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MAOA uVNTR and Early Physical Discipline Interact to Influence Delinquent Behavior

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Abstract

Background—A functional polymorphism in the promoter region of the monoamine oxidizing gene *monoamine oxidase A* (MAOA) has been associated with behavioral sensitivity to adverse environmental conditions in multiple studies (e.g., Caspi et al. 2002, Kim-Cohen et al. 2006). The present study investigates the effects of genotype and early physical discipline on externalizing behavior. We expand on the current literature in our assessment of externalizing, incorporating information across multiple reporters and over a broad developmental time period, and in our understanding of environmental risk.

Method—This study uses data from the Child Development Project, an ongoing longitudinal study following a community sample of children beginning at age 5. Physical discipline before age 6 was quantified using a subset of questions from the Conflict Tactics Scale (Straus 1979). Externalizing behavior was assessed in the male, European-American sub-sample (N=250) by parent, teacher, and self report using Achenbach's Child Behavior Checklist, Teacher Report Form, and Youth Self-Report (Achenbach 1991), at 17 time points from ages 6 to 22. Regression analyses tested the influence of genotype, physical discipline, and their interaction on externalizing behavior, and its subscales, delinquency and aggression.

Results—We found a significant interaction effect between genotype and physical discipline on levels of delinquent behavior. Similar trends were observed for aggression and overall externalizing behavior, although these did not reach statistical significance. Main effects of physical discipline held for all outcome variables, and no main effects held for genotype.

Conclusion—The adverse consequences of physical discipline on forms of externalizing behavior are exacerbated by an underlying biological risk conferred by MAOA genotype.

Keywords

Externalizing behavior; genetics; physical discipline; maltreatment

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Introduction

The social and economic costs of childhood maltreatment are enormous, often directly impacting the criminal justice system, the social welfare system, and the healthcare system. The estimated economic toll of child abuse in the U.S. in 2007 exceeded \$100 billion (Wang & Holton, 2007). Furthermore, many studies demonstrate an association between childhood maltreatment and behavioral problems such as childhood conduct disorder (CD), adult antisocial personality disorder (ASPD), impulsivity, or violent criminal behavior. These observations support the notion that “violence begets violence”, a commonly held belief that is increasingly supported by empirical evidence (Widom, 1989).

Understanding the connection between childhood maltreatment and the subsequent development of behavior problems is complicated by the fact that behavior problems are also known to be genetically influenced. Meta-analyses suggest heritability estimates of ~40–50% for conduct disorder and ASPD (Rhee & Waldman, 2002; Rose, Dick, Viken, Pulkkinen, & Kaprio, 2004). One gene that has received much attention for its effect on pathological or otherwise problematic externalizing outcomes (ASPD, CD, violent arrests, etc.) is *monoamine oxidase A (MAOA)*. MAOA is located on the X chromosome and is involved in the catabolism of monoamines such as dopamine, serotonin, and noradrenaline. Many animal studies demonstrate that increased functioning of dopaminergic and noradrenergic pathways is associated with increases in aggression (Volavka et al. 2004). These catecholamines can affect aggressive behavior in a variety of ways, from their influence on energy and metabolism to their involvement in the central nervous system on arousal, attention, and the neural mechanisms that make possible the manifestation of physical or social aggression (Haller et al. 1998). Further support for the role of monoamines in aggression comes from evidence that pharmacological treatment with drugs acting on these systems attenuates aggressive behavior (de Almeida et al. 2005).

Early evidence for the role of MAOA on human aggression comes from a Dutch kindred in which some males lacked a functional copy of the gene and exhibited mild mental retardation along with aggressive and violent behavior (Brunner et al., 1993). In these men, MAOA was effectively knocked out, since the gene is on the X chromosome and males are hemizygous, having only one copy of the X chromosome. High levels of aggression are also observed in knock-out animal models (Cases et al., 1995).

