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Alcohol Use Disorders and Depression: Protective Factors in the Development of Unique Versus Comorbid Outcomes

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Abstract

Protective factors for young adult alcohol use disorders, depression, and comorbid alcohol use disorders and depression were examined. Participants were recruited from all fifth-grade students attending 18 Seattle elementary schools. Of the 1,053 students eligible, 808 (77%) agreed to participate. Youth were surveyed when they were 10 years-old in 1985 and followed to age 21 years years in 1996 (95% retention). Protective factors were measured at age 14 years years. Young adult disorders were assessed with the Diagnostic Interview Schedule. Alcohol refusal skills, academic skills, school and family bonding, parental rewards, school rewards, and family cohesion at age 14 years years were associated with decreased risk for comorbidity at age 21 years years.

Keywords

Depression; Alcohol Use Disorders; Comorbidity; Adulthood; Adolescence

Alcohol use disorders and major depression are the two most common psychiatric disorders in the United States, with past-year prevalences of approximately 7% and 10%, respectively (Kessler et al., 1994). Both of these disorders are comorbid with other mental health disorders, particularly with each other (Ross & Germanson, 1988). Relative to those with singular diagnoses, individuals diagnosed with concurrent alcohol use disorders and major depression experience greater functional impairment and heightened risk of suicidality (Cornelius et al., 1995; Hanna & Grant, 1997). Despite high prevalence and implications of these comorbid disorders, little research has examined factors that may be protective against the development of alcohol use disorders and depression, as well as their comorbidity. Identifying protective factors will inform the development of preventive interventions that seek to reduce these disorders and their comorbidity.

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Theory provides a framework for organizing the predictors of adult alcohol use disorders and depression. Selection of predictors here was guided by the social development model (SDM) (Catalano & Hawkins, 1996; Hawkins & Weis, 1985), an integrated theory of behavior that combines key constructs from social control (Hirschi, 1969), social learning (Bandura, 1977), and differential association (Cressey, 1955) theories. The SDM hypothesizes that adolescents learn patterns of behavior from socializing units such as family, school, and peers. Socialization is proposed to follow the same processes of social learning, whether it produces prosocial or problem behavior, involving four constructs: 1) perceived *opportunities* for involvement in activities and interactions with others, 2) the degree of actual *involvement* and interaction, 3) the *skills* to participate in activities, and 4) the reinforcement or *rewards* individuals perceive to obtain from their involvements and interactions.

When socializing processes are consistent, a social *bond* develops between the individual and the socializing unit. Social bonds promote, in turn, the adoption of conventional or antisocial *beliefs* and values. It is hypothesized that an individual's behavior will be prosocial or antisocial depending on the predominant behaviors, norms, and values held by those to whom the individual is bonded. Thus, for example, bonds to prosocial socializing units are hypothesized to inhibit problem behaviors through an individual's "stake" in conforming to the norms and values of the socializing unit.

That is, consistent with SDM prosocial behaviors are hypothesized to be promoted, and antisocial behaviors inhibited, when youth experience opportunities for involvement with prosocial others. These two processes provide a context in which youths can experience rewards for prosocial behavior and develop bonds to prosocial others. Thus, the constructs of prosocial rewards and bonding are viewed as more proximal protective factors in relation to antisocial behavior. Another proximal protective factor is the extent to which adolescents have skills to resist antisocial influences and successfully engage in prosocial behaviors. Finally, the SDM highlights the proximal role of belief in the moral order in the development of prosocial behavior and the prevention of antisocial behavior.

Although the SDM was developed to understand deviant behavior, it can also be extended to depression. Kaslow, Brown, and Mee (1994) draw from behavior theory to illustrate a depression-conducive social process. They note that depressed individuals may have social skills deficits that limit the amount of positive reinforcement they receive from others. Poor social skills may also set into motion a "depressogenic" pattern of reinforcement, whereby depressed individuals are poor reinforcers of others, thus reducing their own rates of reciprocal reinforcement. The behavioral perspective of Kaslow, Brown, and Mee (1994) incorporates several proximal components of the SDM, including skills and reinforcement. For example, the SDM hypothesizes that prosocial skills and reinforcement, along with bonding to prosocial others, may counteract depression-conducive social processes, thereby inhibiting the development of depression. The purpose of this prospective longitudinal study is to examine SDM proximal predictors measured during adolescence as protective factors for singular and comorbid alcohol use disorders and depression measured during young adulthood. Thus, our analyses focus on the theoretical constructs of prosocial skills, rewards, bonds, and beliefs.

