whereas neuroticism, DEP and especially GAD load more strongly on the factor.

Once again, the majority of the risk for SAD was unique to this disorder and not shared in common with the other phenotypes. On the other hand, nearly half of the additive genetic (46%) and over one-third (37%) of the shared environmental effect on neuroticism was shared with the common factor. Most (85%) of the unique environmental effect on neuroticism was unique to this phenotype.

All of the familial effects on GAD were shared with the common factor, whereas 70% of the nonshared environmental effect on GAD was unique to this disorder. Similarly, most of the additive genetic (64%) and shared environmental (84%) effects on

DEP were shared with the common factor, whereas 75% of the nonshared environmental effect was unique to DEP.

Each of the three models fit the data well as indicated by Akaike's Information Criterion (AIC). AIC values for the Cholesky (-8430.664), Independent Pathway (-8438.961) and Common Pathway (-8445.309) models were well below zero. The Common Pathway model emerged as the most parsimonious model, with the lowest AIC value, indicating that the constraints imposed by this model do not significantly worsen model fit.

DISCUSSION

Comorbidity

Consistent with other studies, these analyses revealed that anxiety and depression in this sample were positively associated. However, this study contributes a new perspective, in that the relationship of SAD in childhood and anxiety and depression in adolescence and early adulthood has not been extensively investigated. These results demonstrate that although SAD was not as strongly associated with the other phenotypes as, for example, GAD, it was modestly related to each of the other internalizing disorders and neuroticism. The fact that this disorder was assessed differently from the others (i.e. mother report vs. self report) suggests that this is likely a conservative estimate of the association of SAD with later anxiety and depression as well as neuroticism.

Genetic Vulnerability to Anxiety and Depression

The results of the univariate genetic analyses in this study are consistent with previous studies examining the etiology of mother reported SAD. Once again, a strong role for additive genetic influences was found. This has been consistent across all studies of SAD using mother report and all but one behavior genetic study of SAD based on self-report. Additionally, this study found that shared environmental effects could not be discounted. This is particularly interesting given that historically, shared environmental effects have been less consistently detected in studies of psychopathology (e.g. Cronk et al., 2002; Eaves et al., 1997; Silove, et al., 1995). In a previous paper on this topic, it was found that paternal absence and socioeconomic disadvantage were two aspects of that shared environment of twins that contributed significantly to liability to SAD in this

sample (Cronk et al., 2004). There are likely countless other environmental variables that could comprise the important shared environmental risk for SAD.

The findings for DEP and GAD were similar to one another, yet distinct from the findings for SAD. Consistent with previous studies of GAD and OAD, liability for GAD in this sample was determined by additive genetic and nonshared environmental factors. Approximately 40% of risk was attributable to genetic influences. Similarly, 35% of risk for DEP was accounted for by genetic factors, with the remainder attributable largely to nonshared environmental factors. In both cases, shared environmental influences could be dropped from the models, consistent with the majority of studies in the literature. These findings replicate the existing literature in showing moderate familiality of depression and generalized anxiety and that this familiality is due to genetic risk.

The univariate genetic analyses revealed greater familiality of separation anxiety disorder than for either generalized anxiety disorder or depression. This could mean a number of things. First, it may reflect the fact that mothers are rating both twins' separation anxiety disorder symptoms whereas twins are reporting only on their own generalized anxiety and depression symptoms. To the extent that mothers are rating twins more alike, it would result in greater familiality and specifically, greater estimates of shared environmental influences than would be seen if twins rated their own individual symptoms. Second, this finding could reflect a greater role for genetic influences in internalizing symptoms in childhood, and a decreased role for these influences in adolescence and early adulthood. Such a trend has found support in the literature (e.g. Eley & Stevenson, 1999; Topolski et al., 1997). However, other studies of internalizing psychopathology have found an opposite trend (e.g. Feigon et al., 2001) whereas still

others have found no age effects on internalizing symptoms (e.g. Eaves et al., 1997; Thapar & McGuffin, 1995). Third, SAD could represent a form of internalizing disorder distinct from DEP and GAD. Specifically, SAD may index fear, akin to panic disorder or other paroxysmal forms of anxiety, whereas DEP and GAD may index more general distress or negative emotionality (Krueger, 1999). This interpretation is also supported by the stronger associations of neuroticism with DEP and GAD than with SAD.

Shared Liability for Internalizing Disorders

The least restrictive model, the Cholesky model, revealed that the majority of the comorbidity between separation anxiety and depression and between separation anxiety and generalized anxiety was accounted for by shared environmental risk common to the three disorders. These relationships have not been reported in the literature and represent new insights into the nature of the relationship of separation anxiety disorder with other forms of internalizing psychopathology. At the same time, results from the Cholesky model were similar to results found by others (e.g. Kendler et al., 1992b) in demonstrating that nearly half of the association between depression and generalized anxiety disorder is attributable to shared genetic risk for both disorders. As more restrictive models were fit to the data (i.e. the independent and common pathway models), these proportions varied. However, the most parsimonious model, the common pathway model, revealed that the majority (nearly 60%) of the association among the three forms of psychopathology was attributable to shared genetic risk. This finding is consistent with previous studies of depression and generalized anxiety disorder and extends that work to include risk for separation anxiety disorder.