Genetic variants such as that in the Dutch family are quite rare; however, a variable number tandem repeat (VNTR) polymorphism located ~1.2 kilobases upstream of the coding region of MAOA (in the promoter region) is common and is associated with variation in transcript levels. Alleles of the VNTR with 2, 3, or 5 repeats are associated with lower levels of MAOA mRNA than are alleles with 3.5 or 4 repeats (Sabol, Hu, & Hamer, 1998). The relevance of this polymorphism to behavior has recently become the focus of much research after the finding that the MAOA uVNTR (for upstream variable number tandem repeats) moderates an association between childhood maltreatment and behavioral outcomes in adulthood (Caspi et al., 2002). Caspi and colleagues found that males hemizygous for low-activity alleles were more likely to be disposed toward violent behavior, have diagnoses of ASPD, or have violent crime convictions, than were males hemizygous for high-activity alleles. Importantly, this effect was observed only under conditions of childhood maltreatment, such that males with low-activity alleles were more sensitive to the detrimental effects of maltreatment than were males with high-activity alleles. These findings indicate a gene-by-environment interaction (GxE), whereby individuals with different genotypes exhibit varying levels of sensitivity to environmental conditions. Furthermore, recent evidence from a twin study indicates that latent genetic effects on externalizing outcomes can be more influential in the presence of environmental adversity

(Hicks, South, Dirago, Iacono, & McGue, 2009). This may provide one explanation for why children's long-term outcomes are differentially affected by the experience of maltreatment.

Attempts at replication of the original findings by Caspi and colleagues have been mixed, due at least in part to variation in measures of both the predictive variable (childhood maltreatment) and the outcome variable (antisocial or aggressive behavior). In most cases, researchers have focused on extreme levels of both the environmental risk and the behavioral outcome, and have limited their outcome assessment to adults. Another possible explanation for these inconsistencies is the presence of an as-yet-unidentified third variable influencing outcome, which could be environmental or genomic in nature.

The definition of "environmental risk" varies widely among replication attempts. Although many studies include only cases of physical abuse or neglect in their "risk" category (Huang et al., 2004; Huizinga et al., 2006; Weder et al., 2009; Widom & Brzustowicz, 2006; Young et al., 2006), others have included social indicators as risk factors, such as the child's residence type (single or multi-family, rented vs. owned, (Nilsson et al., 2006)); a variety of "early traumatic life events" which included whether a parent was absent for extended periods, parental mental health, and physical handicaps (Frazzetto et al., 2007); still another constructed a life history scale including information on family structure, education, and social status (Reif et al., 2007).

Further complicating our understanding of the dynamics between MAOA uVNTR genotype and the environment are variations in samples and outcome measures. Some samples have emphasized people with psychiatric disorders (Huang et al., 2004; Young et al., 2006), whereas many others represent the wider community. Outcome measures are frequently dichotomous, such as a diagnosis of conduct disorder or antisocial behavior, or a history of violent crime; although continuous measures, such as scores on scales of aggressive behavior or emotional problems, have also been employed. Despite vast heterogeneity in study design, it is worth noting that significant GxE interactions have been identified using both community and case/control populations, using a variety of measures of environmental risk, and with different behavioral outcomes.

Of the previously published replication attempts, our study design is most similar to that of Kim-Cohen and colleagues (2006), which assessed a community-based sample and incorporated prospective maternal and teacher reports of child outcome behavior. Furthermore, the authors constructed a continuous outcome variable that they termed a "composite mental health problem scale" that was composed of several scales from Achenbach's Child Behavior Checklist (Achenbach, 1991). Kim-Cohen *et al.* used a binary measure of environmental risk that reflected more extreme levels of physical harm than used here. The authors reported significant interactions between MAOA genotype and environmental harm on both the ADHD subscale and the composite mental health scale.