Prior research has identified a number of social developmental factors that *increase* risk for alcohol involvement among adolescents and young adults. These include poor family management and inconsistent discipline, association with drug-using peers, school failure, and neighborhood disorganization (Hawkins, Catalano, & Miller, 1992). However, studies addressing these relationships have used a variety of alcohol use outcomes (e.g., frequency, age of initiation), with relatively few applying diagnostic criteria for alcohol abuse or

dependence disorders. Moreover, few have highlighted the role of prosocial protective factors in social development.

Research also has identified social developmental constructs such as poor maternal care, lack of parent-child bonding, and corporal punishment that increase risk for depression among adolescents and young adults (Reinherz, Paradis, Stashwick, & Fitzmaurice, 2003; Straus & Kantor, 1994; Vogel, Stiles, & Nordahl, 1997; Zeng & Rinholm, 1989). However, protective factors have not been emphasized, and the effect of certain social developmental constructs, such as beliefs in the moral order, have not received much attention.

Little research has focused on how alcohol use disorders, depression, and both outcomes combined may be predicted by malleable social developmental factors experienced earlier in life (Robins & Price, 1991). Moreover, few investigators have attempted to disentangle factors that uniquely predict either alcohol use disorders or depression alone versus the comorbidity of both disorders.

Salloum and colleagues (1995) studied adult psychiatric patients diagnosed with depression, alcohol use disorders, or both. They discovered that individuals with comorbid disorders or alcohol use disorders were more likely to report histories of academic problems, as well as behavioral, discipline, and social problems, as compared with those with depression only.

These types of studies suggest unique etiological pathways for depression alone versus depression combined with alcohol disorders. However, these studies typically have relied on clinical samples of depressed individuals, and rarely include an “alcohol use disorder only” group. Moreover, clinical studies are likely to over-represent individuals with severe and comorbid disorders, and to suffer from problems of recall distortion associated with the use of retrospective methods. To address these shortcomings, prospective longitudinal research with general population samples is needed to understand the specific factors experienced in adolescence that decrease risk for depression, alcohol use disorders, or both during adulthood. Further, to isolate the effect of social developmental factors experienced during adolescence on later alcohol use disorders and depression, prior manifestations of these outcomes, such as early depressive symptoms and early alcohol and other drug problems, should be statistically controlled (Kessler & Magee, 1993).

Studies addressing comorbid versus singular alcohol use disorders and depression also should attend to the influence of gender. Research has indicated significant gender disparities in prevalence rates of both pure and comorbid manifestations of alcohol use disorders and depression. It is commonly reported that males experience higher rates of alcohol use disorders, whereas females experience higher rates of depressive disorders (Kessler et al., 1996). Gender differences also have been reported for comorbid outcomes. Kessler and his colleagues found that females were four times more likely than males to experience comorbid alcohol use disorders and depression (Kessler et al., 1997). Because predictors of singular versus comorbid outcomes may be different for males and females, we examined gender moderation in predictive analyses.

This study examines the effects of adolescent social developmental protective factors on alcohol use disorders only, on depression only, and on the co-occurrence of these outcomes at age 21 years. Prospective longitudinal data from a general population sample are used, with multiple social developmental factors assessed across domains at age 14 years and outcomes assessed at age 21 years. Prior alcohol and other drug problems and anxious/depressive symptoms at age 13 years are statistically controlled, and interactions with gender are examined for each predictor. In general, it is expected that the protective factors will be negatively related to the outcomes and that stronger relationships

will be observed for comorbid than singular disorders, due to the heightened level of psychopathology associated with comorbidity.

Method

Participants

Participants were 808 children and their caretakers (83% of whom were the child's mother) involved in the Seattle Social Development Project (SSDP) (Hawkins, Von Cleve, & Catalano, 1991; Peterson, Hawkins, Abbott, & Catalano, 1994). Participants were recruited in the fall of 1985 from all fifth-grade students attending 18 Seattle elementary schools serving high-crime neighborhoods ($N = 1,053$). From these 1,053 children, 808 (77%) children and their parents consented to take part in a longitudinal study. Of the 808 study participants, 51% ($n = 412$) were male, 46% ($n = 372$) were white, 24% ($n = 195$) were African American, 21% ($n = 170$) were Asian American, 6% ($n = 45$) were Native American, and the remaining 3% ($n = 26$) were of other ethnic backgrounds. Fifty-two percent ($n = 423$) of the participants were from low-income families as measured by participation in the federal free and reduced school lunch program in grades 5, 6, or 7.

