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Interactions between Early Parenting and a Polymorphism of the Child's Dopamine Transporter Gene in Predicting Future Child Conduct Disorder Symptoms

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Abstract

Mounting evidence suggests that genetic risks for mental disorders often interact with the social environment, but most studies still ignore environmental moderation of genetic influences. We tested interactions between maternal parenting and the variable number tandem repeat (VNTR) polymorphism in the 3′ untranslated region (UTR) of the dopamine transporter gene in the child to increase understanding of gene-environment interactions involving early parenting. Participants were part of a 9-year longitudinal study of 4–6-year-old children who met criteria for attention-deficit/hyperactivity disorder (ADHD) and demographically matched controls. Maternal parenting was observed during standard mother-child interactions in wave 1. The child's conduct disorder (CD) symptoms 5–8 years later were measured using separate structured diagnostic interviews of the mother and youth. Controlling for ADHD symptoms and child disruptive behavior during the mother-child interaction, there was a significant inverse relation between levels of both positive and negative parenting at 4–6 years and the number of later CD symptoms, but primarily among children with two copies of the 9-repeat allele of the VNTR. The significant interaction with negative parenting was replicated in parent and youth reports of CD symptoms separately.

Keywords

gene-environment interaction; dopamine transporter gene; maternal parenting; conduct disorder

It is likely that genetic vulnerabilities are necessary but insufficient for the development of mental disorders (Caspi & Moffitt, 2006; Reiss & Neiderhiser, 2000). Rather, it is probable that the association of genetic polymorphisms with mental disorders is usually conditional upon particular experiences (Meaney & Szyf, 2005; Neiderhiser, 2001). Nonetheless, most molecular genetic studies continue to ignore the potential moderating role of the environment.

The importance of studying gene-environment interaction (GxE) in the mental health field has been clearly articulated and a number of studies have been carried out (Caspi & Moffitt, 2006; Rutter, Moffitt, & Caspi, 2006). To add to our understanding of gene-environment interactions that may be involved in CD, the present study tested potential interactions between parenting in early childhood and a well-characterized polymorphism in predicting CD symptoms that has not heretofore been tested for such interactions, the VNTR polymorphism in the 3′ UTR of the dopamine transporter gene (DAT1) in child.

Rigorous standards for conducting tests of GxE using candidate genes have emerged in recent years (Hunter, 2005; Mill & Petronis, 2007; Moffitt, Caspi, & Rutter, 2005):

1. Biological plausibility

In studies of GxE, candidate genes, environments, and phenotypes should be selected on the basis of a plausible biological model of environmental moderation of gene expression. The dopamine transporter is a solute carrier protein on presynaptic neurons that plays a key role in the regulation of dopaminergic neurotransmission by removing dopamine from the synapse after release (Bertolino et al., 2006). Because the 3′ UTR VNTR polymorphism is not in a coding region of DAT1, it does not regulate the transcription of DNA into messenger RNA (mRNA). Rather, polymorphisms in the 3′ UTR influence the export of mRNA from the cell nucleus, the stability of mRNA, and the translation of mRNA into amino acids and ultimately proteins (Conne, Stutz, & Vassalli, 2000).

Although much remains to be learned, a great deal has been learned in the last 5 years about the potential biological functionality of the DAT1 VNTR. *In vitro* studies of the expression of the DAT1 VNTR using reporter gene assays and transient transfection in mostly non-human mammalian cell lines have not been helpful, however (Fuke et al., 2001; Michelhaugh, Fiskerstrand, Lovejoy, Bannon, & Quinn, 2001; Mill, Asherson, Browes, D'Souza, & Craig, 2002; Mill, Asherson, Craig, & D'Souza, 2005; VanNess, Owens, & Kilts, 2005). These studies mostly found significant functional differences among the DAT1 genotypes, but in inconsistently varying directions. Brookes et al. (2007) argued that these studies are inconclusive because most of the cell lines did not normally express dopamine and the various clonings were not analogous to the location of the 3′ polymorphism *in vivo*.

The only published *ex vivo* study failed to find significant differences associated with the DAT1 VNTR in dopamine transporter availability in postmortem midbrain tissue from deceased older adults (Brookes et al., 2007). Because the availability of the dopamine transporter protein in the striatum declines steadily with age (van Dyck et al., 2005), however, differences in the expression of the dopamine transporter may be difficult to detect in older adults.

In contrast, three *in vivo* studies of healthy adults using single photon emission computed tomography (SPECT) found consistent evidence of significantly more striatal DAT protein in persons with at least one 9-repeat allele of the DAT1 VNTR (Jacobsen et al., 2000; van de Giessen et al., 2009; van Dyck et al., 2005). A study of 14 abstinent alcoholics and 11 controls reported the opposite result (Heinz et al., 2000), but this could reflect changes in

striatal DAT protein associated with long-term alcohol use or rebound during abstinence (Heinz & Goldman, 2000).

The strongest biological evidence regarding the DAT1 VNTR comes from *in vivo* studies using functional magnetic resonance imaging (fMRI) of differences among genotypes in blood-oxygen-level dependent (BOLD) activation of dopamine-rich fronto-striatal areas of the brain involved in mood, reward, and executive functions. Six studies consistently found greater BOLD activation in the striatum and/or basal ganglia during reward-based, go/no go inhibition, or working memory tasks in participants with the at least one 9-repeat allele of the DAT1 VNTR (Bertolino et al., 2009; Congdon, Constable, Lesch, & Canli, 2009; Dreher, Kohn, Kolachana, Weinberger, & Berman, 2009; Durston et al., 2008; Forbes et al., 2009; Schott et al., 2006). Similarly, four studies consistently found greater activation in regions of the frontal cortex during working memory and cognitive interference tasks in participants with at least one 9-repeat allele (Bertolino et al., 2006; Bertolino et al., 2008; Bertolino et al., 2009; Brown et al., 2010). A fifth study found a nonsignificant trend in the same direction (Caldu et al., 2007).

Neither the SPECT nor fMRI studies provide direct evidence on the biological functionality of the DAT1 VNTR genotype, however, as the significant differences could be due to another polymorphism in strong linkage disequilibrium with the DAT1 VNTR. Nonetheless, these studies suggest that persons with at least one 9-repeat allele have a greater density of the dopamine transporter protein and are more reactive in fronto-striatal networks during tasks activating reward and executive control processes. Unfortunately, the *in vivo* studies cited above did not clearly distinguish between the 9/9 and 9/10 3′ DAT1 VNTR genotypes. As a result, it is not possible to determine if the significant results reflect additive or nonadditive genetic models.