Here, we expand on the current literature in two important ways: by considering an operational definition of maltreatment that has not been previously used in the context of the reported gene-environment interaction, thereby extending our understanding of potential environmental risk, and by testing for effects across a broader developmental time period than in past work. We investigate the behavioral effect of variation in levels of physical discipline, in addition to extremes of maltreatment. Furthermore, we incorporate multiple reports of externalizing behavior, which is normative at some levels, as it is reported by parents, teachers, and selves, and manifests in early childhood (age 6) through early adulthood (age 22). Much of the previously published research has relied on retrospective reports of behavior and/or has limited the behavioral outcome of interest to pathologically aggressive or antisocial behavior in adults. We report findings from a prospective,

longitudinal, community sample of 250 Caucasian males who are part of the Child Development Project (Dodge, Bates, & Pettit, 1990). The effects of childhood maltreatment on behavior have been reported previously for this sample. Dodge, Bates, and Pettit (1990) reported adverse effects of pre-kindergarten physical maltreatment on classroom aggressive behavior. Lansford and colleagues (2002) found that aggression levels, reported at age 16, were nearly twice as high in individuals who had been maltreated before kindergarten as in non-maltreated children. Abused children were also more likely to be arrested as late juveniles or early adults (Lansford et al., 2007). Here we test for interaction effects between childhood maltreatment and MAOA uVNTR genotype in relation to a composite measure of externalizing behavior spanning childhood through early adulthood.

Method

Sample

Participants in the Child Development Project (CDP) were recruited from three sites (Nashville, TN; Knoxville, TN; and Bloomington, IN) during pre-registration for kindergarten in 1987 and 1988. To represent the late-enrolling segment of the population, additional children were recruited at the beginning of the school year. Families were recruited from multiple schools within these sites. The sample was selected to be typical of a range of socioeconomic status (SES) groups, with an effort made to include individuals from at-risk neighborhoods. Over half of the participants lived with single or divorced parents before adulthood. Approximately 75% of parents who were approached agreed to participate in the longitudinal study. The initial sample size included 585 children (52% male, 81% European American, 17% African American, 2% other ethnic groups). Participants provided informed consent at each wave of data collection, and the project was approved by the IRB of Duke University, Indiana University, and Auburn University.

Data collection began prior to kindergarten (around age 5), has been conducted annually, and is ongoing. Currently, approximately 485 individuals continue to participate. These participants are from backgrounds characterized by slightly higher SES than those who have discontinued participation, although they do not differ from the attrited population on other measures.

DNA Collection and Genotyping

DNA was collected from 452 CDP participants from February 2006 to July 2007 via saliva samples using Oragene collection kits, under the supervision of a trained interviewer. Individuals received \$20 and provided separate consent for their participation in the genotyping component of the project. DNA samples were labeled anonymously and sent to Washington University, St. Louis for extraction and genotyping. Genotyping for the MAOA-uVNTR locus was performed as in Caspi et al. 2002 with slight differences in primer sequence (available upon request). Alleles were categorized as “high activity” or “low activity” as in Sabol et al. 1998, i.e. alleles of 2, 3, or 5 repeats were categorized as “low” while those of 3.5 or 4 repeats were categorized as “high.” Because MAOA is an X-linked gene and unequivocal information about X-inactivation at this locus is not available, we limited our analyses to males. Furthermore, we conducted our analyses on only the subsample of European American males (maximum N=250) in an effort to minimize concerns about population stratification since allele frequencies are known to differ across ethnicities. DNA was available for 186 European American males (74.4% of total), all of whom were successfully genotyped. Consistent with other estimates of allele frequencies at this locus (Caspi et al., 2002; Cohen et al., 2003), 68.8% of our sample was hemizygous for a high activity allele and 31.2% was hemizygous for a low activity allele.

Phenotypes

Externalizing Behavior—The CDP collected data on externalizing behavior using Achenbach's Child Behavior Checklist (CBCL), Youth/Young Adult Self Report (YSR/YASR), and Teacher Report Form (TRF). We had available and utilized maternal reports on externalizing from the CBCL for every year at ages 6–17 (12 consecutive years); teacher reports for ages 6–13 (8 consecutive years); and self-reports for ages 12, 14–17, and 19–22 (9 non-consecutive years). Although behavioral data were available for children at age 5, that wave of reports was excluded from latent factors to avoid temporal overlap with our environmental risk measure, which was assessed up to and including age 5 (see below).

