Shared Genetic Influences on Negative Emotionality and Major Depression/Conduct Disorder Comorbidity

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Objective: To investigate whether genetic contributions to major depressive disorder and conduct disorder comorbidity are shared with genetic influences on negative emotionality.

Method: Primary caregivers of 2,022 same- and opposite-sex twin pairs 6 to 18 years of age comprised a population-based sample. Participants were randomly selected across five regions in Tennessee, with stratification by age and geographic location. Face-to-face structured interviews were conducted with the primary caregiver of a representative sample of twins.

Results: After accounting for genetic influences on negative emotionality, genetic influences on major depressive disorder/conduct disorder comorbidity were nonsignificant, but only in male twins. Specifically, 19% of the variance in the two disorders was accounted for by genetic factors shared with negative emotionality in male twins. Although the full hypothesis could not be tested in female twins, 10% to 11% of the variance in the two disorders was also accounted for by genetic factors shared with negative emotionality. Common shared environmental and nonshared environmental influences were found for major depressive disorder/conduct disorder comorbidity in male and female twins.

Conclusions: Negative emotionality represents an important dispositional trait that may explain genetic influences on major depressive disorder/conduct disorder comorbidity, at least for boys. Models of major depressive disorder/conduct disorder comorbidity must simultaneously measure common and specific genetic and environmental factors for a full understanding of this phenomenon. Gender differences require specific research attention in dispositional factors and developmental progression. J. Am. Acad. Child Adolesc. Psychiatry, 2011;50(8):818–827.

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Robust findings have emerged for substantial comorbidity between major depressive disorder (MDD) and conduct disorder (CD) in childhood and adolescence. Comorbidity of MDD and CD has severe and far-reaching implications, because children with comorbidities have more impairment across specific and global domains than children with MDD or CD alone. This pairing has garnered increased research attention, not only because of its prevalence but also because it highlights a connection between disorders that are typically conceptualized under different psychopathologic domains. Specifically, MDD is typically conceptualized as a form of internalizing psychopathology, whereas CD is conceptualized as a form of externalizing psychopathology. Although within-domain comorbidity (e.g., anxiety and mood disorders) is widely accepted as common, cross-domain comorbidity represents a more surprising manifestation that warrants close attention.

Multiple explanations for this comorbidity have been proposed. Studies investigating hypotheses of developmental progresses have provided mixed evidence, with some finding that early CD predicts later MDD, and others finding no evidence of this progression and/or suggesting that MDD is a precipitating factor for later CD. One study found evidence for gender differences in the context of developmental progression, with limited support for early delinquency leading to later depression in adolescent boys but robust evidence for bidirectional influences of delinquency and depression in ad-
olescent girls. The present study tested a novel explanation for MDD/CD comorbidity that has not yet been tested empirically—the possibility that common underlying genetic factors influence the two disorders via shared dispositional characteristics.

COMMON CAUSAL FACTORS OF MDD/CD COMORBIDITY

In addition to hypotheses of developmental progression, other explanations that have received substantial attention focus on shared etiologic factors. This work has identified common causal factors that potentially exert their influence externally (often referred to as risk factors) and those that exert their influence through characteristics of the child (often referred to as vulnerability factors). MDD and CD share many of the same environmental risk factors. Common risk factors such as conflict, rejection, and family risk have received some support as a potential explanation for MDD/CD comorbidity. One study found that two thirds of the variance in MDD/CD comorbidity could be explained by shared risk factors such as marital conflict and peer relations. Another study provided evidence that once the common influence of psychosocial impairment is accounted for, early CD no longer predicts later MDD. Taken together, this work provides support for a strong role of risk factors in accounting for MDD/CD comorbidity.

Studies of risk factors often imply but do not conclusively demonstrate environmental influences; however, often risk factors cannot easily be attributed to environmental or genetic mechanisms of risk transmission. For example, parental psychopathology has received much support as a risk factor for MDD/CD comorbidity, but it could convey risk genetically and/or environmentally. Thus, studies examining risk and vulnerability factors outside the context of a genetically informative investigation may have difficulty differentiating risk from vulnerability.