This multi-ethnic urban panel was tracked and interviewed over an 11-year period through 1996, when respondents averaged 21 years of age. Data used in the present study were collected through surveys of the panel and their caretakers when youths were ages 13 and 14 years and from panel respondents at age 21 years years. Survey participation rates for the panel averaged over 94% of the original sample at each survey point during the last five waves of interviews. Respondents received monetary compensation for their participation. The Human Subjects Review Committee at the University of Washington approved the study and participants were treated in accordance with American Psychological Association guidelines (American Psychological Association, 2001). No adverse events were reported in the course of this study.

Measures

Alcohol use disorders—Past year DSM-IV (American Psychiatric Association, 1994) Alcohol Abuse and Dependence were assessed at age 21 years years using a modified version (McGee et al., 1990) of the Diagnostic Interview Schedule (DIS) (Robins, Helzer, Croughan, Williams, & Spitzer, 1981). These outcomes were then combined such that the “alcohol use disorders” outcome included individuals who met criteria for Alcohol Abuse and/or Alcohol Dependence.

Depression—Past year depression was assessed at age 21 years years using the DIS. Participants were classified as “depressed” if they met the following criteria: 1) depressed mood over the past year for at least 2 weeks and/or a lack of interest in previously pleasurable activities over the past year for at least 2 weeks; 2) a response of either “yes sometimes” or “yes definitely” (versus “no”) for having experienced at least four of the following seven symptoms nearly every day within the same 2-week period: changes in weight or appetite, sleep-related difficulties, moving or talking slowly, fatigue, guilt or worthlessness, poor concentration or indecision, and thoughts of suicide or death; and 3) the above problems interfered with daily life or everyday activities to at least a moderate degree.

Social developmental protective factors—Social developmental protective factors were measured at age 14 years years, which corresponds to Grade 8 for students progressing routinely through school. The survey at this age measured numerous predictors across multiple domains yet was early enough, developmentally, to allow identification of malleable predictors that could be targets for preventive intervention. Analyses included 10

indicators of four proximal social developmental protective processes. Specifically, there were four measures of prosocial skills. One item asked respondents about their skills to resist offers to participate in antisocial activity, whereas another asked about skills to refuse offers to drink alcohol. A scale assessing social skills was created from six items (e.g., “talks out of turn” and “showing off or clowning,” $\alpha = .91$) drawn from the Teacher Report Form of the Child Behavior Checklist (Achenbach, 1991). Finally, two items asked respondents about their grades in school as a measure of academic skills ($\alpha = .72$).

Three measures of prosocial rewards were included in the analyses. A scale assessing the extent to which parents reward good behavior in their children was created from three adolescent-report items (e.g., “My parents notice when I am doing a good job and let me know about it,” $\alpha = .63$). Four additional adolescent-report items were used to create a rewarding school experiences scale (e.g., “My teachers are fair in dealing with students,” $\alpha = .60$). Five items asked respondents to report the amount of cohesion within their family (e.g., “Our family gets along well with each other,” $\alpha = .73$).

Measures of “bonding” with school and family were included in the analyses. Eight self-report items were used to create a school bonding scale (e.g., “I like school” and “Most mornings I look forward to going to school,” $\alpha = .75$). Five self-report items were used to create a family bonding scale (e.g., “Do you share your thoughts and feelings with your mother,” $\alpha = .65$). Finally, a belief in the moral order scale was created from 11 self-report items that asked respondents to indicate how wrong they think it is to participate in antisocial activities, such as skipping school without an excuse and writing graffiti on a building ($\alpha = .85$).

Descriptive statistics of the SDM protective factors across gender groups were examined, and tests of mean differences were conducted. Of the 10 predictors considered, 4 showed statistically significant differences, indicating that females displayed higher levels of refusal skills, social skills, academic skills, and beliefs in the moral order than males (results are not presented in full to conserve space, but are available on request).

