Genotypes Do Not Confer Risk For Delinquency ut Rather Alter Susceptibility to Positive and Negative Environmental Factors: Gene-Environment Interactions of BDNF Val66Met, 5-HTTLPR, and MAOA-uVNTR

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Abstract

Background: Previous evidence of gene-by-environment interactions associated with emotional and behavioral disorders is contradictory. Differences in findings may result from variation in valence and dose of the environmental factor, and/or failure to take account of gene-by-gene interactions. The present study investigated interactions between the brain-derived neurotrophic factor gene (BDNF Val66Met), the serotonin transporter gene-linked polymorphic region (5-HTTLPR), the monoamine oxidase A (MAOA-uVNTR) polymorphisms, family conflict, sexual abuse, the quality of the child-parent relationship, and teenage delinquency.

Methods: In 2006, as part of the Survey of Adolescent Life in Västmanland, Sweden, 1 337 high-school students, aged 17–18 years, anonymously completed questionnaires and provided saliva samples for DNA analyses.

Results: Teenage delinquency was associated with two-, three-, and four-way interactions of each of the genotypes and the three environmental factors. Significant four-way interactions were found for BDNF Val66Met x 5-HTTLPR x MAOA-uVNTR x family conflicts and for BDNF Val66Met x 5-HTTLPR x MAOA-uVNTR x sexual abuse. Further, the two genotype combinations that differed the most in expression levels (BDNF Val66Met Val, 5-HTTLPR LL, MAOA-uVNTR LL [girls] and L [boys] vs BDNF Val66Met Val/Met, 5-HTTLPR S/LS, MAOA-uVNTR S/SS/LS) in interaction with family conflict and sexual abuse were associated with the highest delinquency scores. The genetic variants previously shown to confer vulnerability for delinquency (BDNF Val66Met Val/Met x 5-HTTLPR S x MAOA-uVNTR S) were associated with the lowest delinquency scores in interaction with a positive child-parent relationship.

Conclusions: Functional variants of the MAOA-uVNTR, 5-HTTLPR, and BDNF Val66Met, either alone or in interaction with each other, may be best conceptualized as modifying sensitivity to environmental factors that confer either risk or protection for teenage delinquency.

Keywords: brain-derived neurotrophic factor, gene-environment interaction, juvenile delinquency, monoamine oxidase, serotonin plasma membrane transport proteins
Introduction

Teenage delinquency is a ubiquitous problem in industrial countries. Delinquency is positively associated with sexual abuse and family conflict (Widom, 1989), and negatively associated with parental warmth and support (Hoeve et al., 2008). While some evidence shows that specific genotypes, in interaction with childhood adversity, confer vulnerability for delinquency, several studies have failed to replicate these candidate gene-by-environment interactions (cG × E). The failure to replicate cG × E associations with behavioral disorders may be explained by the contribution of many genes with small effects (Duncan, 2013; Rutter et al., 2009), differences in the designs of studies, statistics, outcome variables, the investigated environmental factors and their dose and valence, and gene-by-gene interactions (G × G; Boyce and Ellis, 2005; Belsky and Pluess, 2009; Comasco et al., 2013; Goldman and Rosser, 2014). cG that interact with environmental events may not confer risk for behavioral disorders but rather increase sensitivity to the environment (Belsky and Pluess, 2009; Hankin, 2011). Investigations of cG × E have the potential to identify protective factors even among individuals exposed to adverse environments (Keller, 2014). Additionally, investigating interactions of multiple genes and environmental factors may provide further insight into the underlying neurobiological mechanisms. The present study aimed to replicate cG × E associated with delinquency, and investigate the interactions of two and three genotypes with negative and positive environmental factors. A general population sample of 1 337 adolescents was studied, which would detect moderate cG × E with 20% power. Three environmental factors were examined: family conflict, sexual abuse, and child-parent relationship. Each was indexed by a continuous variable to measure dosage.

Childhood maltreatment is a complex phenomenon. A recent review has suggested that the occurrence of abuse may be more important than the form, severity, or duration of the abuse. It is unclear why specific forms of abuse correlate more in some studies than in others (Briere and Jordan, 2009). However, it is clear that many child abuse victims experience more than one occurrence of abuse and different forms of abuse and that they are at greater risk of re-victimization in adolescence and adulthood. As there are cumulative effects of all of the different aspects of childhood trauma, it is difficult to ascertain the specific association between one form of childhood abuse and consequences in adulthood (Briere and Jordan, 2009). Thus, a measure of one form of childhood abuse likely indexes other forms of abuse, and thereby it is taken into account in statistical models.