The externalizing scale includes two components: the aggression scale, consisting of 20 items (e.g. "Cruelty, bullying, or meanness to others", "Screams a lot", "Threatens people"); and the delinquency scale, consisting of 13 items (e.g. "Swearing or obscene language", "Steals outside the home", "Uses alcohol or drugs"). Items are scored according to the point values 0 ("not true"), 1 ("sometimes or somewhat true"), or 2 ("very or often true"). Cronbach's alpha values for scores of delinquency, aggression, and externalizing ranged from 0.92–0.96 for maternal reports, 0.79–0.89 for teacher reports, and 0.90–0.92 for self-reports. An individual's score on each of the three scales is simply the sum of the points for each item on the scale; and the externalizing score is the sum of the aggression and delinquency scores. We also conducted secondary analyses examining each of the subscales separately.

To capitalize on the availability of data across multiple time points, we constructed a single overall factor score as described below that incorporates behavioral data from all three reporters— mother, teacher, and self – across all available time points. We also computed latent externalizing factors for each of three reporters separately, for secondary analyses to examine consistency of effects across informants.

Physical discipline—Physical discipline was assessed using items from the Conflict Tactics Scale (CTS) (Straus, 1979). We used mothers' reports of their own and their spouse's behavior toward their child as it related to physical interactions/discipline. Six items were included in our measure of physical discipline: whether a parent 1) threw, smashed, hit, or kicked something; 2) threatened the child; 3) threw something at the child; 4) pushed, grabbed, or shoved the child; 5) hit or tried to hit the child; and 6) hit or tried to hit the child with something. Each item was assessed for each of two age ranges (ages 1–4 and ages 4–5), and for both parents. "Risk" was inferred if the mother indicated that an item had occurred more than once per month, and 1 point was given each time this criterion was met, such that a target child could receive a score of 4 points per item if both parents had engaged in that behavior more than once per month during both age ranges. The highest possible score for "physical discipline" was 24, and the highest score observed in the sample was 19. Readers should note that this variable is different from a measure of discipline previously reported for the Child Development Project sample (Lansford, Deater-Deckard, Dodge, Bates, & Pettit, 2004).

Statistical Analysis

MPlus version 5.0 (Muthen & Muthen, 1998–2007) was used to conduct all statistical analyses. MPlus uses maximum likelihood estimation, enabling us to retain participants for whom only incomplete data are available. We first constructed latent factors for overall externalizing behavior, aggression, and delinquency, using data from multiple sources across multiple points in time, as described above. We then regressed each of these outcome phenotypes onto genotype, physical harm, and a product term reflecting interaction between

these two variables. Males carrying a high-activity allele are coded as “0” and those carrying a low-activity allele are coded as “1”.

Results

Physical Discipline

Scores for the physical discipline risk factor ranged from 0–19, out of a possible 24. The mean score for the sample was 3.02 ± 0.247 , with a median score of 2. To test for gene-environment correlation, we assessed whether genotype classes differed significantly in their level of physical harm, and found that they did not ($r = -0.004$, $p = 0.977$): the mean score for those carrying the high-activity allele was 3.00 ± 0.334 , and for those carrying the risk (low-activity) allele the mean was 2.98 ± 0.499 . Furthermore, there were no differences between genotyped and non-genotyped individuals in physical discipline score ($F = 0.031$, $p = 0.861$), overall externalizing behavior ($F = 1.125$, $p = 0.290$), aggressive behavior ($F = 1.105$, $p = 0.315$), or delinquency ($F = 0.503$, $p = 0.479$).