Control variables—Gender, alcohol and other drug problems, and anxious/depressive symptoms at age 13 years were included as control variables. Alcohol and other drug problems was a summed scale containing 13 self-report questions assessing whether or not various problems (e.g., trouble with police, health problems) had ever occurred due to alcohol, marijuana, or other drug use ($\alpha = .89$). Eighteen Teacher Report Form items (e.g., “unhappy, sad, depressed” and “worries;” $\alpha = .83$) were used to create the Anxious/Depressed scale of the Child Behavior Checklist (Achenbach, 1991).

Analysis

Analyses were conducted using multinomial logistic regression (two-tailed), which is an extension of logistic regression to the analysis of categorical dependent variables with more than two categories (Long, 1997). Prior to analysis, the value of the dependent variable representing “none” (for those not experiencing alcohol use disorders, depression, or concurrent alcohol use disorders and depression) was selected as the reference category to which the probability of membership in the remaining categories was compared. Because the purpose of this study was to isolate theoretically distinct but intercorrelated SDM predictors as potentially important protective factors, separate logistic regressions were run for each protective factor at age 14 years, controlling for gender, alcohol and other drug problems at age 13 years, and anxious/depressive symptoms at age 13 years. This approach is useful for identifying individual protective factors that can serve as promising targets of prevention efforts.

Each regression resulted in three odds ratios for the predictor: 1) the odds ratio for experiencing an alcohol use disorder only versus neither disorder; 2) the odds ratio for experiencing depression only versus neither; and 3) the odds ratio for experiencing comorbid alcohol use disorders and depression versus neither of these at age 21 years years. This is conceptually similar to conducting three separate logistic regressions for each predictor (i.e., comparing the reference category to each of the remaining categories). However, multinomial logistic regression conducts the regressions simultaneously and provides an estimate of overall fit for the model based on the likelihood ratio chi-square test. Logistic regressions that included a variable representing the interaction of gender with each predictor also were conducted. Listwise deletion of missing data was used in the analyses, resulting in an analysis sample of 754 cases.

Results

Prevalence of Disorders at Age 21 years Years

At age 21 years years, 19.1% ($n = 144$) of the sample met criteria for alcohol use disorders only, and 11.9% ($n = 90$) met criteria for depression only; 7.8% ($n = 59$) of the sample had both alcohol use disorders and depression. In contrast, 61.1% ($n = 461$) of the sample did not meet criteria for either alcohol use disorders or depression at age 21 years years. Alcohol disorders were more prevalent among males (26.9%, $n = 102$) than females (11.2%, $n = 42$) and comorbid disorders were somewhat more prevalent among males (9.0%, $n = 34$) than females (7.6%, $n = 25$). Depression was more prevalent among females (15.7%, $n = 59$) than males (8.2%, $n = 31$) and having no disorder was more prevalent among females (66.4%, $n = 249$) than males (55.9%, $n = 212$), $\chi^2(3) = 38.03$, $p < .001$.

Regression Outcomes

Table 1 presents adjusted odds ratios and 95% confidence intervals associated with each social developmental predictor at age 14 years years for each of the three outcomes: alcohol use disorders, depression, and comorbid alcohol use disorders and depression versus none of these outcomes at age 21 years, controlling for gender, alcohol and other drug problems, and anxious/depressive symptoms at age 13 years. Note that each multinomial logistic regression model displayed a statistically significant chi-square test, indicating a good fitting model with the presence of one or more significant predictors.

Predicting Alcohol Use Disorders Versus Neither Disorder

As shown in Table 1, gender significantly predicted alcohol use disorders versus neither disorder. Males were more than two-and-a-half times more likely than females ($OR = 2.75$) to experience alcohol use disorders as opposed to experiencing neither disorder. Alcohol and other drug problems at age 13 years did not predict alcohol use disorders without co-occurring depression at age 21 years. Ten social developmental protective factors were examined at age 14 years; of these, 2 significantly predicted alcohol use disorders without co-occurring depression. Those with alcohol-related refusal skills at age 14 years had a significantly lower probability of alcohol use disorders at age 21 years ($OR = .78$). Those with higher levels of belief in the moral order also had a lower risk of alcohol use disorders at age 21 years ($OR = .70$).