Monoamine oxidase A (MAOA) is a key enzyme in the catabolism of monoamines, especially serotonin. Meta-analysis (Byrd and Manuck, 2013) indicates a robust association of the short and less-active allele (MAOA-uVNTR-S) with antisocial and criminal behavior among males exposed to adversity in childhood, signifying the need for broad-scale cG × E studies (Goldman and Rosser, 2014). Other studies identify associations between an interaction of the long, high-activity allele (MAOA-uVNTR-L) and childhood adversity and antisocial behavior among females (Sjoberg et al., 2007; Aslund et al., 2011), and aggressive behavior (Manuck et al., 2000; Beitchman et al., 2004) and violent crime (Tikkanen et al., 2009) among males. We reported that adolescent males carrying MAOA-uVNTR-S showed increased levels of delinquency when reared in adverse family environments and decreased levels of delinquency when reared in positive family environments (Nilsson et al., 2006; Oreland et al., 2007).

The brain-derived neurotrophic factor (BDNF) gene expresses BDNF in the brain. The BDNF Val66Met polymorphism is functional, with lower BDNF expression associated with the Met allele (Hong et al., 2011). BDNF modulates neuronal plasticity (Calabrese et al., 2009) and is implicated in the regulation of hypothalamic-pituitary-adrenal axis (HPA) response to stress (Wichers et al., 2008; Colzato et al., 2011), with carriers of the Met variant showing increased responsiveness. Some (Oades et al., 2008; Wagner et al., 2010), but not all (Tsai et al., 2005), studies report associations of BDNF Val66Met with aggression. A study of adolescents showed that carriers of BDNF Val66Met Met/Met who affiliated with aggressive peers displayed more aggressive behavior than did carriers of the Val/Val variant (Kretschmer et al., 2013).

Some studies (Retz et al., 2004; Cerra et al., 2005), but not all (Sakai et al., 2007), have reported an association between the short (S) less-active allele of the serotonin transporter, 5-HTTLPR, and aggression, impulsiveness, and conduct disorder. The interactions of 5-HTTLPR with delinquent peers (Vauhn et al., 2009) and family socioeconomic status (Aslund et al., 2013) are associated with antisocial phenotypes. In studies of three samples, among individuals homoygous for the S allele, exposure to negative parenting was associated with low positive affect, while exposure to positive parenting was associated with high positive affect (Hankin et al., 2011).

There is contradictory evidence of epistatic mechanisms being modified by environmental factors (Belsky and Pluess, 2009; Stoltenberg et al., 2012). In a study of male offenders, violence was associated with MAOA-uVNTR-S, whereas childhood adversity impacted later life violence only if 5-HTTLPR-S was present (Reif et al., 2007).

The present study examined associations of delinquency with: (1) the high and low activity alleles of MAOA-uVNTR, BDNF Val66Met, and 5-HTTLPR genotypes; (2) two-, three-, and four-way interactions of each genotype with family conflict and sexual abuse; and (3) two-, three-, and four-way interactions of each of these genotypes with the child-parent relationship, adjusting for family conflict and sexual abuse.

Method

Additional methodological information is presented in the Supplementary Material (S1).

Participants

The present study was part of the Survey of Adolescent Life in Västmanland, a medium-sized county in Sweden, undertaken in 2006. All students in the second year of high school (age 17–18 years) were asked to complete a questionnaire during class or, if absent, on the subsequent day and to provide a saliva sample for DNA extraction. Participation was anonymous and voluntary. A total of 2 263 students, 77.3% of the target population, completed the questionnaires, and 2 131 provided saliva samples. DNA was successfully extracted from 1 414 participants. The final sample included 661 boys and 676 girls (Aslund et al., 2009).