A meta-analytic review found that children with at least one copy of the 10-repeat allele of the DAT1 3′ VNTR exhibit higher levels of ADHD symptoms than children with no 10-repeat copies, but the effect was quite small (Gizer, Ficks, & Waldman, 2009). CD is another plausible behavioral target for studies of the DAT1 3′ VNTR because it is substantially heritable (Rhee & Waldman, 2002) and there is extensive evidence that the dopaminergic system plays a key role in aggression (de Almeida, Ferrari, Parmigiani, & Miczek, 2005; Laakso et al., 2003), reward (Schultz, 2010), and executive processes (Bertolino et al., 2008; Bertolino et al., 2009). The few previous studies of the association between the 3′ DAT1 VNTR and CD have yielded inconsistent results, however, with three studies not finding a significant main-effect association (Burt & Mikolaiewski, 2008; Schulz-Heik et al., 2008; Sonuga-Barke et al., 2009), one study finding lower levels of antisocial behavior in youth with the 9/9 genotype (Guo, Roettger, & Shih, 2007), and one study finding higher levels of antisocial behavior in persons with the 9/9 genotype (Gerra et al., 2005).

Notably, however, a recent cross-sectional study of 708 European/Caucasian males between the ages of 5 and 17 with ADHD reported an interaction between maternal expressed positive emotion and the 9-repeat allele of the DAT1 3′ VNTR in predicting adult ratings of child conduct problems (Sonuga-Barke et al., 2009). Specifically, greater positive expressed emotion was more strongly associated (inversely) with child conduct problems among children with at least one 9-repeat allele. This suggests the important hypothesis that genotypes of the DAT1 3′ VNTR have not been found to be consistently associated with CD in previous studies because the association is moderated by parenting. That is, the association between DAT1 and CD may have been obscured when important aspects of the environment were not considered.

No other studies of GxE involving parenting and DAT1 have been reported, but an interaction was found between alleles of the dopamine D4 receptor gene (DRD4) and maternal insensitivity toward infants in predicting externalizing behavior at 39 months (Bakermans-Kranenburg & van IJzendoorn, 2006). Similarly, an interaction was found between alleles of DRD4 and maternal parenting quality in predicting maternal ratings of temperamental sensation seeking in toddlers (Sheese, Voelker, Rothbart, & Posner, 2007). These studies also suggest that relations between genetic variants that influence dopaminergic functioning and maladapative child behavior are moderated by maternal parenting.

Much remains to be learned about environmental influences on the expression of the 3′ DAT1 VNTR, but some of the transcription factors are known (Wang, Michelhaugh, & Bannon, 2007) and one study of rats found that maternal deprivation during early rearing significantly reduced expression of the dopamine transporter protein (Zhu et al., 2010). This finding is consistent with robust evidence that variations in maternal parenting can robustly influence the expression of many genes in non-human animals (Meaney & Szyf, 2005; Szyf, McGowan, & Meaney, 2008).

In addition, significant interactions in the cross-sectional association of parenting and latent genetic influences estimated from human twin and sibling correlations (i.e., not from genotypes measured at the molecular level) with adolescent antisocial behavior suggest that as negative parenting increases the proportion of variance in the phenotype attributed to genetic factors decreases (Feinberg, Button, Neiderhiser, Reiss, & Hetherington, 2007). In addition, there is replicated evidence of interactions between retrospectively reported harsh parenting and other genetic polymorphisms, including a long-short promoter variant of the serotonin transporter gene (5HTTLPR), in predicting depression (Caspi et al., 2003; Uher & McGuffin, 2008) and a polymorphism of the monoamine oxidase-A (MAO-A) gene in predicting aggression (Caspi et al., 2002; Kim-Cohen et al., 2006). The latter is potentially relevant to the present study because MAO-A regulates the availability of several neurotransmitters, including dopamine.

It is possible that parenting influences the expression of dopamine system genes and other genes through two related processes (Cole et al., 2007; Meaney & Szyf, 2005). First, DNA is typically quiescent and does not produce mRNA until transcription factors or related proteins bind with promoter sequences. Because stress hormones, such as cortisol, moderate gene expression and are strongly influenced by the social environment (Cole et al., 2007), the social environment can regulate gene expression. Consistent with this view, correlational studies in humans suggest that harsh parenting is associated with elevated cortisol in offspring (Tarullo & Gunnar, 2006). Second, DNA is wound around nucleosomes in the form of chromatin, the structure of which also determines availability of DNA to transcription factors. It has now been established that variations in maternal care can influence the number of bound methyl groups, which influences availability of DNA to transcription through regulation of chromatin structure (Meaney, Szyf, & Seckl, 2007). For example, variation in early maternal parenting in rodents causes enduring changes in chromatin structure, which influences gene expression, a phenomenon known as epigenetic programming (Meaney & Szyf, 2005). Thus, it is plausible to hypothesize that maternal parenting moderates the association between DAT1 and CD.

2. Measurement

Informative tests of GxE require that candidate environments and phenotypes be measured reliably, validly, and in developmentally appropriate ways. Furthermore, the environment and phenotype should be measured by different informants to avoid common method confounds. When studying child and adolescent mental disorders, valid assessments

typically require multiple informants (Hart, Lahey, Loeber, & Hanson, 1994). Findings of GxE are strengthened, however, when separately replicated in the reports of multiple informants (Moffitt et al., 2005). Consistent with these recommendations, the candidate environment of maternal parenting was based on direct observations coded by trained observers in the present study whereas symptoms of CD were independently measured using parent and youth reports.

3. Prospective designs

Prospective longitudinal designs avoid recall biases and can rule out reverse causation. For these reasons, they are a minimum requirement for strong tests of GxE (Kraemer, 2010). In the present study, maternal parenting was measured when the children were 4–6 years old and CD was measured in four annual assessments when they were 9–14 years old. Unlike cross-sectional studies, measuring parenting well before measuring the phenotype rules out the possibility that the child's adolescent CD symptoms influenced parenting at 4–6 years, rather than vice-versa (reverse causation). Nonetheless, child disruptive behavior during the mother-child interaction at 4–6 years was controlled in this study to minimize chances that parenting and later CD were correlated solely because early manifestations of CD influenced early maternal parenting through child effects (Jaffee et al., 2004; Lytton & Romney, 1991).

4. Gene-environment correlation

Tests of GxE can be difficult to interpret in the presence of gene-environment correlation (rGE) (Eaves, Silberg, & Erkanli, 2003; Rathouz, Van Hulle, Rodgers, Waldman, & Lahey, 2008). Therefore, the youth's genes and the candidate environment must either be uncorrelated or rGE must be taken into account in analyses. Therefore, we test for rGE prior to testing GxE.