Linear effects of physical discipline

We initially tested whether only extreme levels of physical discipline were associated with increases in externalizing behavior. If this were the case, we would expect a non-linear trend in externalizing as a function of harm exposure, such that externalizing does not increase appreciably at low to medium levels of environmental risk but accelerates beyond some threshold of risk. To address this possibility, we assessed the fit of linear and quadratic models and found no significant improvement in fit with the addition of a quadratic term ($R^2 \Delta = .000$; $F_{\Delta 1,247} = 0.008$, $p = 0.930$). This finding suggests that externalizing behavior increases in a linear manner even at high levels of environmental risk exposure.

Latent Factors of Externalizing Behavior and its Components

Overall externalizing behavior—Parameter estimates and significance values for all analyses are provided in Table 1. There were no main effects of genotype on the overall latent factor for externalizing behavior ($p = 0.255$). We did observe a significant main effect of physical discipline ($p < 0.001$), such that increases in such aggression were associated with increases in externalizing behavior. The effect of GxE interaction was not statistically significant ($p = 0.135$), but the effect was in the expected direction; i.e., as levels of physical discipline increased, males carrying the risk allele had higher mean latent externalizing factor scores than did their counterparts who did not carry the risk allele (Figure 1A). These trends were also evident in main effects models run separately by genotype, in which the parameter estimate (β) of physical discipline was higher for males carrying a low-activity allele ($\beta = 0.473$) than for those carrying a high-activity allele ($\beta = 0.333$).

Aggressive behavior—We next decomposed externalizing behavior into aggressive and delinquent behavior. Results for aggressive behavior were comparable to those for overall externalizing: There was no significant main effect of genotype ($p = 0.309$). Physical discipline was significantly and positively associated with aggressive behavior ($p < 0.001$). The GxE interaction was not significant ($p = 0.275$), but trends were again in the expected direction (Figure 1B), with higher parameter estimates for the effect of physical discipline on males carrying risk alleles ($\beta = 0.367$ vs. $\beta = 0.253$).

Delinquency—There were no significant main effects of genotype on delinquency ($p = 0.287$). Environmental risk was significantly and positively associated with delinquency ($p < 0.001$). We detected a significant ($p = 0.015$) GxE effect in the expected direction, such that males carrying the risk allele were more sensitive to the effects of environmental risk than were males carrying the high-activity allele (Figure 1C). As expected, this pattern is

reflected by the fact that the effect of physical discipline on males carrying the risk allele ($\beta=0.124$) is nearly twice the effect on males carrying the high-activity allele ($\beta=0.065$).

Because detection of GxE interaction effects can be sensitive to scale (Eaves, 2006), we performed natural log transformations on our measure of environmental risk and conducted the regression analyses again. The results were not qualitatively different: the interaction effect remained significant ($p=0.033$), suggesting that these GxE effects are robust to variable scaling.

Analyses of Separate Reports of Behavior

Our primary analyses employed latent factors that were constructed using reports from mothers, teachers, and adolescents/young adults. To examine whether a particular reporter drove the findings associated with the overall latent factor scores, we performed a series of secondary analyses wherein latent factors were derived separately for each reporter, again incorporating all time-points for which data were available from a given reporter.

We first assessed the degree of correlation between factor scores derived from different reporters. Scores were significantly correlated across all factors ($p<0.01$ for all pairwise comparisons), with the lowest correlations between self-reported and teacher-reported behavioral measures; these correlations ranged from 0.19 to 0.35. Correlations between maternal and teacher reports generally exceeded 0.5. This variation in correlations among reporters could be due to the difference in age ranges used for each reporter (see Materials and Methods). In addition, factor scores on the two component scales were significantly correlated within each reporter ($r=0.808$ for maternal reports, $r=0.778$ for teacher reports, and $r=0.622$ for self reports; $p<0.001$ for all correlations).