One interesting pattern across the multivariate genetic model-fitting analyses was

the relatively low association of risk for separation anxiety disorder with risk for generalized anxiety disorder and depression. Across all three models, genetic and environmental risk for separation anxiety disorder was almost exclusively unique to this disorder and not shared with the other phenotypes. One potential interpretation of this finding could be that because this phenotype was assessed by mother report, whereas the remaining phenotypes were assessed by self report, this unique etiology reflects differences in measurement. However, another interpretation of these findings suggests that separation anxiety disorder indexes a form of internalizing psychopathology which is distinct from, though associated with, the other internalizing disorders assessed in this study. According to Krueger's model, generalized anxiety disorder and depression are indicators of the Distress subfactor of internalizing psychopathology. It is possible that separation anxiety disorder is an indicator of the Fear subfactor in this model, and represents a more paroxysmal form of anxiety. This interpretation would be consistent with previous findings in the literature that separation anxiety disorder in childhood is a significant predictor of panic disorder in adulthood (e.g. Biederman et al., 2005; Gittelman, & Klein, 1984). Although methodological limitations make it impossible to clarify these interpretations, these results represent an important first step in extending Krueger's model to child and adolescent forms of internalizing psychopathology.

Neuroticism and Internalizing Psychopathology

The results of this study replicate previous findings in the literature of significant associations of neuroticism with depression and anxiety. This study extends that work by including separation anxiety disorder, which was significantly associated with neuroticism as well. This association is likely a conservative estimate of the relationship

between separation anxiety and neuroticism given that each was assessed by different reporters and the assessment points were approximately one year apart.

The univariate model fitting results for neuroticism are also consistent with previous findings of significant heritability for this trait. As with previous studies, in this sample variability in neuroticism was explained by additive genetic and nonshared environmental influences. Given the association with the internalizing disorders and the significant heritability of neuroticism in this sample, it was a strong candidate to explain shared genetic risk among the internalizing disorders as hypothesized. These analyses indicated that, in fact, neuroticism does share substantial common genetic risk with each of the internalizing disorders. In fact, estimates from the independent pathway model indicate that between 69-80% of the association of neuroticism with anxiety and depression is attributable to shared genetic factors. Results from the common pathway model show that neuroticism loads highly on the internalizing factor with depression and generalized anxiety. The association of neuroticism with separation anxiety disorder was less strong than the associations of neuroticism with depression and generalized anxiety disorder. This may indicate that neuroticism is an index of general distress, similar to generalized anxiety and depression, whereas separation anxiety is an index of paroxysmal anxiety.

The Cholesky model fitting results indicate that after accounting for genetic risk shared in common with neuroticism, less than half of the genetic risk for depression and nearly one-third of genetic risk for generalized anxiety disorder remained. On the other hand, 99% of genetic risk for separation anxiety disorder remained after accounting for risk shared with neuroticism. These findings support the hypothesis that neuroticism

accounts for some of the shared genetic risk for depression and generalized anxiety, but not for separation anxiety disorder.

The finding that the Common Pathway model provided the most parsimonious fit to the data was an interesting finding. This model is a subset of both the Independent Pathway and Cholesky models, with more restrictions than the other two. The Common Pathway model constrains the proportion of variance attributable to common genetic, shared environmental and unique environmental factors across each of the four measured phenotypes (McArdle & Goldsmith, 1990). These constraints are particularly interesting given the apparent uniqueness of SAD relative to the other phenotypes. In the Cholesky model, the least restrictive multivariate model, over half of the correlations between SAD and Neuroticism (56%), between SAD and DEP (54%) and between SAD and GAD (51%) are accounted for by common shared environmental factors. However, the Common Pathway model constrains these proportions to 12% and remains the most parsimonious fit to the data, suggesting that shared environmental factors do not contribute as strongly to the relationships among the measured phenotypes, including SAD, as a common genetic liability.

The common underlying genetic risk for the internalizing disorders and neuroticism is interesting. This finding suggests that a common genotype may be expressed in different phenotypic forms, for example DEP or GAD. Different environmental factors may contribute to the different phenotypes to varying degrees, but the common genetic risk increases the likelihood of co-occurrence of these different phenotypes within individuals. For example, the experience of parental loss has been found to be a risk factor for both SAD and DEP (Cronk et al., 2004; Eley & Stevenson,

200). Therefore, children who experience this risk factor are at increased risk of developing both SAD and DEP, resulting in increased comorbidity between these disorders. The findings of the current study suggest that twins who are characterized by a particular genotype (i.e. exposure to "genetic risk factors") are at increased risk for multiple forms of internalizing disorders as well as development of features of neuroticism.

Limitations

This study is characterized by several limitations. First, one of the primary limitations is the inability to parse out method variance from the models due to the use of different respondents for the different phenotypes. Because separation anxiety disorder was assessed via mother report whereas depression and generalized anxiety disorder were assessed via self report, it is unclear to what extent differences reflect method bias. This problem would be easily solved by incorporating self reports of separation anxiety and/or mother reports of generalized anxiety and depression. There is traditionally little agreement among raters of child and adolescent psychopathology, particularly for internalizing psychopathology (Choudhury, Pimentel, & Kendall, 2003; Foley et al., 2004; Foley et al., 2005; Grills & Ollendick, 2003). It cannot be assumed that the findings from this study generalize to studies based on self reported separation anxiety or mother reported generalized anxiety and depression.