Genotyping

The MAOA-uVNTR polymorphism was analyzed as previously described (Nilsson et al., 2011). The genotypes were defined as...
either short (S), with 2 or 3 copies of MAOA-uVNTR; S (boys)/ SS (girls), long (L), with 3.5, 4, or 5 (Deckert et al., 1999) copies of MAOA-uVNTR L (boys)/LL (girls), or LS for girls if heterozygous for a long and a short allele. In the final analysis, there were no individuals with the rare 2-allele repeats, and only eight girls and five boys with the 5-repeat variant of MAOA-uVNTR, due to missing values for other variables (Supplementary Table S1). The BDNF Val66Met polymorphism, which consists of a single-nucleotide polymorphism G/A substitution at codon 66 (Val66Met), was analyzed as described previously, Val/Val versus Val/Met or Met/Met (Comasco et al., 2013). The Met allele is less active than the Val allele. The soluble carrier family 6 (SLC6A4, neurotransmitter transporter), serotonin transporter-linked promoter region (5-HTTLPR) polymorphism, which consists of an insertion/deletion that creates a short 14 repeat (S) or a long 16 repeat (L) allele, was analyzed as described previously (Aslund et al., 2009). Both the BDNF Val66Met and 5-HTTLPR genes were in Hardy-Weinberg equilibrium (p = 0.113 and p = 0.209 respectively), as was the MAOA-uVNTR gene among girls (p = 0.886).

Environmental Factors

Six questions assessed the adolescent’s perception of his/her family. Scores for responses were summed to create a family-conflict score that ranged from 0 to 6. Sexual abuse was assessed by three items. Scores were summed to create a sexual-abuse score that ranged from 0 to 9 (Nilsson et al., 2011). The participant’s perception of his/her relationship with his/her parents was assessed by four items. Answers were summed to create a child-parent relationship score that ranged from 4 (a poor relationship) to 90 (Aslund et al., 2011).

Delinquency

Delinquency was measured by 14 questions. Responses were summed to create a total delinquency score that ranged from 0 to 90 (Aslund et al., 2011).

Alcohol Consumption, Symptoms of Depression, and Attention Deficit Hyperactivity Disorder

Swedish adolescent versions of self-report instruments were used to assess alcohol consumption (AUDIT-C, Nilsson et al., 2011); depression (Depression Self-Rating Scale [DSRS]; Aslund et al., 2009); and attention deficit hyperactivity disorder (ADHD; ADHD Self-Report Scale [ASRS]; Kessler et al., 2005; Sonnby et al., 2010).

Ethics

The study was approved by the Regional Ethical Review Board of Uppsala University, Dr.nr 2005:375. Oral informed consent was obtained from the participants.

Statistical Analysis

Univariate comparisons were computed using chi-squared tests, t-tests, and analyses of variance. Main and interaction effects of genetic and environmental factors were analyzed by general linear models (GLMs). GLMs were validated using Poisson log-linear models. Only significant main and interaction terms are included and reported for the final models. A p-value < 0.05 was considered significant for main effects, and p < 0.10 was considered significant for interaction effects (Fleiss, 1986).

A model-dependent realistic analysis (Hawking and Mlodinow, 2010) was performed, taking possible genetic plasticity and susceptibility into account. Analyses were based on the assumption that associations of a genotype with the outcome would change when another related genotype or a positive or negative environmental factor was entered into the statistical model.

Results

Table 1 presents characteristics of the sample. Greater proportions of girls than boys reported a conflicted family environment, having experienced sexual abuse, and a good relationship with their parents. Sex was not associated with genotype, with the exception of MAOA-uVNTR. Scores for delinquency were significantly higher among boys than among girls, as was the proportion of individuals reporting any delinquency. There were no differences in scores for family conflict, sexual abuse, and child-parent relationship by genotype, nor any differences in delinquency by genotype. Participants from families with conflict (t = -8.434, p < 0.001) and those who had experienced sexual abuse (t = -6.796, p < 0.001) reported higher delinquency scores, whereas participants with a positive child-parent relationship reported lower delinquency scores (t = -6.688, p < 0.001). There was a strong positive association between family conflict and sexual abuse (boys: γ = 0.426, p < 0.001; girls: γ = 0.440, p < 0.001), a strong negative association between family conflict and child-parent relationship (boys: γ = -0.437, p < 0.001; girls: γ = -0.540, p < 0.001), and a weaker negative association between child-parent relationship and sexual abuse (boys: γ = -0.202, p = 0.054; girls: γ = -0.334, p < 0.001). In univariate analyses, neither the MAOA-uVNTR (p = 0.083), 5-HTTLPR (p = 0.525), nor BDNF Val66Met (p = 0.628) genotype was associated with delinquency (not shown in tables).