Maternal reports of externalizing and its components—The results from analyses of the factor scores derived from maternal reports of overall externalizing, aggression, and delinquency mapped quite closely onto the results from analyzing the latent factor scores derived from all three reporters. In regression analyses, no main effects of genotype were observed, but level of physical discipline was significantly and positively associated with all three behavioral outcomes ($p<0.01$ in all cases) and significant GxE effects were observed for delinquent behavior ($p<0.026$) but not for general externalizing or aggression, although in the same predicted direction.

Teacher reports of externalizing and its components—Results from regressions using teacher-report-derived factor scores were also qualitatively similar to those in our primary analyses. We did not observe main effects of genotype; the main effect of physical discipline was significant ($p<0.001$ for delinquency and $p=0.001$ for aggression and externalizing). The effect of GxE on delinquent behavior did not achieve statistical significance ($p=0.0804$) although there was a trend in the expected direction. GxE effects on general externalizing and aggression were not statistically significant.

Self-reports of externalizing and its constituents—Analyses using factor scores derived from self-reports differed considerably from the previously described results. There were no significant effects of genotype, environment, or their interaction on any of the three behavioral outcomes. These analyses suggest that the effects observed with the overall latent factor scores are primarily influenced by maternal and teacher reports of behavior.

Discussion

We examined the effects of genotype and variation in early physically harsh parenting on externalizing behavior, which was measured as a function of multiple reports of aggression

and delinquency from early childhood through early adulthood. Our findings support the existence of a GxE interaction between MAOA uVNTR genotype and harsh parenting; specifically, males carrying a low-activity risk allele are more sensitive to the effects on their delinquency of physical discipline than are males who do not carry the risk allele.

Our findings are consistent with previous reports that individuals who carry a risk allele and are subject to childhood adversity are more likely to exhibit problematic behavioral outcomes. Caspi and colleagues (2002) first identified this gene-by-environment interaction in the context of childhood maltreatment and adult outcomes such as a propensity toward violence or antisocial behavior. It has since been found in both community samples and in samples enriched for psychiatric disorders; among individuals experiencing physical and/or psychosocial stressors; and it has been shown to influence violent criminal behavior, aggression, and psychiatric problems. There have also been a number of failures to replicate the original findings (e.g., (Prichard, Mackinnon, Jorm, & Easteal, 2008; Reif et al., 2007; Young et al., 2006).

To our knowledge, this is the first report of an interaction between MAOA and environmental stress that studied a continuous measure of physical environmental risk and the resulting influence on problematic behavioral outcomes. We assessed whether externalizing behavior increases linearly as a function of physical discipline exposure. That is, do we observe increases in externalizing only at high levels of discipline, or does the behavioral outcome increase gradually alongside increases in physical discipline? Our results indicate that the relation between these variables is indeed linear; individuals experiencing high levels of discipline are more likely to exhibit problematic levels of externalizing, but there does not appear to be a “threshold” of discipline experience beyond which the pattern of externalizing changes. However, we did conduct preliminary analyses using a binary measure of maltreatment, which was defined as an intentional strike that left physical marks for at least 24 hours. The statistical power of these analyses was limited, and no significant GXE effects were detected, although interaction effects were in the expected direction.

Furthermore, most previous studies have relied upon adult-based behaviors, such as criminal convictions or antisocial personality disorder, or on retrospective accounts of adolescent behavior; in contrast, we incorporate prospective measures of externalizing behavior from multiple reporters and demonstrate that the effects of GxE are evident with respect to behavior spanning childhood through early adulthood. Another strength of our study is the inclusion of prospective reports of behavior from multiple reporters. Self-reported aggression and delinquency are far higher than maternal and teacher reports, a discrepancy that is more pronounced in the reports of covert delinquent behavior than in reports of overt aggression. Similarly, Collishaw and colleagues (2009), using a different scale of problematic behavior, also reported that youth self-reports of emotional and conduct difficulties were higher than parent or teacher reports. Our findings seem to be driven primarily by maternal reports; perhaps mothers are more aware of their child’s delinquent behavior than are teachers, to the degree that such behavior might be more frequent outside of school. That self-reports contribute little to our findings is interesting: perhaps adults are more inclined to rate a child’s behavior in the context of what is developmentally appropriate and how it compares to that of his/her peers, whereas a child’s notion of his/her own behavior lacks contextual and developmental perspective. Child reports of externalizing behavior may reflect more adolescent “bravado”; in this developmental period, it may be more common for youths to engage in a multitude of low-level externalizing behaviors, potentially obscuring GxE interaction effects, which would still be evident in teacher and parent reports of delinquency that reflect more understanding of what varies from normative adolescent externalizing.