Genetically informative designs have also offered support for common vulnerability factors as a potential explanation for MDD/CD comorbidity. One family study of twin and non-twin siblings found significant common influences on depression and antisocial behavior co-occurrence from additive genetic, shared environmental, and nonshared environmental factors. How-

A SPECTRUM MODEL APPROACH TO MDD/CD COMORBIDITY

The spectrum model is one proposed model of personality-psychopathology relations that positions personality as a core feature of psychopathologic comorbidity. A spectrum conceptualization is related to a common cause approach, such that shared causal factors are presumed to exert influence on an underlying core dimension that manifests in potentially distinct phenotypes. From a spectrum approach, phenotypic differentiation of disorders may be conceptualized as quantitative differences on underlying core dimensions. Considering MDD/CD comorbidity, one good candidate for such an underlying core dimension is negative emotionality (NE). NE has been linked to many forms of childhood psychopathology and already has...
been proposed as a potential common feature for explaining MDD/CD comorbidity, although not yet tested empirically. High levels of NE are associated with MDD and CD individually. MDD-/CD comorbidity may show an even greater association with NE than each disorder on its own. In addition, NE is significantly influenced by genetic factors. Support for the spectrum conceptualization would be found if the genetic influences on MDD/CD comorbidity were entirely shared with genetic influences on NE, a hypothesis that previous research has not yet examined. The present study was designed to provide the first empirical test of this proposal.

THE PRESENT STUDY
In the present study, the goal was to build a comprehensive framework of common and specific etiologic factors influencing MDD symptoms, CD symptoms, and MDD/CD symptom comorbidity, specifically in the context of NE as an overarching common factor. The following research questions were addressed.
1. Evidence for common etiologic factors in MDD/CD symptom comorbidity was examined. Common genetic and nonshared environmental influences underlying this comorbidity were expected. Based on the literature examining common environmental risk factors, common shared environmental influences also were expected, although this hypothesis was more exploratory given conflicting evidence from previous genetically informative studies.
2. Assuming that common genetic factors for MDD/CD symptom comorbidity were found, the role of NE was examined. The hypothesis was that accounting for common genetic influences on NE, MDD symptoms, and CD symptoms would eliminate remaining genetic influences on MDD/CD symptom comorbidity.
3. Given the ambiguity in previous research regarding gender differences in MDD/CD comorbidity, specific hypotheses regarding gender were not made. Evidence for gender differences was examined throughout the study.

METHOD
Participants
Participants included primary caregivers of 2,022 twin pairs in the Tennessee Twin Study. The Tennessee Twin Study was comprised of a sample of twins who were born in Tennessee and living in one of the five Metropolitan Statistical Areas (Bristol, Chattanooga, Knoxville, Memphis, and Nashville) during the time of the study. Participants were randomly selected (stratified by age and geographic location) from addresses provided by the Tennessee Department of Health for eligible families and thus were representative of the sampled areas. Participating caregivers were primarily biological mothers (90.8%, 7.5% biological fathers, 0.5% stepmothers, and 1.2% grandmothers). Written informed consent was obtained from all participating caregivers. More information about the Tennessee Twin Study is provided by Lahey et al.

The twins were 6 to 17 years old at the time of recruitment, but some turned 18 before being interviewed (mean = 11.71, standard deviation = 3.31). The present sample included roughly equivalent numbers of female twin pairs (monozygotic female twins n = 386, 19.1% of overall sample; dizygotic female twins, n = 339, 16.8% of overall sample), male twin pairs (monozygotic male twins, n = 366, 18.1% of overall sample; dizygotic male twins, n = 330, 16.3% of overall sample), and dizygotic opposite-sex twin pairs (n = 601, 29.7% of overall sample). The twins' ethnic background was indicated by the caregiver (24.0% black, 71.4% white, 4.6% other). Zygosity was assigned using a questionnaire on the physical similarities between the twins. Ambiguous cases were resolved using 12 polymorphic DNA markers obtained from cheek swabs. After data collection, twins were randomly designated as twin 1 or twin 2. Based on the gender-specific nature of the following hypotheses, dizygotic opposite-sex twins were recoded nonrandomly (male = twin 1; female = twin 2).

Measurements
Negative emotionality was assessed using the Child and Adolescent Dispositions Scale (CADS). The CADS measures three broad dispositional traits (NE, prosociality, and daring) and was developed based on a theoretical model for the development of CD, with explicit attempts made in measurement development to prevent items from overlapping with DSM-defined symptoms. The CADS was administered to caregivers as a structured interview, requiring caregivers to rate how well each item describes their child on an ordinal scale from 1 (not at all) to 4 (very much). Evidence regarding the reliability and validity of the CADS can be found in Lahey et al.