A second limitation of this study is the time difference in the assessment of neuroticism. In this case, neuroticism was assessed approximately one year after the three internalizing disorders. An expansive personality literature demonstrates that personality traits, including neuroticism, change throughout the lifespan, becoming more

stable with increased age and that consistency does not peak until adulthood (Caspi & Roberts, 1999; Roberts & DelVecchio, 2000). Therefore, had neuroticism been measured at the same time, it is possible that different patterns of relationships with the internalizing disorders may have been found. However, by measuring both internalizing disorders and neuroticism at the same time, correlations could be artificially inflated due to state effects on reporting. Although studies have demonstrated that neuroticism traits predispose to internalizing disorders such as depression, there has also been some evidence of a "scar effect," whereby the experience of depression results in increases in neuroticism scores (Kendler et al., 1993a). In this study, because neuroticism was measured only after twins and their mothers reported on internalizing psychopathology, it is not possible to assess the degree to which the experience of anxiety and depression in twins in this sample influenced reporting of neuroticism at the later assessment point.

Third, mother reports of separation anxiety in this sample are retrospective and all of the recall biases and difficulties pertaining to retrospective reporting apply to this study as well. In some cases, where twins were in their early twenties, mothers were reporting on symptoms that may have been present up to 17 years previously. Finally, this sample consists exclusively of female twin pairs. There has been substantial evidence suggesting that the etiological mechanisms for anxiety and depression differ for boys and girls (e.g. Eaves et al., 1997). Therefore, the results of this study cannot be assumed to generalize to male populations.

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Table 1

Assessment Timeline

Time 1	Time 2 (1 year follow-up)
Parent Interview [12-22]	
SAD (DICA-P)	
Twin Interview [12-22]	*Twin Questionnaire [13-23]*
DEP (DICA-C)	Neuroticism (JEPQ)
GAD (DICA-C)	

Note. DEP=depression; DICA-C=Diagnostic Interview for Children and Adolescents (Child Version); DICA-P=Diagnostic Interview for Children and Adolescents (Parent Version); GAD=generalized anxiety disorder; JEPQ=Junior Eysenck Personality Questionnaire; SAD=separation anxiety disorder. [] denotes age range of twins at each assessment.

Table 2

Prevalence of Disorders

	Total Sample (N=4,320)	Age Group 1 [12-14] (N=1,478)	Age Group 2 [15-17] (N=1,109)	Age Group 3 [18-20] (N=775)	Age Group 4 [21-23] (N=43)
Separation Anxiety					
Symptoms	12.2%	12.4%	11.5%	11.0%	2.3%
Full Diagnosis	4.4%	4.3%	4.5%	4.2%	0%
Depression					
Symptoms	14.7%	9.5%	22.4%	30.3%	30.2%
Full Diagnosis	6.7%	2.6%	10.5%	16.9%	11.6%
Generalized Anxiety					
Symptoms	14.1%	13.1%	19.0%	25.4%	20.9%
Full Diagnosis	1.6%	0.9%	2.2%	3.7%	0%

Table 3

Logistic Regression Coefficients and Odds Ratios Predicting Phenotypes from Age

	Linear (Age)		Quadratic (Age^2)	
	Coefficient	Odds Ratio	Coefficient	Odds Ratio
SAD	0.01	1.01		
DEP	0.23*	1.26*	-0.03*	0.97*
GAD	0.14*	1.15*		
Neuroticism	-0.05	0.95		

Note. * p<.001; SAD = 3+ symptoms of Separation Anxiety Disorder; DEP = 5+ symptoms of Depression; GAD = excessive worry in multiple domains and 3 physical symptoms of Generalized Anxiety Disorder

Table 4

Associations Among Phenotypes ^

	SAD	DEP	GAD	Neuroticism*
SAD		2.14	1.85	1.47
DEP	.24		5.10	1.87
GAD	.19	.52		2.22
Neuroticism	.20	.34	.42	

Note. Correlations are listed below the diagonal and odds ratios (predicting phenotypes listed down the first column from the phenotypes listed across the first row) are listed above the diagonal. *Odds ratios are computed using a standardized Neuroticism variable. ^Correlations among disorder categories are tetrachoric correlations, whereas correlations with neuroticism are polychoric correlations; All correlations and odds ratios are significant ($p<.05$); SAD = 3+ symptoms of Separation Anxiety Disorder; DEP = 5+ symptoms of Depression; GAD = excessive worry in multiple domains and 3 physical symptoms of Generalized Anxiety Disorder

Table 5

MZ and DZ Twin Correlations for Internalizing Disorders and Neuroticism

	Total Sample (N=4,320)		Age Group 1 [12-14] (N=1,478)		Age Group 2 [15-17] (N=1,109)		Age Group 3 [18-20] (N=775)	
	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
SAD	.92*	.66*	.86*	.68	.95*	.62	.96*	.65
DEP	.44	.27	.39	.30	.35	.27	.38	.05
GAD	.41	.23	.47	.18	.35	.33	.33	.02
Neuroticism	.46*	.27*	.46	.30	.44	.32	.51	.17

Note. There were not sufficient numbers of twin pairs to compute twin correlations for the four phenotypes in Age Group 4. * = significantly different from zero ($p < .05$).