Although our primary analyses used overall latent externalizing factors, we were interested in whether the genotype-by-discipline interaction effect varied across time. Maternal reports were available for the broadest age range (6–17), so we conducted post-hoc analyses to test for GxE at each age (data not shown). The pattern of results suggested consistency of the gene-by-environment interaction effect across this age range. It is plausible that one would find enhanced or reduced effects of the interaction at other ages, and this would be interesting to explore in future research.

Although our GxE effects were statistically significant only with delinquent behavior, we did observe a consistent pattern in the expected direction for aggression and externalizing as well. This suggests that the lack of statistical significance might be due to power rather than to the absence of effect; our data do not support a qualitative difference in GxE effects on delinquency versus aggression. It is worth noting that endorsement of items indicative of delinquent behavior was far lower than endorsement of aggressive behavior, even after weighting scores to account for the fact that the aggressive behavior scale has more items than the delinquency scale. These findings parallel the differences observed among the raters in the sense that the GxE effect was only significant when analyzing the less frequently endorsed behavior (i.e., delinquency). This may suggest that the finding is most apparent among more extreme externalizing behavior.

Physical discipline was a significant and positive predictor of nearly every measure of externalizing behavior. Previous studies using the entire CDP population have reported higher levels of externalizing (Keiley, Howe, Dodge, Bates, & Pettit, 2001; Lansford et al., 2007), internalizing (Keiley et al., 2001), and a variety of other problematic behavioral outcomes (Lansford et al., 2007) in children who were physically maltreated before the age of 5 than in those who were not maltreated. While our results are in agreement with previous findings that early maltreatment can lead to later problematic behavior, they also indicate that levels of physical discipline that might be considered mild or moderate can actually create an environmental risk. Clearly, exposure to an adverse environment such as harsh physical discipline is a powerful risk factor for problematic behavior throughout development. Such environmental risk is exacerbated by the presence of the biological vulnerability conferred by low-activity alleles at this locus. The neurobiological ramifications of low levels of MAOA transcript are still unknown, and much work remains to be done to elucidate the mechanisms by which transcript variation influences behavior.

We did not detect a main effect of genotype on externalizing, nor is there evidence of gene-environment correlation. This demonstrates that in the absence of environmental risk, males with a “risky” genotype are no more likely to exhibit problematic behavior than are their counterparts carrying a high-activity allele. Furthermore, they are not more likely to experience physical discipline through passive, active, or evocative means related to their genotype. In other words, children’s genotypes are not associated with their rearing environment, which is in part a product of their parents’ heritable characteristics (via passive gene-environment correlation); their genotype does not elicit an environmental response such as harsher discipline (evocative correlation); and their genotype does not influence an active selection of environmental circumstances (via active correlation).

We focused on males in this report because there is uncertainty regarding the analysis of female data due to questions surrounding how X-inactivation affects MAOA in females. However, we did conduct secondary analyses on females wherein heterozygotes were included and coded as a separate genotype class, as has been done previously (Prom-Wormley et al., 2009). We did not detect main effects of genotype, and main effects of physical discipline were not consistently statistically significant. Furthermore, we did not detect significant GxE effects. However, trends in the direction of effect were opposite that

observed in males: that is, high-activity alleles conferred risk in conjunction with increasing levels of physical discipline, while females homozygous for low-activity alleles exhibited lower levels of externalizing behavior with increasing environmental risk. These results are consistent with previous studies in females (Nilsson, Wargelius, Sjöberg, Leppert, & Orelund, 2008; Prom-Wormley et al., 2008; Wakschlag et al., 2009). Larger, more highly powered studies, focused on females, along with enhanced biological understanding of how X-inactivation affects MAOA, will be necessary to better understand the meaning of these findings.