Symptoms of CD and MDD were assessed using the Child and Adolescent Psychopathology Scale (CAPS). The CAPS items reflect stem questions used in the Diagnostic Interview Schedule for Children Version IV (DISC), with an expanded response format from the dichotomous option in the Diagnostic Interview Schedule for Children Version IV to a more descriptive ordinal scale ranging from 1 (not at all) to 4 (very much). Caregivers were asked to consider how
descriptive each item was of their child and how severe and frequent the emotion or behavior was during the previous 12 months. Only the DSM-IV CD and MDD symptoms were used in the present study. Evidence regarding the reliability and validity of the CAPS can be found elsewhere. \textsuperscript{30} Caregivers completed the CADS and CAPS in sequence for each twin during a home visit. Items were randomized and administered in counterbalanced order (forward or backward) for both measures.

Statistical Analysis
Scores for all three dimensions (NE, MDD, and CD) represent the average response on a 4-point item for that dimension after a rescaling of the items to a 0 (not at all) to 3 (very much) format. All structural equation models were fit in MPlus version 5.2\textsuperscript{32} using the mean- and variance-adjusted maximum likelihood estimator, which accounts for non-normality in the diagnostic data. All variables were regressed on age before the model-fitting analyses and the residualized scores used in the present study.

Bivariate and multivariate models were fit according to the classic twin design (for more detailed information, see Plomin et al.\textsuperscript{33}). Twin studies capitalize on the genetic differences between monozygotic twins (who share 100% of their genetic material) and dizygotic twins (who share approximately 50% of their segregating genes) to decompose variance, reflecting genetic and environmental influences. Specifically, these models differentiate between phenotypic variance and covariance that is attributable to additive genetic influences, shared environmental influences, and nonshared environmental influences. Shared environmental influences reflect environmental factors that affect the two twins in similar ways, whereas nonshared environmental influences reflect factors that differentially affect co-twins. The nonshared environmental component also contains estimated measurement error. All models were fit using a Cholesky decomposition and were tested for the presence of gender differences.

RESULTS
Descriptive statistics and twin correlations for NE, MDD, and CD are presented in Table 1. All correlations appeared to be higher in monozygotic than in dizygotic twins, consistent with evidence for additive genetic influences. One exception was CD in female twins, which evidenced correlations much more comparable in size for monozygotic and dizygotic female twins, pointing to a large role for shared environmental influences. Bivariate and multivariate Cholesky models were fit with and without sex differences. Specifically, models without sex differences constrained all parameter estimates to be equal for male and female twins and were compared with models with sex differences, which allowed all parameter estimates to freely vary between male and female subjects. In all cases, models allowing sex differences showed superior fit; thus, results from sex difference models are presented. Guidelines for interpreting model fit indices were used according to Hu and Bentler.\textsuperscript{34}

Before testing the full spectrum hypothesis, evidence for shared genetic contributions to MDD/CD comorbidity had to be established. The full bivariate Cholesky model allowing sex differences fit well (comparative fit index [CFI] = 0.98; root mean square error of approximation [RMSEA] = 0.03; Figures 1 and 2). In accordance with the study goals, the primary interest concerned etiologic influences on MDD/CD comorbidity. Male and female twins showed significant influences on MDD/CD comorbidity from shared environmental factors and nonshared en-

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Note: Data are presented as mean (standard deviation). All correlations significant at p < .01. Means reflect average item endorsement given a 4-point response scale. DZF = dizygotic female; DZM = dizygotic male; DZOS = dizygotic opposite sex; MZF = monozygotic female; MZM = monozygotic male; T1 = twin 1; T2 = twin 2.
for male and female genders and both are presented for completeness. The multivariate Cholesky model allowing for gender differences showed good fit (CFI = 0.99, RMSEA = 0.03; Figures 3 and 4). Only parameters reflecting additive genetic and nonshared environmental influences are presented in Figures 3 and 4; shared environmental influences were omitted for clarity.

However, in male twins, findings for shared environmental influences supported common shared environmental influences on NE (accounting for 10% of the variance in NE), CD (accounting for 6% of the variance in CD), and MDD (accounting for 16% of the variance in MDD). In female twins, findings for shared environmental influences supported common shared environmental influences on NE (accounting for 9% of the variance in NE) and MDD (accounting for 40% of the variance in MDD). After accounting for these common shared environmental influences, no remaining shared environmental influences were estimated for MDD/CD comorbidity. To obtain model convergence, the path for unique shared environmental influences on MDD was constrained to zero, consistent with
findings in the bivariate Cholesky model. In addition, no unique shared environmental influences on CD remained after accounting for the common shared environmental influences described earlier.