The results of the multivariate GLM models are presented in Table 2. The observed power in the final model, after the removal of all nonsignificant interaction effects, was between 0.509 and 1.0 for all interaction effects. Scores for both family conflict and sexual abuse were more strongly associated with delinquency among boys than among girls. The interactions between BDNF Val66Met and family conflict and sexual abuse were both significantly associated with delinquency, as were the interactions of 5-HTTLPR and MAOA-uVNTR with family conflict. Furthermore, delinquency was significantly associated with three-way interactions of BDNF Val66Met × 5-HTTLPR × family conflict and BDNF Val66Met × 5-HTTLPR × sexual abuse, as were the interactions of BDNF Val66Met × MAOA-uVNTR × family conflict and BDNF Val66Met × MAOA-uVNTR × sexual abuse. The interaction of 5-HTTLPR × MAOA-uVNTR × family conflict was also significantly associated with delinquency. Finally, four-way interactions (BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × family conflict and BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × sexual abuse) were significantly associated with delinquency.

An illustration of the model adjusted for the main and interaction effects is not possible to draw in two dimensions. Instead, Figure 1 shows that in carriers of all combinations of genotypes (range: n = 63–358), delinquency scores varied as a function of family conflict, sexual abuse, and child-parent relationship (t = 1.0 for all interaction effects). Scores for both family conflict and sexual abuse were more strongly associated with delinquency among boys than among girls. The interactions between BDNF Val66Met and family conflict and sexual abuse were both significantly associated with delinquency, as were the interactions of 5-HTTLPR and MAOA-uVNTR with family conflict. Furthermore, delinquency was significantly associated with three-way interactions of BDNF Val66Met × 5-HTTLPR × family conflict and BDNF Val66Met × 5-HTTLPR × sexual abuse, as were the interactions of BDNF Val66Met × MAOA-uVNTR × family conflict and BDNF Val66Met × MAOA-uVNTR × sexual abuse. The interaction of 5-HTTLPR × MAOA-uVNTR × family conflict was also significantly associated with delinquency. Finally, four-way interactions (BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × family conflict and BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × sexual abuse) were significantly associated with delinquency.
The results are presented in Table 2. Generally, the results were similar to those of the GLM, although in the Poisson log-linear model the associations of delinquency with the interaction terms of sex and the two environmental factors were not significant, whereas the sex × genotype interactions were significant for all three genotypes. Notably, according to the $\chi^2$ and $p$-values, there was a strong sex difference in the effect of MAOA-uVNTR, and substantially weaker sex differences for BDNF Val66Met, 5-HTTLPR, and a weak association was detected for the interaction of BDNF Val66Met and 5-HTTLPR, and in interaction with BDNF Val66Met, 5-HTTLPR, and MAOA-uVNTR.

Although the analyses presented in Figure 1 suggested that the absence of family conflict and the absence of sexual abuse were associated with low levels of delinquency, regardless of the combinations of genotypes, the quality of the child-parent relationship was entered into the final model to test for susceptibility to environmental influence. The results are presented in Table 3. In the GLM, the child–parent relationship interacted with BDNF Val66Met and with BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR after adjusting for family conflict and sexual abuse. The observed power of the interaction was 0.998. Notably, according to the F-values, entering the child-parent relationship into the model strengthened most of the other interaction terms with family conflict and sexual abuse. The results of the Poisson log-linear model indicated that the child-parent relationship interacted with all tested combinations of two genotypes. The results are illustrated in Figure 2. As predicted by the differential susceptibility hypothesis, carriers of the genotype combination that would be expected to have the highest risk for delinquency in an adverse environment according to the sum of the sensitivity alleles (BDNF Val66Met Val/Met, 5-HTTLPR S, and MAOA-uVNTR S) showed the lowest delinquency scores if they reported a positive relationship with their parents.

