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Role of Genotype in the Cycle of Violence in Maltreated Children

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We studied a large sample of male children from birth to adulthood to determine why some children who are maltreated grow up to develop antisocial behavior, whereas others do not. A functional polymorphism in the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (*MAOA*) was found to moderate the effect of maltreatment. Maltreated children with a genotype conferring high levels of *MAOA* expression were less likely to develop antisocial problems. These findings may partly explain why not all victims of maltreatment grow up to victimize others, and they provide epidemiological evidence that genotypes can moderate children's sensitivity to environmental insults.

Childhood maltreatment is a universal risk factor for antisocial behavior. Boys who experience abuse—and, more generally, those exposed to erratic, coercive, and punitive parenting—are at risk of developing conduct disorder, antisocial personality symptoms, and of becoming violent offenders (*1*, *2*). The earlier children experience maltreatment, the more likely they are to develop these problems (*3*). But there are large differences between children in their response to maltreatment. Although maltreatment increases the risk of later criminality by about 50%, most maltreated children do not become delinquents or adult criminals (*4*). The reason for this variability in response is largely unknown, but it may be that vulnerability to adversities is conditional, depending on genetic susceptibility factors (*5*, *6*). In this study, individual differences at a functional polymorphism in the promoter of the monoamine oxidase A (*MAOA*) gene were used to characterize genetic susceptibility to maltreatment and to test whether the *MAOA* gene modifies the influence of maltreatment on children's development of antisocial behavior.

The *MAOA* gene is located on the X chromosome (Xp11.23–11.4) (*7*). It encodes the *MAOA* enzyme, which metabolizes neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA), rendering them inactive (*8*). Genetic deficiencies in *MAOA* activity have been linked with aggression in mice and humans (*9*). Increased aggression and increased levels of brain NE, 5-HT, and DA were observed in a transgenic mouse line in which the gene encoding *MAOA* was deleted (*10*), and aggression was normalized by restoring *MAOA* expression (*11*). In humans, a null allele at the *MAOA* locus was linked with male antisocial behavior in a Dutch kindred (*12*). Because *MAOA* is an X-linked gene, affected males with a single copy produced no *MAOA* enzyme—effectively, a human knockout. However, this mutation is extremely rare. Evidence for an association between *MAOA* and aggressive behavior in the human general population remains inconclusive (*13*–*16*).

Circumstantial evidence suggests the hypothesis that childhood maltreatment predisposes most strongly to adult violence among children whose *MAOA* is insufficient to constrain maltreatment-induced changes to neurotransmitter systems. Animal studies document that maltreatment stress (e.g., maternal deprivation, peer rearing) in early life alters NE, 5-HT, and DA neurotransmitter systems in ways that can persist into adulthood and can influence aggressive behaviors (*17*–*21*). In humans, altered NE and 5-HT activity is linked to aggressive behavior (*22*). Maltreatment has lasting neurochemical correlates in human children (*23*, *24*), and although no study has ascertained whether *MAOA* plays a role, it exerts an effect on all aforementioned neurotransmitter systems. Deficient *MAOA* activity may dispose the organism toward neural hyperreactivity to threat (*25*). As evidence, phenelzine injections, which inhibit the action of monoamine oxidase, prevented rats from habituating to chronic stress (*26*). Low *MAOA* activity may be particularly prob-

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lematic early in life, because there is insufficient *MAOB* (a homolog of *MAOA* with broad specificity to neurotransmitter amines) to compensate for an *MAOA* deficiency (*8*).

Based on the hypothesis that *MAOA* genotype can moderate the influence of childhood maltreatment on neural systems implicated in antisocial behavior, we tested whether antisocial behavior would be predicted by an interaction between a gene (*MAOA*) and an environment (maltreatment). A well-characterized variable number tandem repeat (VNTR) polymorphism exists at the promoter of the *MAOA* gene, which is known to affect expression. We genotyped this polymorphism in members of the Dunedin Multidisciplinary Health and Development Study, a sample without population stratification confounds (*27*). This birth cohort of 1,037 children (52% male) has been assessed at ages 3, 5, 7, 9, 11, 13, 15, 18, and 21 and was virtually intact (96%) at age 26 years.