Table 6

MZ and DZ Probandwise Concordance Rates for Internalizing Disorders

	Total Sample (N=4,320)		Age Group 1 [12-14] (N=1,478)		Age Group 2 [15-17] (N=1,109)		Age Group 3 [18-20] (N=775)	
	MZ	DZ	MZ	DZ	MZ	DZ	MZ	DZ
SAD	.72	.49	.65	.49	.79	.44	.80	.49
DEP	.36	.31	.23	.23	.36	.35	.44	.32
GAD	.34	.29	.33	.20	.33	.36	.36	.29

Note. There were not sufficient numbers of twin pairs to compute probandwise concordance rates for the three internalizing disorders in Age Group 4; SAD = 3+ symptoms of Separation Anxiety Disorder; DEP = 5+ symptoms of Depression; GAD = excessive worry in multiple domains and 3 physical symptoms of Generalized Anxiety Disorder

Table 7

Univariate Model Fitting Results and Parameter Estimates

Phenotype	Parameter Estimates			Best-Fitting Model
	a^2	c^2	e^2	
SAD	.56 (.34-.82)	.36 (.11-.56)	.08 (.05-.13)	ACE
DEP	.35 (.00-.51)	.04 (.00-.36)	.61 (.49-.74)	AE
GAD	.39 (.01-.51)	.00 (.00-.32)	.61 (.49-.73)	AE
Neuroticism	.38 (.17-.52)	.08 (.00-.27)	.54 (.47-.60)	AE

Note. This model was fit to the entire sample; a^2, c^2, e^2 represent estimates of genetic, shared environmental and unique environmental influences, respectively; 95% confidence intervals for parameter estimates given in parentheses; SAD = 3+ symptoms of Separation Anxiety Disorder; DEP = 5+ symptoms of Depression; GAD = excessive worry in multiple domains and 3 physical symptoms of Generalized Anxiety Disorder

Table 8

Multivariate Cholesky Model Fitting Results

	Variance Components			Parameter Estimates											
	a^2	c^2	e^2	A_1	C_1	E_1	A_2	C_2	E_2	A_3	C_3	E_3	A_4	C_4	E_4
Neuroticism	.38	.09	.53	.61	.29	.73									
Separation Anxiety	.53	.38	.08	.05	.36	.07	.73	.50	.28						
Depression	.31	.17	.52	.40	.00	.14	.05	.25	.18	.38	.32	.69			
Generalized Anxiety	.31	.10	.59	.44	.10	.17	.03	.13	.13	.20	.28	.15	.27	.00	.72

Table 9

Proportion of Correlations Attributable to Latent Factors From Multivariate Cholesky Model

		Sources of Correlation		
Bivariate Associations	Correlation^	%A	%C	%E
Neuroticism*SAD	.19	17	56	27
Neuroticism*DEP	.35	70	0	30
Neuroticism*GAD	.42	63	7	29
SAD*DEP	.25	25	51	24
SAD*GAD	.20	22	53	25
DEP*GAD	.52	48	23	29

Note. ^ Correlations are estimated from multivariate model-fitting and are within rounding error of correlations presented in Table 3; SAD = Separation Anxiety Disorder; DEP = Depression; GAD = Generalized Anxiety Disorder

Table 10

Multivariate Independent Pathway Model Fitting Results

	Variance Components			Parameter Estimates													
	a^2	c^2	e^2	A_C	C_C	E_C	A_N	C_N	E_N	A_S	C_S	E_S	A_D	C_D	E_D	A_G	C_G	E_G
Neuroticism	.40	.06	.53	.62	.00	.28	.14	.25	.67									
Separation Anxiety	.56	.36	.08	.23	.18	.12				.71	.57	.26						
Depression	.20	.25	.54	.39	.50	.39							.23	.00	.62			
Generalized Anxiety	.30	.11	.59	.49	.33	.42										.24	.00	.65

Table 11

Multivariate Common Pathway Model Fitting Results

	Variance Components			Parameter Estimates														
	a^2	c^2	e^2	A_C	C_C	E_C	F	A_N	C_N	E_N	A_S	C_S	E_S	A_D	C_D	E_D	A_G	C_G	E_G
Neuroticism	.37	.09	.53	.77	.34	.53	.54	.45	.24	.67									
Separation Anxiety	.56	.35	.08	.77	.34	.53	.30				.71	.58	.24						
Depression	.42	.06	.51	.77	.34	.53	.67							.39	.10	.62			
Generalized Anxiety	.36	.07	.57	.77	.34	.53	.77										.01	.01	.63

Note. F denotes path from common factor to measured phenotype.