5. Sampling

The strongest tests of GxE use data from representative samples or case-control designs (Moffitt et al., 2005; Yang & Khoury, 1997). The present study uses an efficient case-control sample that provides a large number of cases (children with ADHD) and well-matched comparison children (Yang & Khoury, 1997).

6. Statistical analysis

Because violations of the assumption of multivariate normality can result in the "detection" of GxE when none exists (Eaves, 2006), statistical methods must reduce the likelihood of scaling artifacts. Therefore, the present analyses use statistical methods that are not based on the assumption of multivariate normality.

In accordance with these guidelines for rigorous tests of GxE, the present paper reports tests of potential interactions between two dimensions of maternal parenting observed at 4–6 years of age and a VNTR polymorphism in the 3′ UTR of DAT1 in predicting CD symptoms in later childhood. Based on previous studies of the potential importance of early parenting in GxE across species (Caspi & Moffitt, 2006; Meaney, 2006; Rutter, 2008) and the findings reviewed of the association of the 9-repeat allele of DAT1 with dopaminergic functioning and behavior, we predict that maternal parenting will moderate the association between the 9-repeat DAT1 allele and levels of child CD 5–8 years later.

METHODS

Participants

Two cohorts of 3.8–7.0 year old children were recruited in consecutive years in both Chicago and Pittsburgh. Participants all lived with their biological mothers and half initially met DSM-IV diagnostic criteria for ADHD. In Chicago, children with ADHD were recruited from a university child psychiatry clinic. In Pittsburgh, 42% of the children with ADHD were recruited from a university child psychiatry clinic and 58% were recruited through advertisements, but no differences were found between recruitment methods in any demographic variable or measure functional impairment in wave 1 (Lahey et al., 1998). Five potential participants were excluded because of pervasive developmental disorder, mental retardation, or seizure disorder (Lahey et al., 2004). Comparison children were recruited from similar schools and neighborhoods as probands and approximately matched probands on sex, ethnicity, and age. They had never been referred for mental health problems but were not excluded if they met criteria for a disorder other than ADHD. Of 310 eligible participants, 259 parents gave written informed consent after the study was explained; all children gave oral assent. Four children with intelligence scores <70 were later excluded (Lahey et al., 2004).

Demographic and methodologic characteristics of the genotyped participants who were included in the present analyses are presented in Table 1 by genotype group. Similarly, behavioral characteristics of children in the three genotype groups are presented in Table 2.

Measures

Diagnosis of ADHD—Beginning with the initial assessment when the children were 4–6 years old, approximately annual assessments were conducted by trained lay interviewers, except that no assessment was conducted in the fifth year due to lack of funding. The Diagnostic Interview Schedule for Children (DISC) (Shaffer, Fisher, Piacentini, Schwab-Stone, & Wicks, 1993) was administered to mothers in each assessment. Information was obtained from mothers on DSM-IV diagnostic criteria for a range of mental disorders, including symptoms of ADHD and CD during the last 12 months (Lahey et al., 1998). In each annual assessment, the child's main teacher completed the DSM-IV version of the DBD Rating Scale (Pelham, Gnagy, Greenslade, & Milich, 1992) by mail. Following standard procedures (Pelham et al., 1992), teacher-reported ADHD symptoms rated "pretty much" or "very much" were scored as present.

The diagnosis of ADHD in year 1 was based on a combination of parent reports of DSM-IV symptoms in DISC interviews and teacher reports on the DBD. We used the standard method (Pelham et al., 1992) of considering teacher ratings of the DBD of "pretty much" or "very much" as indicating that the symptom was present. In the present sample, parent and teacher reports of the number of ADHD symptoms were moderately correlated (rho = .41 – .59). Because reports of ADHD symptoms from parents and teachers each account for independent variance in measures of functional impairment (Hart et al., 1994), parent and teacher reports were combined at the symptom level as in the DSM-IV field trials (Lahey, Applegate, McBurnett et al., 1994). That is, using the standard procedure for the DISC, ADHD symptoms were considered to be present if each symptom was reported by either teacher or parent (Piacentini, Cohen, & Cohen, 1992).

Impairment was assessed in two ways for the diagnosis of ADHD. First, the parent was asked in the DISC if the child's ADHD symptoms had caused problems (a) at home or with friends, or (b) at school. Second, parents and teachers completed the Impairment Rating Scale (IRS) (Fabiano et al., 2006), in which the child's need for treatment in multiple areas

was rated using 7-point scales ranging from "No problem; definitely does not need treatment" (=0) to "Extreme problem; definitely needs treatment" (=6). Parents rated the child on problems in relations with peers, siblings, and parents, academic progress at school, self-esteem, and impact on the family. Teachers rated problems in relations with classmates and teachers, academic progress, classroom disruption, and self-esteem using the IRS. Both respondents rated the child's overall need for treatment. For the year 1 diagnosis of ADHD, based on previous studies, children were said to be impaired if they received a rating of 3 on at least one IRS scale (Fabiano et al., 2006).

Children were said to exhibit ADHD if they met DSM-IV symptom criteria and exhibited impairment in at least one setting. We relaxed the DSM-IV requirement of impairment in two settings because previous findings based this sample suggested that 4–6 year olds who meet symptom criteria often do not exhibit impairment in school until later ages and the great majority of children who met criteria for ADHD with impairment in one setting later exhibited impairment in two settings (Lahey, Pelham, Loney, Lee, & Willcutt, 2005). In addition, it is not clear why children with serious impairment in one setting should be excluded from the diagnosis. All diagnosed children met the age of onset criterion for ADHD.

Measures of the CD Phenotype—Beginning in wave 6, when the children were 9–11 years old, independent DISC interviews of the mother and child were conducted concurrently. The DISC was administered to the youth in waves 6–9 to query DSM-IV diagnostic criteria for CD and other disorders. Consistent with other studies (Achenbach, McConaughy, & Howell, 1987), symptoms of CD reported by parents and youth were modestly correlated across waves 6–9 (rho = .13 – .24). Following standard procedures (Piacentini et al., 1992), each CD symptom was considered to be present if it was reported by either the parent or the youth in the analyses of combined parent + youth reports, as in the DSM-IV field trials (Lahey, Applegate, Barkley et al., 1994). Because both parent and youth reports of CD symptoms account for independent variance in functional impairment (Hart et al., 1994), the combination of parent and youth reports may provide the most valid measure of CD symptoms, but tests of GxE also were repeated using reports of CD symptoms from each informant separately to test the replicability of findings across informants.