The study offers three advantages for testing gene-environment ($G \times E$) interactions. First, in contrast to studies of adjudicated or clinical samples, this study of a representative general population sample avoids potential distortions in association between variables (*28*, *29*). Second, the sample has well-characterized environmental adversity histories. Between the ages of 3 and 11 years, 8% of the study children experienced "severe" maltreatment, 28% experienced "probable" maltreatment, and 64% experienced no maltreatment (*27*). (Maltreatment groups did not differ on *MAOA* activity, $\chi^2(2) = 0.38$, $P = 0.82$, suggesting that genotype did not influence exposure to maltreatment.) Third, the study has ascertained antisocial outcomes rigorously. Antisocial behavior is a complicated phenotype, and each method and data source used to measure it (e.g., clinical diagnoses, personality checklists, official conviction records) is characterized by different strengths and limitations. Using information from independent sources appropriate to different stages of development, we examined four outcome measures (*27*). Adolescent conduct disorder was assessed according to criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV); convictions for violent crimes were identified via the Australian and New Zealand police; a personality disposition toward violence was measured as part of a psychological assessment at age 26; symptoms of antisocial personality disorder were ascertained at age 26 by collecting information about the study members from people they nominated as "someone who knows you well." A common-factor model fit the four measures of antisocial behavior well (*27*), with factor loadings ranging from 0.64 to 0.74, showing that all four measures index liability to antisocial behavior.

Using moderated regression analysis, we predicted scores on a composite antisocial index comprising the four measures of antisocial behavior (*27*) (Fig. 1). The main effect of *MAOA* activity on the composite index of

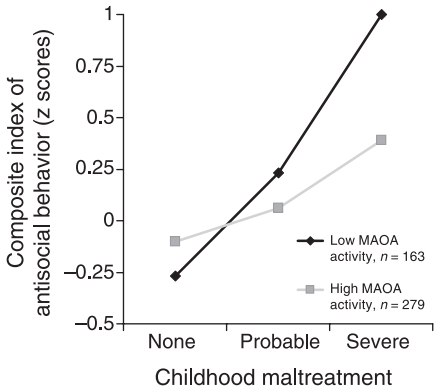

Fig. 1. Means on the composite index of antisocial behavior as a function of *MAOA* activity and a childhood history of maltreatment (*27*). *MAOA* activity is the gene expression level associated with allelic variants of the functional promoter polymorphism, grouped into low and high activity; childhood maltreatment is grouped into 3 categories of increasing severity. The antisocial behavior composite is standardized (*z* score) to a M = 0 and SD = 1; group differences are interpretable in SD unit differences (d).