Figure 1. Neuroticism Medians and Modes

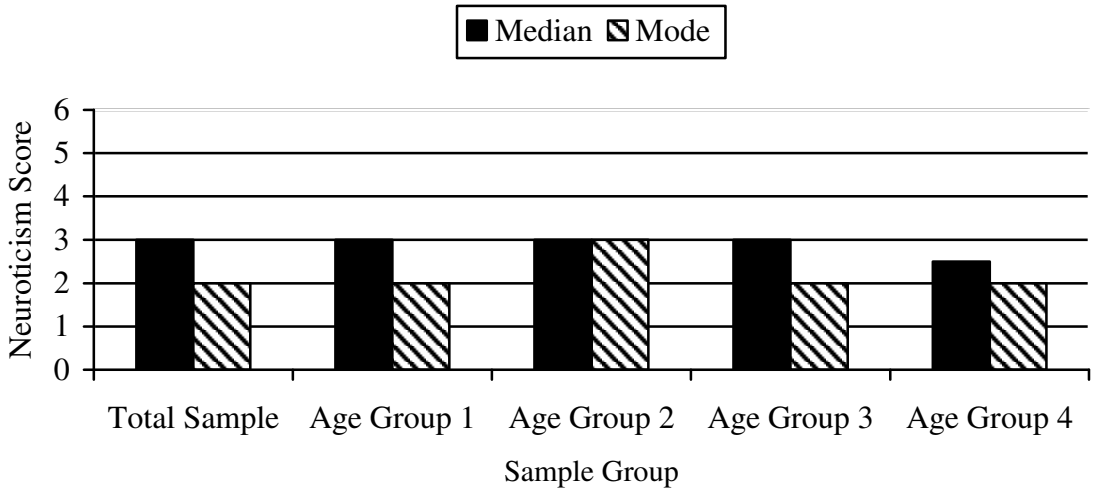
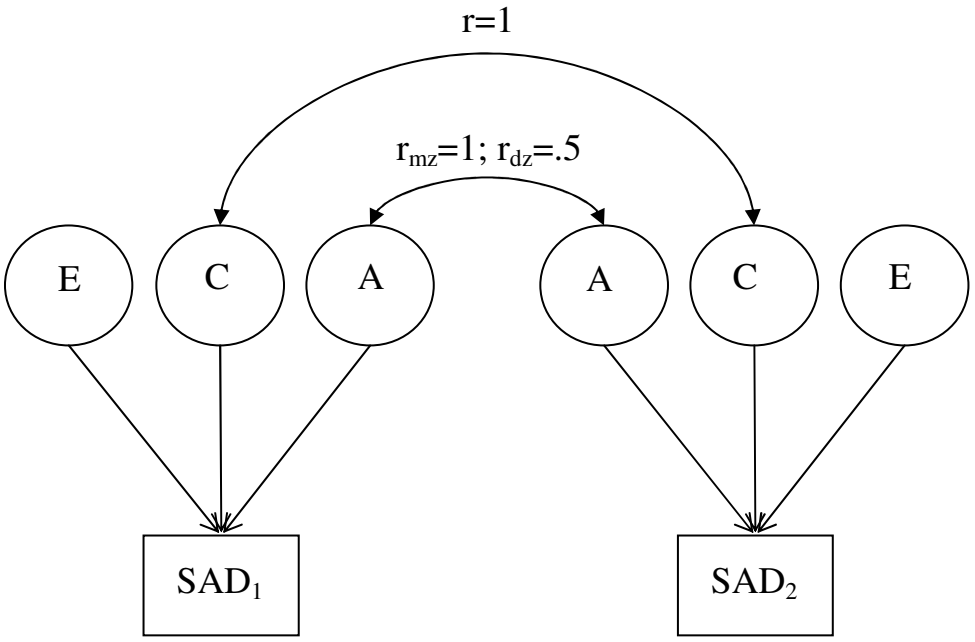
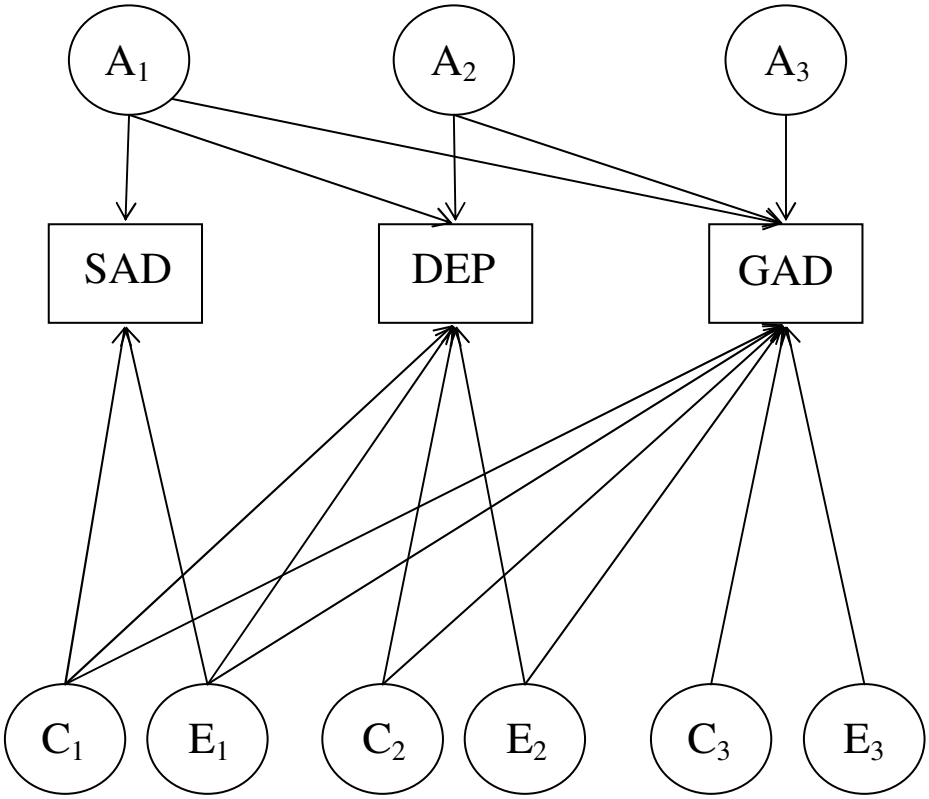


Figure 2. Univariate Model Explaining Variation in Liability for Separation Anxiety Disorder