Observational Measures of Maternal Parenting—During the initial assessment, each mother was videotaped interacting with her child during a structured protocol in a standard room. The room contained chairs, magazines, toys, and a television showing cartoons. After the mother played freely with her child for 10 minutes, an interviewer entered the room and scattered clothes, papers, and empty containers around the room. The interviewer gave the mother a list of instructions and materials. The interviewer quietly reviewed the instructions with the mother before leaving the room. They asked the mother to instruct her child to complete moderately challenging tasks during the remaining 15 minutes: (1) return toys to the shelf; (2) put scattered clothes in a box; (3) place scattered crumpled paper and empty containers in the waste basket; (4) sit at the table and count geometric shapes; (5) copy geometric designs on paper; (6) dust the table with the cloth; (7) work cooperatively with the mother to draw a diagonal line on the Etch-a-Sketch ©; and (8) choose one toy and play quietly while the mother reads and takes a 1 minute telephone call.

Discrete mother and child behaviors were coded continuously from videotapes by coders who were blind to information about the child. Each incident of the child's disruptive behavior and two mutually exclusive categories of parenting behavior were coded using the standardized and well-validated dyadic parent-child interaction coding system (Robinson & Eyberg, 1981). Two global categories of parenting were defined by summing component scores: (1) negative parenting (critical comments, negative commands (i.e., not to do

something), and negative physical contact), and (2) positive parenting (praise, expressions of positive affect, and positive physical contact). No observed instance of negative parenting in this study constituted verbal or physical abuse. The correlation between positive and negative parenting was $r = .12$, $N = 243$, $p = .06$. These categories of maternal parenting behavior have been reported to discriminate children referred for behavior problems from matched healthy controls (Robinson & Eyberg, 1981). Coders were trained to 80% agreement. Coders were supervised with weekly telephone calls and monthly in-person meetings to resolve differences and avoid drift in coding. A primary coder scored all videotapes and a second coder scored a random 30% of the videotapes. Inter-rater reliability was: Cohen's kappa = .90 for negative parenting, .95 for positive parenting, and .92 for total child disruptive behavior (whining, crying, negative physical behaviors, smart talk, yelling, destructiveness, and noncompliance). In the present sample, positive parenting has been found to inversely predict levels of CD symptoms reported by parents and teachers 5–8 years after the initial assessment among children with ADHD in wave 1 (Chronis et al., 2007).

DNA Collection and Genotyping

The 40 base-pair (bp) VNTR polymorphism located in the 3′-untranslated region of the DAT1 gene (SLC6A3) was genotyped from cheek swabs in 186 of the children. Alleles reflect the number of repeated DNA fragments of the 40-bp sequence, almost entirely 9- or 10-repeats. PCR was carried out in a 10 μl volume containing 50 ng of genomic template, 0.5 μM of each primer, one of which was 5′ fluorescently labeled, 200 μM of each dNTP, 1 × PCR buffer, 1.5 mM MgCl2, and 0.3 units of DyNAzyme™ EXT DNA polymerase (Finnzymes Oy, Espoo, Finland), with 0.5 M GC-melt (Clontech, Palo Alto, CA, USA). The DAT1 primer sequences were 5′-NED-TGTGGTGTAGGGAACGGCCTGAG-3′ and 5′ CTTCCTGGAGGTCACGGCTCAAGG-3′.

Samples were amplified on an Applied Biosystems 9700 thermal cycler (Foster City, CA) with an initial 12-minute step to heat-activate the enzyme at 96°C, followed by 45 cycles of a denaturation step of 96°C for 30 s, an annealing step for 45 s at 68°C, and an extension step of 72°C for 3 min, with final extension step at 72°C for 10min. Post PCR products were purified with Sephadex G-50 gel filtration system, and then added to 10 μl of deionized formamide and 0.5 μl of ROX labeled size standard. PCR products were injected and detected by laser-induced fluorescence on an ABI PRISM 3730 Genetic Analyzer. Electropherograms were processed with Genescan software and alleles were called with Genotyper software, blind to all but a consecutively assigned number. DAT1 genotype frequencies met the assumption of Hardy-Weinberg equilibrium ($\chi^2 = 1.14$, $p = .39$).

Data Analysis

Of the 255 children, 72% ($n = 183$) were genotyped and 174 had 9/9, 9/10, or 10/10 genotypes. The percent of parents interviewed in waves 6–9 was 94%, 89%, 85%, and 86%, respectively. The child was interviewed in 97–99% of these assessments during waves 6–9. Families that did not participate in one wave typically participated in the next assessment, but four genotyped children dropped out before wave 6. Seven children did not participate in the mother-child interaction task and one had missing data on family income, resulting in a sample for the present analyses of 162 children.

Associations of DAT1 genotypes with CD were analyzed by coding three dummy variables based on the 9/9, 9/10, and 10/10 DAT1 genotypes. Two of the three dummy variables for DAT1 genotype were entered as predictors, with the 10/10 genotype as the reference group. Two *df* tests were performed in regression models to test for differences among children with the three genotypes and interactions between the parenting measures and genotype,

followed by pairwise post-hoc comparisons. Other tests used 1 *df* unless noted. To normalize the dimensions of positive and negative parenting, the log of each variable plus 1 was taken.

Preliminary cross-sectional tests of year 1 data, including tests of rGE, were conducted using logistic or log-linear regression. Longitudinal tests of associations of genotype and parenting with the repeated measure of the number of CD symptoms reported during the sixth through ninth annual assessments were conducted using longitudinal generalized estimated equations (GEE) applied to log-linear models in SAS GENMOD. An autoregressive (AR1) working covariance structure was assumed, and because the number of CD symptoms was a skewed count variable, the working distribution was specified as Poisson. GEE uses a robust variance estimator for regression coefficient estimates that adjusts for deviations from the specified distribution and covariance structure (Zeger & Liang, 1986).

Sample Bias—To determine if the final sample of 162 included youth was biased, we tested whether included participants differed from the rest of the sample. Log-linear regression revealed that the included and excluded participants did not differ on family income ($\chi^2 = 1.34$, $p = .25$) or CD symptoms ($\chi^2 = 2.78$, $p = .10$) in wave 1, but the youth included in the analyses exhibited more wave 1 ADHD symptoms ($\chi^2 = 4.80$, $p < .03$), requiring this variable to be covaried in all analyses.

Population Stratification—Given the case-control design of this study, we conducted preliminary cross-sectional tests of possible associations between DAT1 genotype and demographic factors that could reflect population stratification of genotypes that confounded tests of association with DAT1 (Lander & Schork, 1994). As reported in Table 1, there were no significant differences among the three DAT1 genotype groups on site, cohort, age, sex, race-ethnicity, or total family income.