We do acknowledge a number of limitations to the current study. The size of our sample is modest, which limits statistical power. Furthermore, our analyses are limited to a Caucasian population due to the small number of minority individuals participating in the Child Development Project; while this reduces the likelihood of population stratification, we cannot say with absolute certainty that no stratification exists. The effects of genotype and physical discipline on externalizing behavior and its components should be explored in larger samples. Finally, due to the highly correlated nature of these analyses, correction for multiple tests is not straightforward and has not been applied to our results.

In summary, we report replication of previous findings of an effect of the interaction between MAOA uVNTR genotype and environmental risk on behavioral outcomes. These results were found using prospective reports of behavior across multiple reporters in a community sample. We extend the current understanding of this effect by demonstrating that an individual's genotype interacts with the environment across a continuous measure of physical discipline to influence levels of delinquent behavior.

Key Points

- Harsh/physically aggressive parenting can influence externalizing behavior, as evidenced by the significant main effects of physical discipline on behavioral outcome.
- Twin studies demonstrate that externalizing behavior is also influenced by genetic factors.
- Our results indicate that MAOA uVNTR genotype and physical discipline interact to influence delinquent behavior. This finding expands on previous research in that the effect is observed early in life and at relatively modest levels of parental aggression; furthermore, the effect is detectable at sub-clinical levels of externalizing behavior.
- Future research should investigate the effects of these genetic and environmental factors on minority populations and in females.

Abbreviations

MAOA uVNTR	monoamine oxidase A upstream variable number tandem repeats
CD	conduct disorder
ASPD	antisocial personality disorder
mRNA	messenger ribonucleic acid
GxE	gene-by-environment
CDP	Child Development Project

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Figure 1. Overall Externalizing Behavior. Mean factor scores for (A) overall externalizing behavior, (B) aggressive behavior, and (C) delinquent behavior are depicted as a function of exposure to physical discipline. Dashed lines represent males hemizygous for a high activity MAOA uVTNR allele and solid lines represent males hemizygous for a low-activity (risk) allele. GxE effects were significant ($p < 0.05$) for delinquent behavior, but not for overall externalizing or aggressive behavior.

Table 1

For each type of latent externalizing factor, parameter estimates (β) and significance levels (p) are given for independent factor main effects and interaction effects.

Reporter	Latent Externalizing Factor Phenotype	Main Effects Models				Interaction Model	
		MAOAGenotype (G)		Physical Discipline (E)		G*E	
		β	p	β	p	β	p
Multiple [/]	Externalizing	0.874	0.255	0.396	<0.001	0.284	0.135
	Aggression	0.627	0.309	0.305	<0.001	0.168	0.275
	Delinquency	0.191	0.287	0.093	<0.001	0.109	0.015
Maternal	Externalizing	0.637	0.398	0.416	<0.001	0.269	0.15
	Aggression	0.469	0.448	0.33	<0.001	0.184	0.233
	Delinquency	0.217	0.241	0.099	<0.001	0.102	0.026
Teacher	Externalizing	1.447	0.197	0.379	0.001	0.159	0.579
	Aggression	1.217	0.216	0.309	0.001	0.076	0.763
	Delinquency	0.208	0.21	0.068	<0.001	0.072	0.081
Self	Externalizing	0.487	0.373	0.063	0.275	-0.033	0.816
	Aggression	0.208	0.572	0.045	0.252	0.051	0.889
	Delinquency	-0.012	0.944	0.024	0.206	-0.013	0.255

[/] This represents the overall factor score derived from reports from mothers, teachers, and target individuals.