The GLM and Poisson models were re-run, omitting the eight females and five males with the 5-repeat variant of the MAOA-uVNTR, and similar results were obtained. Models were adjusted for self-reported ethnicity, symptoms of ADHD, depression, co-occurring ADHD and depression, and alcohol use. Ethnicity was unrelated to delinquency. Symptoms of ADHD and depression and alcohol use were each related to delinquency and increased the explained variance from 29.6% to 42.4%. However, these factors did not modify the effect of BDNF Val66Met Val/Met, 5-HTTLPR S, and MAOA-uVNTR S nor the interactions of these genes with the negative and positive environmental factors associated with delinquency (Supplementary Table S2). In an adjustment for multiple comparisons the corrected $p$-value was 0.0015, indicating that 21 of the 25 statistically significant comparisons remained significant in the final Poisson model testing for susceptibility (Table 3).

Analyses were re-run separately among boys and girls and the results are presented in Supplementary Table S3. Among boys, in all cG × E, cG × cG × E, and cG × cG × cG × E models, the interaction terms with family conflict were significantly associated with delinquency. By contrast, among girls family conflict was only associated with delinquency in interaction with 5-HTTLPR and in interaction with BDNF Val66Met, 5-HTTLPR, and MAOA-uVNTR. Unlike boys, among girls, cG × E and cG × cG × E of BDNF Val66Met and MAOA-uVNTR with sexual abuse and child-parent relationship were associated with delinquency, and a weak association was detected for the interaction of 5-HTTLPR, MAOA-uVNTR, and sexual abuse.

**Discussion**

This is the first study to investigate the interactions of variants of BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR and both environmental risk and protective factors in relation to delinquency. Each of the three genotypes and the three environmental factors were associated with delinquency, as was male sex. Three two-way cG × E were significantly associated with delinquency: BDNF Val66Met × family conflict, 5HTTLPR × family conflict, and MAOA-uVNTR × sexual abuse. Five three-way interactions...
were significantly associated with delinquency: BDNF Val66Met × 5-HTTLPR × family conflict, BDNF Val66Met × MAOA-uVNTR × sexual abuse, 5-HTTLPR × MAOA-uVNTR × family conflict, and 5-HTTLPR × MAOA-uVNTR × sexual abuse. Two four-way interactions were significantly associated with delinquency: BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × family conflict and BDNF Val66Met × 5-HTTLPR × MAOA-uVNTR × sexual abuse. The low-activity allele
of the MAOA-uVNTR gene was associated with delinquency both directly and in interaction with sexual abuse. Furthermore, as predicted by the genetic susceptibility hypothesis (Belsky et al., 2009), the BDNF Val66Met Val/met, 5-HTTLPR S/SS/LS × child-parent relationship interaction was protective against teenage delinquency. If these results are replicated, they suggest that promoting positive child-parent relationships, even among children experiencing adversity, would reduce the risk of teenage delinquency.

Poisson models detected gene by sex interactions for MAOA-uVNTR, BDNF Val66Met Val/Met, and 5-HTTLPR associated with delinquency, with the strongest gene by sex effect for MAOA-uVNTR. We, and others, have previously reported sex differences detected by analyses conducted separately within sex (Sjoberg et al., 2006; Aslund et al., 2009, 2011; Nilsson et al., 2011). However, such analyses only show different directions of the findings in relation to sex, but do not allow for testing whether those differences in direction are statistically significant. In order to analyze sex differences statistically, both the main and interaction terms of sex and the genes of interest must be included in the same model. However, after finding that the sex interaction term was significant, we re-ran analyses separately by sex. These results showed that the interactions of family conflict and genotypes were significantly associated with delinquency among boys, while among girls interactions of the genotypes with sexual abuse and, to some extent, the child-parent relationship, were associated with delinquency.

None of the genotypes showed significant associations with delinquency in univariate analyses, whereas most interaction terms, as well as several gene main effects, were significant in the multivariate models and validated by complementary statistical analyses. Taken together, these results imply a robust deviation from chance findings. The figures serve as illustrations and should be interpreted with caution, as it is not possible to...
construct a two-dimensional figure that describes the complexity of the interaction effects. Figure 1 illustrates an environmental risk model and Figure 2 illustrates an environmental protective model, not adjusted for each other and not statistically investigated. Moreover, the subgroups presented in the figures are not similar to the analyses in the statistical models since the statistical analyses of the environmental factors are based on scaled variables. However, it is important to note the differences in direction of the effect on delinquency depending on the negative and positive environmental factors. These directional differences indicate susceptibility properties of the investigated genotypes.