Tests of Gene-Environment Correlation—Two-df log-linear regressions indicated that DAT1 was not significantly associated with positive parenting ($\chi^2 = 0.26$, $p = .88$) or negative parenting ($\chi^2 = 0.81$, $p = .67$), providing no evidence of gene-environment correlation that could confound tests of GxE (Eaves et al., 2003; Rathouz et al., 2008).

Tests of GxE—All main effects, methodologic and demographic covariates, time (i.e., repeated assessment waves), and whether each interviewer was blind to information about the youth in each wave were controlled in longitudinal tests of GxE in log-linear GEE models. Because the sample is based on a case-control design and was selected to include children with ADHD and demographically matched non-ADHD comparison children, and because ADHD is correlated with CD (Lee et al., 2007), the combined number of wave 1 ADHD symptoms reported by the parent and teacher also was included as a covariate to minimize the influence of "child effects" on early parenting (Lytton & Romney, 1991). For the same reason, the child's disruptive behavior during the mother-child interaction was controlled. All covariates were entered simultaneously with dummy codes for genotype. Interactions between DAT1 genotype and each dimension of parenting were tested in the same model using 2-df tests, followed by post hoc tests when significant at $p < .05$.

RESULTS

Main Effects of Genotype and Maternal Parenting

Parent Report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the main effect of DAT1 genotype was not significant for CD symptoms across waves 6–9 ($\chi^2 = 2.93$, $p = .23$). When positive and negative parenting scores and the child's total disruptive behavior during

the mother-child interaction were added to the main effects models for genotypes, neither positive nor negative parenting were associated with CD symptoms during waves 6–9 at p < .10.

Youth Report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the main effect of DAT1 genotype was significant for CD symptoms across waves 6–9 ($\chi^2 = 12.43$, $p = .005$). Children with the 9/9 genotype reported more CD symptoms across the period from wave 6 (when they were 9–11 years old) through wave 9 (when they were 12–14 years old) than both children with the 10/10 genotype ($\beta = 0.77$, $z = 2.83$, $p < .005$) and children with the 9/10 genotype ($\beta = 0.93$, $z = 3.09$, $p < .005$), but the 9/10 group did not differ from the 10/10 group at $p < .05$. When positive and negative parenting scores and the child's total disruptive behavior during the mother-child interaction were added to the main effects models for genotypes, neither positive nor negative parenting were associated with youth-reported CD symptoms during waves 6–9 at $p < .10$.

Combined Parent+Youth report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the main effect of DAT1 genotype was not significant for CD symptoms across waves 6–9 ($\chi^2 = 3.51$, $p = .17$). When positive and negative parenting scores and the child's total disruptive behavior during the mother-child interaction were added to the main effects models for genotypes, neither dimension of parenting was associated with CD symptoms during waves 6–9 at $p < 10$.

Tests of Gene-Environment Interaction for CD Symptoms

Parent Report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the interaction between negative parenting and DAT1 genotypes was significant ($\chi^2 = 11.99$, $p < .003$). The slope for the 9/9 genotype was significantly more negative than for the 9/10 ($\chi^2 = 10.42$, $p < .002$) and 10/10 genotypes ($\chi^2 = 11.40$, $p < .005$), but the slopes of the 9/10 and 10/10 genotypes did not differ from one another ($\chi^2 = 0.06$, $p = .81$). For positive parenting, the 2-df test of the interaction also was significant ($\chi^2 = 12.25$, $p < .003$). The slope for the 9/9 genotype was significantly more negative than that for the 9/10 ($\chi^2 = 7.62$, $p < .006$) and 10/10 genotypes ($\chi^2 = 12.20$, $p < .0005$), but the slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 0.58$, $p = .46$).

Youth Report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the interaction between negative parenting and DAT1 genotypes was significant ($\chi^2 = 6.15$, $p < .05$). The slope for the 9/9 genotype was significantly more negative than that for the 10/10 genotype ($\chi^2 = 6.11$, $p < .02$), but not significantly different from the 9/10 genotype ($\chi^2 = 1.09$, $p = .30$). The slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 0.81$, $p = .37$). In contrast, the 2-df test of the interaction between positive parenting and DAT1 genotypes was not significant for youth-reported CD symptoms ($\chi^2 = 1.04$, $p = .60$).

Combined Parent+Youth Report of CD Symptoms—Controlling all demographic and methodologic covariates and the child's ADHD symptoms in wave 1, the 2-df test of the interaction between negative parenting and DAT1 genotypes was significant, indicating differences among the genotypes in the slopes of the association between negative parenting and the combined report of CD symptoms ($\chi^2 = 14.48$, $p < .001$). As shown in Table 3, interactions between the 9/9 genotype and parenting were significant (using the 10/10 genotype as the comparison), but interactions with the 9/10 genotype were not. When the

9/10 genotype was used as the comparison in a separate analysis (not tabled), interactions between the 10/10 genotype and positive and negative parenting were not significant, $z =$ 0.28, $p = .23$ and $z = 0.04$, $p = .86$, respectively.

To visualize this interaction, plots of the slopes of negative parenting against the average number of CD symptoms reported during waves 6–9 are presented separately for each genotype in Figure 1. Note that the numbers of CD symptoms across waves 6–9 were averaged solely for the purpose of this illustration; the slopes were generated from longitudinal analyses of the repeated measures of the numbers of CD symptoms in each assessment wave. The same applies to Figure 2 below. As shown in Figure 1, the slope for the 9/9 genotype (slope $\beta = -1.01$, $p < .001$) was significantly more negative ($\chi^2 = 9.87$, $p < .002$) than the slope for the 9/10 genotype (slope $\beta = 0.08$, $p = .68$) and significantly more negative ($\chi^2 = 14.33$, $p < .0002$) than the slope for the 10/10 genotype (slope $\beta = 0.12$, $p = .48$). Slopes of the 9/10 and 10/10 genotypes did not differ from one another ($\chi^2 = 0.03$, $p = .86$).

The 2-df test of the interaction between positive parenting and genotype for the response variable of CD symptoms also was significant ($\chi^2 = 9.82$, $p < .01$). As illustrated in Figure 2, the slope for the 9/9 genotype (slope $\beta = -0.78$, $p < .002$) was significantly more negative ($\chi^2 = 9.82$, $p < .002$) than for the 10/10 genotype (slope $\beta = 0.02$, $p = .92$), but only marginally different ($\chi^2 = 3.62$, $p = .057$) from the 9/10 genotype ($\beta = -0.27$, $p = .26$). The slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 1.41$, $p = .23$).

Diagnostic and Sensitivity Analyses

We conducted two additional sets of analyses to evaluate the robustness of the findings of GxE for the association of maternal parenting and genotype with later symptoms of CD.