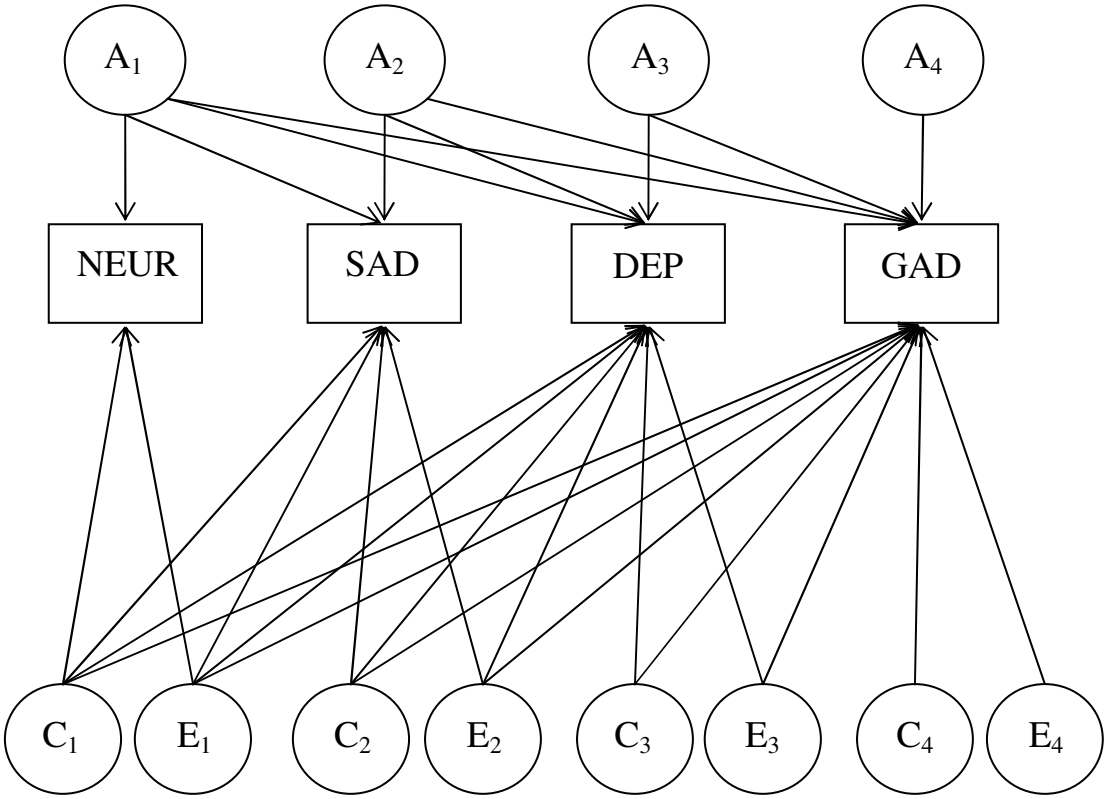
Note. A=additive genetic factor; C=shared environmental factor; E=nonshared environmental factor; r_{dz}=dizygotic twin correlation; r_{mz}=monozygotic twin correlation; SAD_1=separation anxiety disorder in twin 1; SAD_2=separation anxiety disorder in twin 2

Figure 3. Cholesky Model Explaining Covariation Among Separation Anxiety Disorder, Depression and Generalized Anxiety Disorder



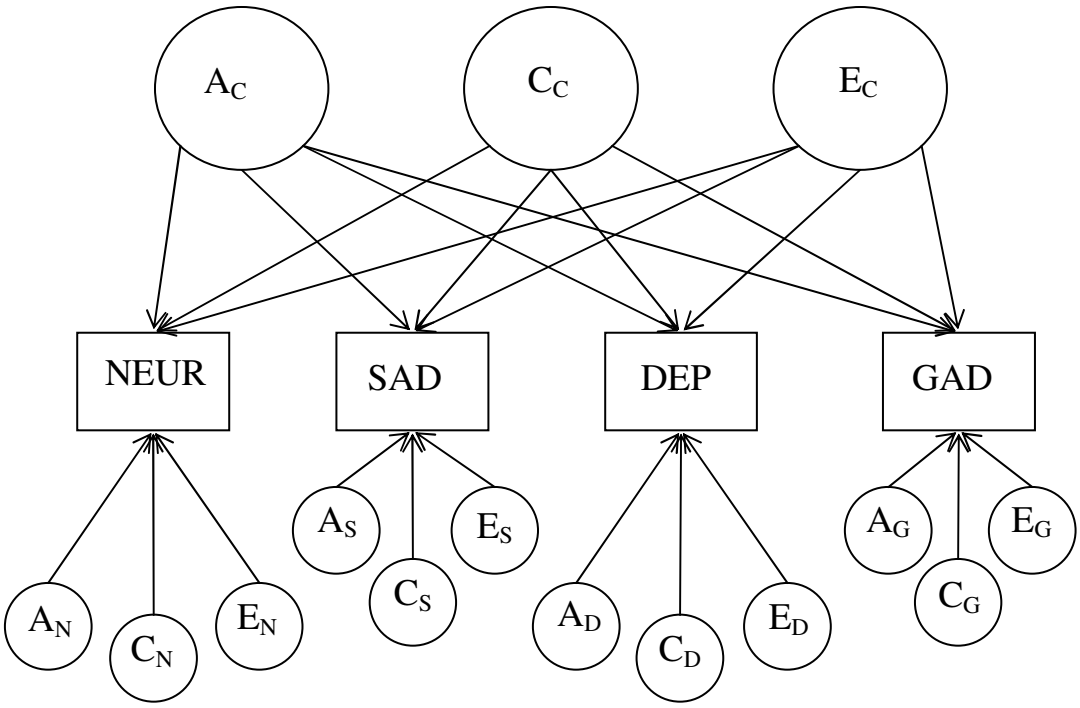
Note. A=additive genetic factor; C=shared environmental factor; DEP=depression; E=nonshared environmental factor; GAD=generalized anxiety disorder; NEU=neuroticism; SAD=separation anxiety disorder