We used a model-dependent realistic analysis (Hawking and Mlodinow, 2010) and investigated cG × cG and cG × E in relation to delinquency, and whether the directions of cG × cG varied depending on the different environmental exposures (Comasco et al., 2013; Reiss et al., 2013). Since there is neither any knowledge about the phenotypic effects of various cG interactions, nor with combinations of cG × cG and cG × E, a directional hypothesis-driven method could not be used. This approach differs from belief-dependent realism that relies on reference anchors or small pieces of information, and emphasizes seeking and confirming evidence supporting existing beliefs (Shermer, 2011). When the genetic and environmental factors are truly interacting, a statistical model will constantly change depending on which factors and interaction terms are included in the analysis. Therefore, it is inappropriate to assume an additive genetic risk model of three susceptibility genes by using a genetic risk score if the cG × cG is unknown. For instance, although previous research has shown that an individual carrying a short 5-HTTLPR genotype is at greater risk for a negative outcome and other research has shown that male carriers of the short MAOA-uVNTR variant exposed to a specific negative environmental factor are also at greater risk for the same negative outcome, this does not mean that carriers of both genotypes are at increased risk, as there is no evidence that the combination of genotypes is additive (Goldman and Rosser, 2014). Rather, interactions might be subadditive (i.e. 2 + 2 = 3) or superadditive (i.e. 2 + 2 = 10; Goldman and Rosser, 2014). In the present study, as expected (Briere and Jordan, 2009), there was a medium to strong association between the environmental factors, which probably affected the cG × E, cG × cG × E, and cG × cG × cG × E. For instance, in the model that included cG × E without adjusting for the cG × cG, none of the cG × E was significant, whereas the models that included adjustment for most of the possible interactions produced significant results. It is important to note that the final interaction term in the model sets the effect of all lower-order interactions. Therefore, some of the two- or three-way interactions will vary depending on the effect of the four-way interaction. Studies of other samples presenting different genetic profiles and different proportions of participants exposed to each environmental factor will detect slightly different associations with the outcome. Therefore, studies of samples with varying genetic profiles and exposures to environmental variables applying multiple models are needed for further understanding of how genes interact with the environment to modify behavior.

The present study, in line with previous findings (Stoltenberg et al., 2012), suggests a non-additive model for cG × E associated with teenage delinquency. Previous studies suggest that the BDNF Val66Met Val/Met allele (Kretschmer et al., 2013), the short and less-active (S) allele of 5-HTTLPR (Retz et al., 2004; Gerra et al., 2005), and the short and less-active MAOA-uVNTR (S) allele (Byrd and Manuck, 2011) are related to delinquency, either directly or in interaction with adversity. However, the present study showed that these genotypes confer susceptibility to the environment, thereby providing either vulnerability or protection against delinquency, depending on the negative and positive environmental influences (Hankin et al., 2011). If these results are replicated, future studies will be needed to determine the neurobiological mechanisms underlying these effects. Males carrying MAOA-uVNTR-S, relative to those with MAOA-uVNTR-L, show reductions in gray matter in the limbic and orbital frontal regions, hyper-responsiveness of the amygdala and hippocampus during aversive recall, and impaired cingulate activation during cognitive inhibition (Meyer-Lindenberg et al., 2006). These neural correlates of the MAOA-uVNTR-S genotype in males occur in regions with the highest density of serotonin receptors. 5-HTTLPR S (Canli et al., 2005; Pezawas et al., 2005) has been associated with limbic structures and functions similar to MAOA-uVNTR-S, specifically...
increased amygdala activation and relatively decreased responsiveness in the cingulate circuitry that regulates the amygdala. Impulsive aggression has been associated with lower serotonergic innervation in the anterior cingulate cortex (Frankle et al., 2005). There are strong functional links between serotonin and BDNF. BDNF moderates HPA activity (Gatt et al., 2009; Shalev et al., 2009), and human (Stoltenberg et al., 2012) and rodent (Ren-Patterson et al., 2005) studies suggest that the low-expressing BDNF Val66Met and 5-HTTLPR genotypes may potentiate each other. Furthering our understanding of the mechanisms by which positive relationships offer protection against teenage delinquency is important and urgent.