Predicting Depression Versus Neither Disorder

Neither alcohol and other drug problems at age 13 years nor gender significantly predicted depression versus neither disorder at age 21 years. Although teacher ratings of high internalizing problems at age 13 years increased risk for depression only, the relationship was not statistically significant due to a high degree of variability in this association. Two of

the 10 social developmental constructs measured at age 14 years significantly predicted depression at age 21. Specifically, both alcohol-related refusal skills and academic skills at age 14 years predicted less risk for depression at age 21 years ($ORs = .75$ and $.72$, respectively).

Predicting Comorbidity Versus Neither Disorder

Males were twice as likely ($OR = 2.04$) as females to experience co-occurring alcohol disorders and depression. Alcohol and other drug problems at age 13 predicted co-occurring alcohol use disorders and depression at age 21 years ($OR = 1.24$). Co-occurring alcohol use disorders and depression were predicted by seven social development protective factors. As with depression alone, both alcohol-related refusal skills and academic skills predicted a reduced likelihood of co-occurring problems at age 21 years ($ORs = .65$ and $.67$, respectively). Similarly, bonding with family ($OR = .48$) and school ($OR = .56$), rewarding school experiences ($OR = .43$), and parents rewarding good behavior ($OR = .62$) were significant protective factors against co-occurring problems at age 21. Finally, family cohesion ($OR = .49$) predicted a decreased risk of experiencing co-occurring problems at age 21.

Gender Differences Across Outcomes

We tested whether each predictor was associated with the outcomes differently for males and females, controlling for age 13 anxious/depressive symptoms and alcohol and other drug problems. No clear patterns emerged from these analyses. Only two predictors showed statistically significant gender interactions. For these, odds ratios were calculated separately for males and females. Unexpectedly, a higher level of antisocial activity-related refusal skills significantly predicted *greater* risk for experiencing alcohol use disorders as opposed to experiencing neither disorder at age 21 years for females ($OR = 1.54$) but not for males. Also, academic skills at age 14 years reduced the likelihood of experiencing co-occurring problems at age 21 years for males ($OR = .40$), but not for females.

Discussion

Overall, more social developmental protective factors predicted comorbid alcohol use disorders and depression at age 21 years than predicted either alcohol disorders alone or depression alone. It is possible that interventions seeking to change these protective factors could have their greatest preventive effects on the co-occurrence of comorbid alcohol use disorders and depression, a combination that produces a greater health burden than either disorder alone. However, it is also possible that additional background characteristics not considered in this study explain the associations. Additional research is needed to examine plausible alternative explanations of the findings.

Each of the social development model protective factors of skills, rewards, and bonds predicted comorbid alcohol use disorders and depression. Specifically, rewarding school experiences, parents rewarding good behavior, family cohesion, alcohol-related refusal skills, academic skills, and bonding to school and family at age 14 years each predicted a significantly reduced risk of experiencing comorbid outcomes at age 21. Preventive interventions that teach parents to reward their children's good behaviors, increase family cohesion, establish solid bonds with their children, and teach their children the skills to refuse alcohol may hold promise for reducing the probability that children will develop comorbid alcohol use disorders and depression during early adulthood. Enhancing academic skills and promoting bonding to school may also protect against comorbid alcohol use and depression disorders.

Significant age 14 years predictors of alcohol use disorders alone at age 21 years included alcohol refusal skills and beliefs in the moral order. This study also found a significant negative association between academic skills as well as alcohol-related refusal skills at age 14 years and adult depression. The protective effect of alcohol refusal skills in relation to depression is noteworthy, and illustrates the importance of examining a broad array of predictors for multifaceted disorders, such as depression. Teaching youth skills to refuse peer influences to use alcohol may protect against depression by facilitating commitments to prosocial peers and, perhaps, by breaking the hypothesized link between substance use and depression (Khantzian, 1985). Randomized prevention trials that manipulate alcohol refusal skills and examine effects on peer bonding, substance use, and depression are needed to better understand these associations.