Figure 4. Cholesky Model Explaining Covariation Among Neuroticism, Separation Anxiety Disorder, Depression and Generalized Anxiety Disorder



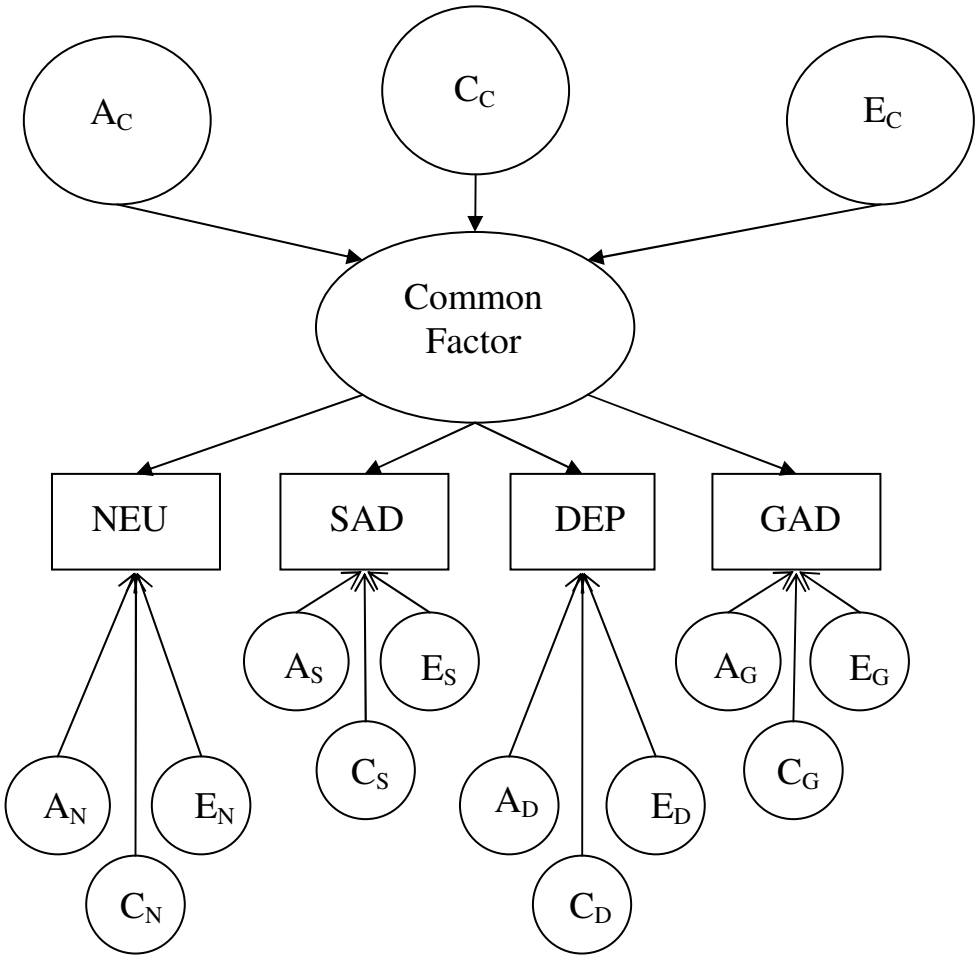
Note. A=additive genetic factor; C=shared environmental factor; DEP=depression; E=nonshared environmental factor; GAD=generalized anxiety disorder; NEU=neuroticism; SAD=separation anxiety disorder

Figure 5. Multivariate Independent Pathway Model Explaining Covariation Among Neuroticism, Separation Anxiety Disorder, Depression, and Generalized Anxiety Disorder



Note. A=additive genetic factor; C=shared environmental factor; DEP=depression; E=nonshared environmental factor; GAD=generalized anxiety disorder; NEU=neuroticism; SAD=separation anxiety disorder

Figure 6. Multivariate Common Pathway Model Explaining Covariation Among Neuroticism, Separation Anxiety Disorder, Depression, and Generalized Anxiety Disorder



Note. A=additive genetic factor; C=shared environmental factor; DEP=depression; E=nonshared environmental factor; GAD=generalized anxiety disorder; NEU=neuroticism; SAD=separation anxiety disorder

Appendix A

Separation Anxiety Disorder Questions

When she was six years or older, did (twin):

- *Often* get very worried and upset when she was away from her parent(s) or away from home, or when she thought she would be going away?
- Worry *a lot* that something bad would happen to her parent(s) or that she would never see you again?
- Did she worry *a lot* that she would get lost or kidnapped and be separated from her parent(s)?
- Did she *often* want to stay home from school, or not go other places, because of her worries about being away from home and away from her parent(s)?
- Was she afraid to be all by herself in a room at home, even if a parent was in the next room?
- When she was away from home, was she so fearful that she had to stay right by a grownup?
- Did she *often* insist on having a parent with her when she went to sleep or refuse to sleep away from home?
- Did she *often* have bad dreams about losing her parents or getting lost or being kidnapped?
- When she had to be away from her parent(s), or thought she would have to be away, would she *often* get headaches or stomach aches or feel sick to her stomach?