The present results have four important implications for the generalizability of most previously-reported cG × E findings. (1) Positive environmental factors are not equal to the absence of adversity. (2) Previous findings may be inconsistent because analyses did not adjust for positive environmental factors. (3) The results of studies of large community samples, which include individuals characterized by multiple risk and protective factors of varying intensity, would be expected to differ from results of studies of high-risk samples exposed to more adverse and fewer protective factors. Consequently, future cG × E studies and meta-analyses of the results of these studies would increase in validity by taking account of both adverse and positive environmental factors. (4) Composite measures of “cumulative genetic plasticity” (a sum of the number of plasticity genotypes; Vaughn et al., 2009) will fail to capture cG × cG and cG × cG × cG × E. The results of the present study suggest that the debate about the validity of cG × E (Duncan and Keller, 2011) and the preferred strategy of genome-wide association studies, rather than studies of candidate genes, merits nuance, as these designs are complementary. Until the mechanisms involved in genetic-phenotypic associations are better understood, studies are needed using various populations with various environmental exposures to provide knowledge about the susceptibility conferred by genes and to prevent the rejection of cG × E interaction models.

Limitations and Strengths

The final sample included only 59% of the target population, mainly due to problems in collecting and extracting DNA. Since participation was anonymous, there was no possibility to obtain more saliva from the individuals with failing DNA analyses. The tri-allelic genotype of 5-HTTLPR and rs2553 was not isolated (Hu et al., 2006). However, the Lg genotype is present among only ~0.9% of the population (Wray et al., 2009), and findings of the effect of the Lg allele on 5-HTT mRNA transcription are contradictory (Philibert et al., 2008). Thus, not measuring this variant probably did not affect the results. In fact, since those individuals would have been re-classified to the susceptibility group, this would have even strengthened the results. Furthermore, models were adjusted for symptoms of ADHD, depression, and alcohol consumption, each of which were related to delinquency and increased the explained variance. However, these variables did not affect the cG × cG × cG × E. We interpret this finding as a further indication of the robustness of the results of the present study. Unfortunately, no data were available on use of medications that could, potentially, affect the results. However, in Sweden at the time the data were collected (2006), very few adolescents age 15–19 years were prescribed medications (i.e. selective serotonin re-uptake inhibitors: boys, 0.15%; girls, 0.34%; methylphenidate: boys, 0.13%; girls, 0.043%; Socialstyrelsen, 2014) indicating that medication influences in the present sample were negligible. Additional limitations of the present study include the combination of BDNF Val66Met Val/Met with Met/Met, 5-HTTLPR SS and LS, and MAOA-uVNTR S (boys), SS, and LS. If there is a true difference between the merged genotypes, the results might have been stronger.

The delinquency variable was skewed such that transformations failed to correct the distribution. Consequently, cG × E was interpreted cautiously, calculations of observed power were computed, and data were additionally analyzed using Poisson log-linear regressions, which are suitable for non-normally distributed ordinal variables. The observed power was generally high, and the Poisson log-linear models yielded results that were similar to those of the GLMs. The sample used was relatively large, included as many girls as boys who were likely post-pubertal (Goldman and Rosser, 2014), included a majority of Caucasians, and was recruited in a county with income, education, and employment levels that are similar to those of Sweden as a whole (SCB, 2009). Environmental factors were indexed by self-reports, the prevalence of adversity was low, and continuous variables were used to capture dosage (Österman et al., 1998). Self-reports of delinquency are recommended, as they have been found to be reliable and valid (Smith and Thornberry, 1995; Tikkanen et al., 2009). The current prevalence of delinquency was similar to that of previous reports in Sweden (Svensson and Ring, 2007).

Finally, despite the use of complementary statistical methods and high statistical power, corrections for multiple comparisons indicated that 21 of the 25 statistically significant comparisons remained significant in the final Poisson model testing for susceptibility. However, it is also important to recognize that Type II errors that exclude true differences due to overly strict corrections are as important to avoid as Type I errors (Perneger, 1998).

Conclusions

Teenage delinquency was associated with interactions between MAOA-uVNTR, 5-HTTLPR, and BDNF Val66Met genotypes and family conflict, sexual abuse, and child-parent relationships. Importantly, a positive child-parent relationship conferred protection against delinquency, even after taking family conflict and sexual abuse into account. Future investigations should measure cG × cG × cG × E while considering the range of positive and negative environmental factors to which the participants have been exposed.

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Statement of Interest

All authors declare no biomedical financial interests or potential conflicts of interest.
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