When controlling for shared genetic influences with NE, no additional shared genetic influences contributed to MDD/CD comorbidity in male twins (Figure 3). Approximately 19% of the variance in CD and MDD reflected shared genetic

FIGURE 3 Multivariate Cholesky model for negative emotionality (NE), conduct disorder (CD), and major depressive disorder (MDD) in male twins. Note: Variance components (i.e., squared standardized parameter estimates) and 95% confidence intervals are presented for additive genetic and nonshared environmental influences only. Shared environmental influences are omitted for clarity. A_{CD} = additive genetic influences on CD; A_{MDD} = additive genetic influences on MDD; A_{NE} = additive genetic influences on negative emotionality; E_{CD} = nonshared environmental influences on CD; E_{MDD} = nonshared environmental influences on MDD; E_{NE} = nonshared environmental influences on negative emotionality.

FIGURE 4 Multivariate Cholesky model for negative emotionality (NE), conduct disorder (CD), and major depressive disorder (MDD) in female twins. Note: Variance components (i.e., squared standardized parameter estimates) and 95% confidence intervals are presented for additive genetic and nonshared environmental influences only. Shared environmental influences are omitted for clarity. A_{CD} = additive genetic influences on CD; A_{MDD} = additive genetic influences on MDD; A_{NE} = additive genetic influences on negative emotionality; E_{CD} = nonshared environmental influences on CD; E_{MDD} = nonshared environmental influences on MDD; E_{NE} = nonshared environmental influences on negative emotionality.
factors with NE in addition to unique genetic influences on each phenotype in male twins. In female twins, approximately 10% to 11% of the variance in CD and MDD reflected shared genetic factors with NE; unique genetic influences on each phenotype were also present. The two phenotypes also showed shared and nonshared environmental influences common to NE. The multivariate Cholesky was again run with the order of MDD and CD switched to ensure that order effects did not account for the findings. The order of NE was not manipulated because it was determined by the primary hypothesis. The model with MDD and CD order switched also found no additional shared genetic influences on MDD/CD comorbidity after accounting for those shared with NE in male twins; the proportion of variance influenced by shared genetic factors with NE was 19% for the two phenotypes.

Although age differences were not a primary goal of the present investigation, the full multivariate Cholesky model was fit separately by split halves of the sample based on age. The model fit well in the child subsample (n = 1,215; ages 6–12 years; CFI = 1.00; RMSEA = 0.00) and the adolescent subsample (n = 807; ages 13–18 years; CFI = 0.98; RMSEA = 0.05). Comparing the pattern of loadings across the two age samples alongside the full sample revealed some differences. Some parameter estimates that were significant in the full sample were nonsignificant in one or both age-based subsamples, potentially reflecting limited power in the reduced groups. Unique shared environmental influences on CD in girls, which were nonsignificant in the full sample, showed significance in the child subsample. Detailed results are available on request from the first author.

**DISCUSSION**

These findings provide the first empirical support for the hypothesis that genetic influences on MDD/CD comorbidity are entirely shared with genetic influences on NE, at least in male twins. Specifically, these findings suggest that approximately one fifth of the additive genetic influences on CD and MDD are shared between the two phenotypes in boys, but this is entirely accounted for by genetic influences on NE, offering the first empirical test of the spectrum hypothesis for MDD/CD comorbidity. Because no evidence of genetic influence on MDD/CD comorbidity was found for girls in the bivariate model, the multivariate spectrum hypothesis could not be tested in girls. For girls, MDD/CD comorbidity was entirely accounted for by shared and nonshared environmental influences. In boys and girls, genetic influences played a larger role in explaining variance in CD than in MDD.

Although not explicitly hypothesized, common shared environmental influences on MDD/CD comorbidity were also entirely shared with common shared environmental influences on NE (in boys and girls), suggesting common shared environmental risk factors for a broader dimension of negative affectivity. Shared environmental influences played a larger role for girls than for boys. For male and female subjects, evidence for nonshared environmental influences on MDD/CD comorbidity remained, even after accounting for NE in the multivariate model.

The multivariate model provides support for a spectrum conceptualization of NE, MDD, and CD in that genetic influences on MDD/CD comorbidity were entirely shared with genetic influences on NE. This suggests that high levels of NE may partly account for high levels of MDD and CD, at least in boys, consistent with theoretical conceptualizations of this relation. These results support previous work by providing confirmatory evidence that some variables confer MDD/CD comorbidity risk through environmental transmission. This builds on previous evidence that shared environmental risk factors influence within-domain comorbidity, suggesting the potential for some risk factors to confer general psychopathology risk beyond risk for within-domain comorbidity.