Whereas the measure of alcohol refusal skills was a common predictor across outcomes, beliefs in the moral order showed specificity of prediction to alcohol use disorders only. Refusal skills may have broad protective effects across internalizing, as well as externalizing problems, whereas prosocial beliefs may not have protective effects for internalizing problems. Based on the results of the SDM, processes of prosocial socialization lead to the adoption of prosocial beliefs and values that, in turn, protect against involvement in rule violating behaviors, such as alcohol misuse. One implication may be that parent-training programs that promote the positive social development of children may have long-term protective effects on externalizing behaviors through the adoption of prosocial beliefs. Combined, these findings suggest that alcohol use disorders and depression have partly overlapping and partly unique etiologies. Interestingly, although gender differences in the rates of disorders were observed, there was little evidence that gender moderated the relationships between the predictors and the outcomes. Similar fundamental protective processes may operate for both young men and young women in relation to alcohol use disorders and depression.

Limitations

Results are based on data collected from a community sample of children from schools serving high-crime neighborhoods of Seattle, and findings may not generalize to other populations. Note, however, that at the time the longitudinal study began, the Seattle school district had instituted a policy of mandatory busing, which resulted in each school having a student body comprised of children from multiple neighborhoods. The reliability of certain measures was somewhat low, which could have contributed to bias in the estimated predictive associations. Also, in order to identify possible SDM predictors of alcohol use disorders alone, depression alone, and comorbid alcohol use disorders and depression, each predictor was included in its own regression equation, increasing the possibility of Type I errors. As noted above, the current analyses did not consider other factors, such as genetic characteristics or cultural identity, that may be important in the prediction of co-occurring alcohol use disorders and depression and might explain associations between the protective factors and the outcomes. Finally, variable coding and model parameterization in the multinomial logistic regression analyses allowed a comparison of each disorder category to the non-disorder category, as guided by the research questions at this stage of inquiry. Future studies comparing the comorbid category and the alcohol use disorders only category or the depression only category will be useful.

Clinical Implications

In summary, the current results suggest that teaching children the skills to succeed in school and to resist alcohol during adolescence may help to reduce later co-occurring alcohol use disorders and depression. These findings also suggest that efforts to increase bonding with school and family during adolescence hold promise for reducing co-occurring alcohol use

disorders and depression in early adulthood. Preventive interventions that strengthen these malleable protective factors could provide a cost-effective way to reduce the burden that accompanies comorbid alcohol use related problems and depression.

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Table 1

Odds ratios comparing specific and comorbid outcome groups with those with neither disorder at age 21 years.

Constructs	Alcohol Use Disorders Only (<i>n</i> = 144 versus 461)	Depression Only (<i>n</i> = 90 versus 461)	Alcohol Disorders and Depression (<i>n</i> = 59 versus 461)
Control Variables (adjusted for one another)			
Anxious/depressive symptoms	.36 (.09-1.39)	3.01 (.90-10.09)	.27 (.03-2.42)
Alcohol and other drug problems	1.03 (.91-1.18)	1.01 (.85-1.19)	1.24*** (1.09-1.40)
Gender	2.75*** (1.72-4.35)	.67 (.36-1.15)	2.04* (1.01-4.17)
Social Development Model Factors (adjusted for controls)			
Skills - refusal to antisocial activities	1.10 (.87-1.38)	1.10 (.82-1.47)	.76 (.54-1.08)
Skills - refusal to alcoholic beverages	.78* (.62-.98)	.75* (.57-.98)	.65* (.46-.92)
Skills - social	.91 (.69-1.21)	1.00 (.68-1.46)	.71 (.48-1.03)
Skills - academic (self-reports)	.98 (.76-1.28)	.72* (.52-.99)	.67* (.44-.99)
Rewards - parents reward good behavior	.88 (.64-1.20)	.79 (.56-1.12)	.62* (.41-.92)
Rewards - rewarding school experiences	1.19 (.84-1.68)	.76 (.50-1.15)	.43** (.26-.69)
Rewards - family cohesion	1.02 (.72-1.45)	.73 (.49-1.09)	.49** (.29-.80)
Bonding - to school	.73 (.50-1.06)	.82 (.52-1.32)	.56* (.32-.98)
Bonding - to family	.99 (.71-1.39)	.92 (.61-1.39)	.48** (.29-.80)
Beliefs - moral order	.70* (.49-.99)	.88 (.56-1.37)	.62 (.36-1.06)

Note. 95% confidence intervals are provided in parentheses. Separate regression equations were conducted for each SDM protective factor, controlling for gender and baseline measures of anxious/depressive symptoms, alcohol and other drug problems, and gender.

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$.