Diagnostic Analyses to Assess the Impact of Highly Influential Participants—
We conducted formal diagnostic analyses to determine if the significant parenting-by-genotype interactions were the result of data from a small number of participants who were highly influential (e.g., outliers). Person-level deletion diagnostics were conducted in GEE (Preisser & Qaqish, 1996) using SAS PROC GENMOD. Standardized cluster-level (i.e., participant-level) delta-beta (DBETACS) statistics were computed for the differences in slopes for positive and negative parenting in the comparisons of the 9/9 to the 9/10 and 10/10 genotypes (i.e., the two contrasts that yielded significant differences in slopes). For any regression coefficient, DBETACS approximates the difference in that coefficient estimated for the full data set, compared to the estimate with each participant removed, standardized by the standard error of the regression coefficient. According to the DBETACS statistics, the data for two participants was found to be notably more influential in one or more of the four slope contrasts (i.e., two comparisons each for positive and negative parenting) than the rest of participants. As shown in Table 4, when the parenting-by-genotype interactions were tested with data from these two participants removed, the results were qualitatively identical to the results based on the full data set.

Specifically, with the two highly influential participants removed, the 2-df test of the interaction between negative parenting and DAT1 genotypes for the combined reports of CD symptoms by parents and youth was significant ($\chi^2 = 14.63$, $p < .001$). The slope for the 9/9 genotype was significantly more negative than for the 9/10 ($\chi^2 = 9.23$, $p = .003$) the 10/10 genotype ($\chi^2 = 13.55$, $p < .0002$), but the slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 0.01$, $p = .93$). For positive parenting, the 2-df test of the interaction also was significant ($\chi^2 = 11.97$, $p < .003$). The slope for the 9/9 genotype was significantly more negative than the 9/10 ($\chi^2 = 5.01$, $p < .03$) and the 10/10 genotypes ($\chi^2 = 11.94$, $p < .0005$), but the slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 2.11$, $p = .15$).

Additionally, in a more conservative test of the robustness of the present findings, we removed all seven participants with absolute DBETACS values > ±.20 from the models testing GxE for positive or negative parenting and found qualitatively identical evidence for both parenting-by-genotype interactions.

Tests of GxE among Only Children with ADHD in Wave 1—Although the number of participants was small (n =87), the interactions between genotype and positive and negative parenting were found with one exception among only those children who met criteria for ADHD in wave 1. Based on combined reports of CD symptoms by parents and youth, the 2-df test of the interaction between negative parenting and DAT1 genotypes was significant ($\chi^2 = 12.32$, $p < .003$). The slope for the 9/9 genotype was only marginally more negative than for the 9/10 ($\chi^2 = 3.36$, $p = .067$), but was significantly more negative than for the 10/10 genotype ($\chi^2 = 11.36$, $p < .001$); slopes of the 9/10 and 10/10 genotypes did not differ significantly ($\chi^2 = 1.25$, $p = .26$). For positive parenting, the 2-df test of the interaction also was significant ($\chi^2 = 6.32$, $p < .05$). The slope for the 9/9 genotype was significantly more negative than for the 9/10 ($\chi^2 = 5.16$, $p < .03$) and 10/10 genotypes ($\chi^2 = 5.21$, $p < .03$), but the slopes of the 9/10 and 10/10 genotypes did not differ ($\chi^2 = 0.26$, $p = .61$).

DISCUSSION

The present findings support the general hypothesis that variations in early parenting interact with the child's genetic characteristics to influence the development of antisocial behavior (Caspi et al., 2002). Specifically, these findings suggest that the predictive association between maternal parenting at 4–6 years of age and the child's CD symptoms 5–8 years later varies as a function of the child's 9-repeat allele of the DAT1 3′ VNTR and vice-versa. Specifically, the inverse association between both negative and positive parenting and future CD symptoms was significantly stronger among children with two copies of the 9-repeat allele than children with other genotypes in the present study. This finding is consistent with the findings of a previous cross-sectional study showing a stronger association between parental expressed emotion and conduct problems among children with at least one copy of the 9-repeat allele of the DAT1 3′ VNTR (Sonuga-Barke et al., 2009).

Only 7% of the current sample had the 9/9 genotype, but for at least two reasons the present findings do not necessarily mean that there are no genetic influences on sensitivity to early parenting associated with the DAT1 3′ VNTR in the 93% of children who did not have the 9/9 genotype. Although the present findings are consistent with a recessive effect of the 9-repeat allele on responsiveness to both positive and negative parenting, the systematic changes in slopes shown in Figures 1 and 2 raise the possibility that a larger sample might reveal significantly more negative slope in the 9/10 than the 10/10 group, consistent with an additive model of genetic transmission associated with the number of 9-repeat alleles. That is, there may be a smaller but significant influence of the 9-repeat allele on sensitivity to parenting in child with only one copy of the allele.

The use of two informants on CD symptoms in the present study provided important advantages. There is evidence that the combination of parent+youth reports of CD symptoms provides the most valid data on child CD symptoms (Bird, Gould, & Staghezza, 1992; Hart et al., 1994; Jensen et al., 1999). Nonetheless, it is important that the significant interaction between genotype and negative parenting was replicated in parent and youth reports of CD separately, even though the interaction involving positive parenting was not. Thus, the replication across informants provides stronger support for the interaction with negative parenting than for positive parenting.

The present findings suggest that different parenting styles may be adaptive for children with different DAT1 VNTR genotypes. If this hypothesis is confirmed in future studies, it would be important to know exactly which aspects of parenting are related to adaptive outcomes among children with each DAT1 genotype and why. As defined in the present study, both positive and negative parenting were inversely related to future CD symptoms in the present study, but primarily among children with two copies of the 9-repeat alleles of the DAT1 3′ VNTR. This suggests that, for reasons not currently understood, both warm and constructive (positive) parenting and parental prohibitions and critical corrective feedback (negative parenting) may function to reduce risk for CD among children with the 9/9 genotype. It is important to note that none of the negative parenting observed in the present study was outside the "normative" range and constituted abusive parenting. Because negative parenting within and outside the normative range appear to be related differently to maladaptive outcomes in children (Jaffee et al., 2004), the present findings do not challenge the hypothesis that child maltreatment fosters antisocial behavior (Caspi et al., 2002).

Given the highly consistent evidence from the *in vivo* studies reviewed above showing that persons with the 9-repeat allele of the DAT1 3′ VNTR exhibit greater availability of the dopamine transporter and greater functional reactivity in fronto-striatal networks, the present findings could indicate that variations in dopaminergic functioning in brain systems implicated in reward processes and executive control moderate children's response to early maternal parenting. Therefore, additional strong studies of the interaction of multiple aspects of early maternal parenting with the full range of genetic polymorphisms that influence dopamine system functioning are needed. Consistent with this view, two previous studies have found interactions between a polymorphism of the dopamine reception gene, DRD4, and parenting on child behavior (Bakermans-Kranenburg & van IJzendoorn, 2006; Sheese et al., 2007). A full understanding of genetic influences on sensitivity to parenting will require the systematic study of all of those polymorphisms related to dopamine and far more.