In these results, models allowing for gender differences showed superior fit to models with no gender differences. Specifically, shared genetic influences on MDD/CD comorbidity were not found for female twins, precluding investigation of the spectrum hypothesis. One genetically informative study on this topic supported evidence for potential gender differences, whereas another did not. The small number of genetically informative studies on this topic limits the generalizability of specific gender differences. Thus, the present study offers an important extension in this regard, particularly in the use of dizygotic opposite-sex twins, which provides a powerful methodologic approach to potential gender differences. Previous research has suggested that common risk factors may show
gender-specific differentiation in resultant MDD and CD phenotypes. MDD/CD comorbidity in boys may be more strongly influenced by early CD leading to peer rejection and school impairment, which leads to subsequent MDD. Other work has found no gender differences in the strength of associations between MDD/CD comorbidity and adjustment. It is possible that etiologic profiles and developmental trajectories differ somewhat for girls and boys, even if resulting consequences from MDD/CD comorbidity are the same between genders. More studies allowing for better differentiation of findings by gender, such as the present study, are needed.

Research is currently moving toward a better understanding of specific environmental factors implicated in gender differences for MDD/CD comorbidity. For example, some research has found that family coercion processes increased risk for depression and antisocial behavior, but only in girls, with some divergent findings. In addition, parent–child conflict was higher in families who had an adolescent girl with CD than in those with an adolescent boy with CD, suggesting that female CD behaviors lead to increased environmental risk. In the context of the present study, this may suggest that shared environmental factors play a larger role in influencing MDD/CD comorbidity in girls, whereas genetic factors play a larger role in influencing MDD/CD comorbidity in boys. It is also possible that the presence of a gene-environment interplay in girls may be masking the magnitude of genetic influences. These findings highlight the importance of continued examination of gender differences.

Several limitations of this research should be discussed. The validity of twin studies rests on important tenets and assumptions of twin study methodology; a comprehensive overview of this methodology can be found elsewhere. Such assumptions include the equal environments assumption—the assumption that greater genetic similarity does not result in more similar environmental influences on the phenotype of interest. In addition, an important assumption of twin studies is that the empirical results from such research are generalizable to the broader population. Previous studies in this area, such as those using twins and nontwin siblings, broaden the present interpretation of these findings. In addition, the present work focused on additive genetic influences rather than interactive influences between genes and environment. Given the compelling previous work on developmental progressions of MDD and CD, which has often implicated dynamic relations between child and environmental variables, incorporating gene-by-environment interactions will be particularly important in future work in this area.

Another limitation of this work is the reliance on parent report and a structured interview format. Use of self-report data may partly explain the conflicting findings of shared environmental influences in genetically informative studies to date, because self-report data may result in lower estimates for shared environmental parameters. The addition of other informants and methods of data collection will be important in future work to determine the generalizability of these findings. However, it is important to note that these findings were largely consistent with previous studies using different informants and methods. In addition, lower prevalence rates of antisocial behavior in girls often result in decreased power to examine gender differences in epidemiologic samples. In the present study, decreased variability may have resulted in non-significant genetic influences on MDD/CD comorbidity for girls, particularly in light of the findings that a substantial portion of genetic influences on NE was common to MDD and CD in girls. Future work with more specialized samples, such as high-risk or clinical girls, may have better power to investigate etiologic pathways toward MDD/CD comorbidity in girls. Further, this study employed a large sample of twins across a wide age range. Although age was controlled in these analyses, future studies with more focal age groups should investigate potential moderation of these influences across development.

In conclusion, these data provide the first empirical support for a specific common causal pathway by which additive genetic factors influence MDD/CD comorbidity in boys, specifically through the dispositional trait of NE. Broad dimensions of individual difference characteristics are measurable in young life and show significant heritability. Thus, they represent an important psychological foundation against which later disorder develops. In the present study, such dispositions may also serve as a unifying feature by helping to organize seemingly disparate results and outcomes in a theoretical framework. Further, these results point to the important role of shared...
and nonshared environmental influences in contributing to MDD/CD comorbidity for males and females. Future work should continue to disentangle the role that environmental risk factors play in this comorbidity, keeping in mind that the same risk factor may have different effects across groups such as gender. As researchers continue to search for specific genes and environmental risk factors implicated in disorder development, research questions must be framed in broader conceptualizations of comorbidity that highlight common and unique factors.

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