- Did (twin) have three or more of these problems occur together for a period of a month or longer?
- Did these worries cause problems for (twin) at school?
- Did these worries cause problems for how (twin) got along with other children?
- Did (twin)'s worries cause problems at home?
- Did (twin) see a doctor, counselor or any other professional about these worries?
- Did *you* ever talk to a doctor, counselor, or other professional about (twin)'s worries?

Appendix B

Depression Questions

Initial Screen:

- Has there ever been two weeks or more when you were depressed or down most of the day nearly every day?

- How about feeling sad, blue, low, or gloomy most of the day, nearly every day, for two weeks or more?

- Has there ever been two weeks or more when you were a lot less interested in most things or unable to enjoy the things you used to enjoy, most of the day nearly every day?

- Has there ever been two weeks or more when most of the day, nearly every day you were a lot more irritable or angry than usual?

At least one affirmative response to the questions above was required to proceed to the depression section of the interview:

Can you tell me about a period of time in your life that stands out as the worst? During this period of time, most of the day, nearly every day for at least two weeks:

- Were you feeling depressed or down?

- Were you a lot less interested in most things, or unable to enjoy the things you used to enjoy?

- Were you feeling a lot more irritable or angry than usual?

  During that period of time when you were (depressed/less interested/irritable), nearly every day for at least two weeks:

  - Did you gain or lose as much as 2 lbs a week or 10lbs altogether when you were not trying to?

  - Was your appetite very different from your usual appetite?

  - Did you have trouble with your sleep or sleeping much more than usual?

  - Were you fidgety or restless?

  - Did you talk or move much more slowly than usual?

  - Were you a lot more tired than usual, as if you had no energy?

- Did you feel that everything was your fault or that you were a bad person?
- Did you feel that you were a failure or worthless?
- Did you have trouble thinking or concentrating?
- Was it hard to make decisions about everyday things – for example, what to wear, or whether or not to go out with your friends?
- Did you frequently think about death or dying?
- Did you frequently think about taking your own life, or wish you were dead?
- Did you try to take your own life?
- Did you make a plan to take your own life?

- Was there a period of at least two weeks when 5 or more problems including "felt depressed or down most of the day," "felt a lot more irritable than usual, most of the day," or "felt a lot less interested in most things, or unable to enjoy the things you used to enjoy" occurred together nearly every day?
- During this time, did you seek or receive help from a psychiatrist, other doctor, psychologist, counselor, social worker, clergyman or other professional?
- During this time, were you hospitalized for depression?
- Did you find that you couldn't (do your job, school work/take care of your house or someone else/other) as well as usual?
- Did you have trouble in any other area of your life, such as your relationships or leisure activities?

Appendix C

Generalized Anxiety Disorder Questions

Initial Screen:

- Have you ever been *worried a lot about things before they happened*: for example, about starting school in the fall, going to a party, or going to see a doctor?

- Have you ever *worried a lot about not being able to do things as well as you wanted to*? That includes jobs, school work, sports, social, or other activities?

- Have you *worried a lot about little things that you've done in the past*, like something you've said that might have been taken the wrong way?

- Have you *worried a lot about how you looked, what you said, or how you acted in front of your friends*? I'm talking about feeling that way *a lot*, more than most of your friends.

At least one affirmative response to the questions above was required to proceed to the depression section of the interview:

At times when you were anxious, nervous or worried, did:

- You feel physically restless – couldn't sit still?

- You feel keyed up or on edge?

- You get tired easily?

- You have trouble concentrating or with your mind going blank?

- You feel irritable?

- Your muscles often feel tense, sore or achy?

- You have trouble falling asleep or staying asleep?

- Has there ever been a period of *six months* or more when you were worried or anxious *most* days?

- Has there ever been a period of six months or more when you were worried and had at least one problem such as (feeling physically restless, feeling keyed up/on edge, getting tired easily, having trouble concentrating/mind going blank, feeling irritable, muscles feeling tense/sore/achy, or having trouble falling/staying asleep) most days?

- Did these worries, anxieties or problems ever interfere with work or studying?

- Did these worries, anxieties or problems ever interfere with your activities or relationships with friends or family?

- Did you ever talk to a psychiatrist, other doctor, psychologist, counselor, social worker, clergyman, or other professional about these worries, anxieties or problems?

Appendix D

Neuroticism Questions

1. Do you often feel guilty about things you've said or done?
2. Do lots of things annoy you?
3. Do you ever feel "just miserable" for no good reason?
4. Do you often feel life is very dull?
5. Do you worry about awful things that might happen?
6. Are your feelings rather easily hurt?
7. `Do you often feel "fed-up?"
8. Do you worry for a long while if you feel you have made a fool of yourself?
9. Do you sometimes feel life is just not worth living?
10. Do you often feel lonely?
11. Do you sometimes feel specially cheerful and at other times sad without any good reason?
12. Do you often need kind friends to cheer you up?

VITA

Nikole J. Cronk was born January 30, 1973, in Billings, Montana. After attending public schools through the eighth grade, she graduated from Billings Central Catholic High School in Billings in 1991. She has received the following degrees: A.B. in Psychology from Harvard University, graduating Cum Laude in General Studies (1995); M.A. in Psychological Studies from Seton Hall University (1996); M.A. in Clinical Psychology from the University of Missouri – Columbia (2001); Ph.D. in Clinical Psychology from the University of Missouri – Columbia (2006). Upon completion of her doctoral degree, she accepted a postdoctoral fellowship at Linden Oaks Hospital at Edward in Naperville, Illinois.