It is important to note that a previous analysis of data from the current longitudinal study found positive parenting in year 1 to inversely predict parent and teacher ratings of CD symptoms across years 2–8 among children who met criteria for ADHD in year 1 (Chronis et al., 2007). In the partially overlapping subset of the sample used in the present analyses, however, the trend towards inverse prediction of parent and youth reports of CD symptoms across years 6–9 did not reach significance at p < .05. This could reflect a greater protective role of early positive parenting among children with ADHD and/or variations in the role of positive parenting according to genotypes of the DAT1 3′ VNTR, which is associated with ADHD in this sample (Chronis et al., 2007).

Limitations

It is important to emphasize that the statistical power of the present study was quite limited. Although the probability of Type I errors (i.e., rejecting a null hypothesis that should not be rejected) is controlled by selecting the alpha level and not by the sample size, caution is warranted in interpreting gene-environment interactions detected in smaller samples (Risch et al., 2009) for several reasons. First, smaller samples are more prone to Type II errors (i.e., the failure to detect small effect sizes). Second, although we carefully evaluated the contributions of outliers in the present analyses, smaller samples are more vulnerable to their effect. Third, it is less likely that small samples adequately represent the population, raising the risk of incorrect estimation of population effect sizes. Fourth, confidence intervals around effect sizes estimated in smaller samples tend to be wider. The latter two issues are problematic because the size of adequate replication samples must be based on the lower bound of the effect size estimated in the original sample, and the lower bound tends to be lower and potentially misestimated in smaller samples. This is an important practical issue as the replication samples used in previous genetic research often have had insufficient

statistical power to detect the lower bound of the original effect size, likely leading to inconsistent replications (Chanock et al., 2007; Salanti, Sanderson, & Higgins, 2005).

The present analyses were based on a plausible biological model of GxE, identified robust interaction effects, used strong prospective methods with the environmental moderator and outcome variables being measured by different informants and methods, and replicated the findings of significant interactions with negative parenting in this sample using data on CD from two different informants. In addition, although the sample size for the present study was modest, the fact that the finding of GxE survived two kinds of diagnostic and sensitivity analyses (dropping the most influential participants and limiting the sample to those with a diagnosis of ADHD in wave 1), increases confidence in these findings. Nonetheless, like all such studies, these findings require replication in other samples.

Acknowledgments

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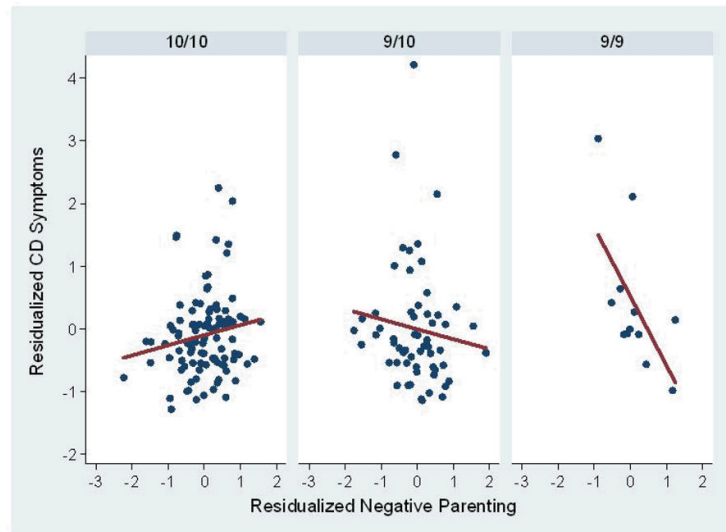
Figure 1.
Partial residual plots and regression lines based on linear regression for the association between negative parenting and conduct disorder symptoms reported by parents and youth among children with the three DAT1 genotypes. The mean of the number of CD symptoms in waves 6–9 in each genotype group was taken solely for the purpose of these illustrations; the longitudinal statistical analyses were performed on the unaveraged repeated measures of CD across waves 6–9. In these plots, negative parenting and the mean number of CD symptoms across waves 6–9 are residualized on site, cohort, age in wave 1, the child's sex and race-ethnicity, the child's number of ADHD symptoms in wave 1, and the child's disruptive behavior during the mother-child interaction task.
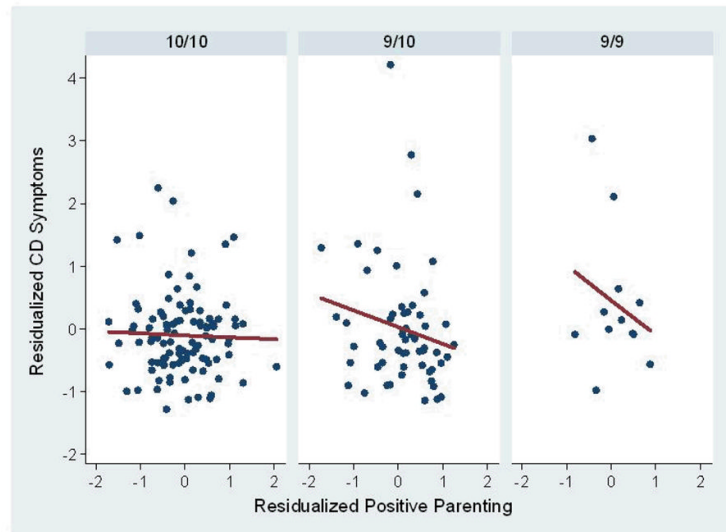
Figure 2.
Partial residual plots and regression lines to illustrate the results of linear regression of the association between positive parenting and conduct disorder symptoms across study waves 6–9 reported by parents and youth among children with the three DAT1 genotypes. The mean of the number of CD symptoms in waves 6–9 in each genotype group was taken solely for the purpose of these illustrations; the longitudinal statistical analyses were performed on the unaveraged repeated measures of CD across waves 6–9. In these plots, positive parenting and the mean number of CD symptoms across waves 6–9 are residualized on site, cohort, age in wave 1, the child's sex and race-ethnicity, the child's number of ADHD symptoms in wave 1, and the child's disruptive behavior during the mother-child interaction task.

Table 1

Demographic and Methodologic Characteristics in Wave 1 of the 169 Children Included in Statistical Analyses Grouped by their DAT1 3′ VNTR Genotype

	9/9	9/10	10/10	χ^2	df
n =	12	54	96		
Site (% Chicago)	58.3	42.6	36.5	2.33	2
Cohort (% cohort 1)	50.0	37.0	35.4	0.97	2
Age in years (mean, SD):	5.6 (0.7)	5.1 (0.7)	5.2 (0.7)	1.79	2, 162
Sex (% female):	8.3	18.5	13.5	1.11	2
Race-Ethnicity:				0.61	4
Non-Hispanic White (%)	75.0	70.4	67.7		
African-American (%)	16.7	24.1	26.0		
Hispanic and Other (%)	8.3	5.6	6.2		
Total family income (mean, SD):	59,292 (43,078)	39,537 (30,358)	44,755 (34,409)	2.81	2, 162

Note: Comparisons of differences among the three genotype groups on nominal variables were made using χ^2; tests of differences among the groups on variables that were not nominal (family income and counts of CD symptoms in wave 1) were made using Poisson regression. No comparisons of genotype groups significant at p < .05.

Table 2

Behavioral and Parenting Characteristics of the 169 Children Included in Statistical Analyses by Genotype

	9/9	9/10	10/10	χ^2 (df = 2)
n =	12	54	96	
Child's Behavioral Characteristics in Wave 1				
Diagnosis of ADHD (%)	58.3	59.3	46.9	2.34
Number ADHD symptoms (mean, SD)	8.8 (6.6)	10.6 (6.0)	8.3 (7.2)	3.86
Number CD symptoms (mean, SD)	1.3 (2.1)	1.3 (1.8)	0.8 (1.4)	4.15
Child disruptive behavior during observation	9.2 (11.1)	14.1 (14.6)	13.8 (16.0)	1.06
Maternal Parenting in Wave 1				
Positive parenting during observation	13.4 (10.2)	13.1 (9.6)	12.5 (10.4)	0.91
Negative parenting during observation	9.8 (10.4)	12.5 (13.1)	11.2 (9.6)	0.81
CD Symptoms During Outcome Waves				
Number CD symptoms in wave 6 (mean, SD)	1.3 (1.5)	0.9 (1.6)	0.4 (1.0)	7.30[*]
Number CD symptoms in wave 7 (mean, SD)	1.0 (1.8)	0.7 (1.2)	0.4 (0.8)	4.93
Number CD symptoms in wave 8 (mean, SD)	0.9 (1.2)	0.4 (1.0)	0.4 (0.7)	3.31
Number CD symptoms in wave 9 (mean, SD)	0.8 (1.4)	0.5 (1.1)	0.5 (1.0)	1.20

[*]
p < .05

Note: ADHD symptoms = number of symptoms reported by either the parent or the teacher; CD symptoms = number of symptoms reported in each assessment wave by either the parent or the youth (combined); comparisons of differences among the three genotype groups on binary variables were made using χ^2; tests of differences among the groups on variables that were not dichotomous (family income and counts of CD symptoms in wave 1) were made using log-linear regression.

Table 3

Results of Longitudinal Generalized Estimating Equations for Covariates, Genotypes, Parenting, and Genotype × Parenting Interactions Predicting Conduct disorder Symptoms During Assessment Years 6–9

Predictor	β	95% Confidence Interval	z	p <
Time (assessment waves)	−0.07	(−0.18 – 0.04)	−1.21	.23
Site	−0.34	(−0.93 – 0.25)	−1.13	.26
Cohort	−0.45	(−0.94 – 0.05)	−1.77	.08
Child's age in year 1	0.24	(−0.09 – 0.57)	1.42	.16
Child's sex	−0.54	(−1.09 – 0.02)	−1.89	.06
ADHD symptoms in year 1	0.08	(0.03 – 0.14)	3.10	.002
Family income (log)	−0.15	(−0.35 – 0.05)	−1.47	.15
African American[1]	−0.19	(−0.78 – 0.40)	−0.63	.53
Other ethnic group[1]	0.16	(−0.54 – 0.87)	0.46	.65
Parent interviewer blind	−0.05	(−0.42 – 0.32)	−0.25	.81
Youth interviewer blind	0.07	(−0.24 – 0.38)	0.47	.65
9/9 genotype[2]	4.86	(3.40 – 6.32)	6.52	.0001
9/10 genotype[2]	0.91	(−0.49 – 2.31)	1.27	.21
Positive parenting[3]	0.02	(−0.28 – 0.31)	0.10	.92
Negative parenting[3]	0.12	(−0.21 – 0.45)	0.71	.48
Child disruptive behavior[3]	0.014	(−0.01 – 0.02)	0.89	.38
9/9 × positive parenting	−0.80	(−1.30 – −0.30)	−3.13	.002
9/9 × negative parenting	−1.13	(−1.71 – −0.549)	−3.79	.0002
9/10 × positive parenting	−0.28	(−0.75 – 0.18)	−1.19	.24
9/10 × negative parenting	−0.04	(−0.482 – 0.40)	−0.17	.87

[1]Contrast group is non-Hispanic white

[2]Contrast group is 10/10 genotype

[3]Observed during mother-child interaction task in year 1

Note: Conduct disorder symptoms = number of symptoms reported in each assessment wave by either the parent or the youth (combined); estimates of main effects for genotype and parenting are changed by the presence of interaction terms; all tabled tests of significance used 1 df.

Table 4

Slopes within genotypes and tests of genotype-by-parenting interactions for the number of conduct disorder symptoms during waves 6–9 for the full sample and when highly-influential participants were dropped

Full Sample

| | Slopes | | | | | | Interactions with Genotype | | | | | | |
| | 9/9 | | 9/10 | | 10/10 | | 2-df test | | 9/9 vs. 9/10 | | 9/9 vs. 10/10 | | 9/10 vs. 10/10 | |
	β	$p <$	β	$p <$	β	$p <$	χ^2	$p <$	χ^2	$p <$	χ^2	$p <$	χ^2	$p <$
Positive Parenting														
	−0.78	.002	−0.27	.26	0.02	.92	9.82	.01	3.62	.057	9.82	.002	1.41	.23
Negative Parenting														
	−1.01	.001	0.08	.68	0.12	.48	14.48	.001	9.87	.002	14.33	.0002	0.03	.86
Two Highly-Influential Participants Removed from Sample														
Positive Parenting														
	−0.84	.004	−0.18	.46	−0.01	.97	8.67	.02	4.78	.03	8.60	.004	0.54	.47
Negative Parenting														
	−0.47	.04	0.31	.07	0.09	.58	9.68	.008	9.03	.003	6.44	.02	1.10	.30

Note: Conduct disorder symptoms = number of symptoms reported in each assessment wave by either the parent or the youth (combined).