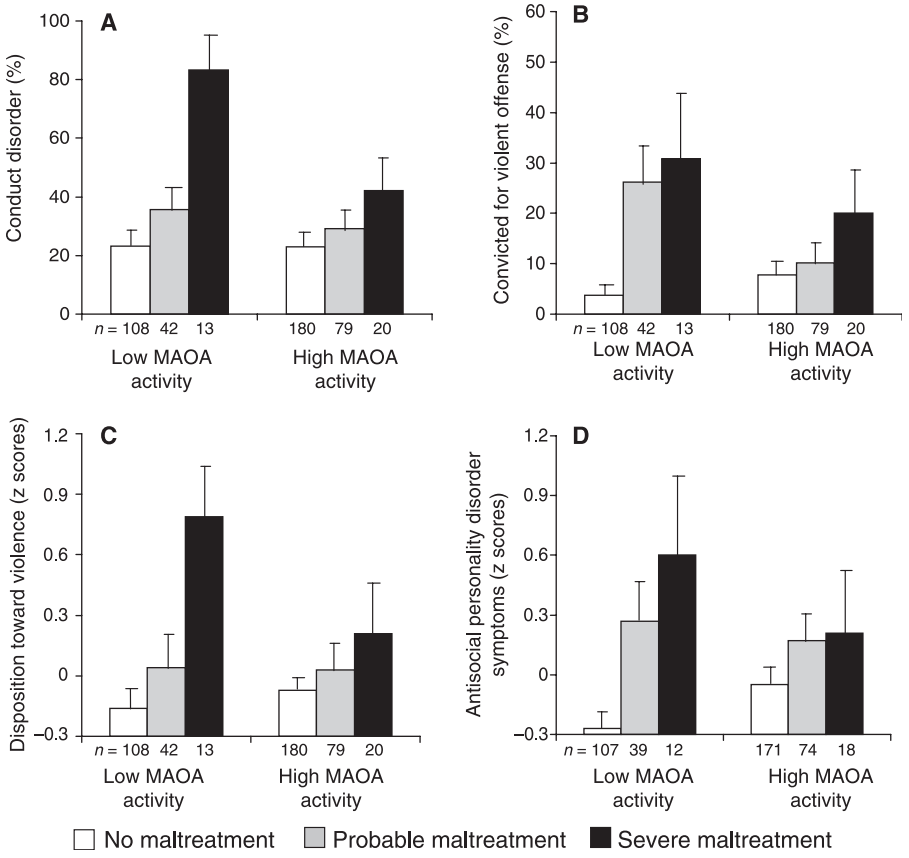

Fig. 2. The association between childhood maltreatment and subsequent antisocial behavior as a function of *MAOA* activity. (**A**) Percentage of males (and standard errors) meeting diagnostic criteria for Conduct Disorder between ages 10 and 18. In a hierarchical logistic regression model, the interaction between maltreatment and *MAOA* activity was in the predicted direction, $b = -0.63$, SE = 0.33, $z = 1.87$, $P = 0.06$. Probing the interaction within each genotype group showed that the effect of maltreatment was highly significant in the low–*MAOA* activity group ($b = 0.96$, SE = 0.27, $z = 3.55$, $P < 0.001$), and marginally significant in the high-*MAOA* group ($b = 0.34$, SE = 0.20, $z = 1.72$, $P = 0.09$). (**B**) Percentage of males convicted of a violent crime by age 26. The $G \times E$ interaction was in the predicted direction, $b = -0.83$, SE = 0.42, $z = 1.95$, $P = 0.05$. Probing the interaction, the effect of maltreatment was significant in the low–*MAOA* activity group ($b = 1.20$, SE = 0.33, $z = 3.65$, $P < 0.001$), but was not significant in the high *MAOA* group ($b = 0.37$, SE = 0.27, $z = 1.38$, $P = 0.17$). (**C**) Mean *z* scores (M = 0, SD = 1) on the Disposition Toward Violence Scale at age 26. In a hierarchical ordinary least squares (OLS) regression model, the $G \times E$ interaction was in the predicted direction ($b = -0.24$, SE = 0.15, $t = 1.62$, $P = 0.10$); the effect of maltreatment was significant in the low–*MAOA* activity group ($b = 0.35$, SE = 0.11, $t = 3.09$, $P = 0.002$) but not in the high *MAOA* group ($b = 0.12$, SE = 0.07, $t = 1.34$, $P = 0.17$). (**D**) Mean *z* scores (M = 0, SD = 1) on the Antisocial Personality Disorder symptom scale at age 26. The $G \times E$ interaction was in the predicted direction ($b = -0.31$, SE = 0.15, $t = 2.02$, $P = 0.04$); the effect of maltreatment was significant in the low–*MAOA* activity group ($b = 0.45$, SE = 0.12, $t = 3.83$, $P < 0.001$) but not in the high *MAOA* group ($b = 0.14$, SE = 0.09, $t = 1.57$, $P = 0.12$).

antisocial behavior was not significant (b = 0.01, SE = 0.09, t = 0.13, P = 0.89), whereas the main effect of maltreatment was significant (b = 0.35, SE = 0.07, t = 4.82, $P <$ 0.001). A test of the interaction between *MAOA* activity and maltreatment revealed a significant $G \times E$ interaction (b = –0.36, SE = 0.14, t = 2.53, P = 0.01). This interaction within each genotype group showed that the effect of childhood maltreatment on antisocial behavior was significantly weaker among males with high *MAOA* activity (b = 0.24, SE = 0.11, t = 2.15, P = 0.03) than among males with low *MAOA* activity (b = 0.68, SE = 0.12, t = 5.54, $P <$ 0.001).

We conducted further analyses to test if the $G \times E$ interaction was robust across each of the four measures of antisocial behavior that made up the composite index. For all four antisocial outcomes, the pattern of findings was consistent with the hypothesis that the association between maltreatment and antisocial behavior is conditional, depending on the child's *MAOA* genotype ($G \times E$ interaction P = 0.06, 0.05, 0.10, and 0.04, respectively). For adolescent conduct disorder (Fig. 2A), maltreated males (including probable and severe cases) with the low–*MAOA* activity genotype were more likely than nonmaltreated males with this genotype to develop conduct disorder by a significant odds ratio (OR) of 2.8 [95% confidence interval (CI): 1.42 to 5.74]. In contrast, among males with high *MAOA* activity, maltreatment did not confer significant risk for conduct disorder (OR = 1.54, 95% CI: 0.89 to 2.68). For adult violent conviction (Fig. 2B), maltreated males with the low–*MAOA* activity genotype were more likely than nonmaltreated males with this genotype to be convicted of a violent crime by a significant odds ratio of 9.8 (95% CI: 3.10 to 31.15). In contrast, among males with high *MAOA* activity, maltreatment did not confer significant risk for violent conviction (OR = 1.63, 95% CI = 0.72 to 3.68). For self-reported disposition toward violence (Fig. 2C) and informant-reports of antisocial personality disorder symptoms (Fig. 2D), males with the low–*MAOA* activity genotype who were maltreated in childhood had significantly elevated antisocial scores relative to their low-*MAOA* counterparts who were not maltreated. In contrast, males with high *MAOA* activity did not have elevated antisocial scores, even when they had experienced childhood maltreatment.

These findings provide initial evidence that a functional polymorphism in the *MAOA* gene moderates the impact of early childhood maltreatment on the development of antisocial behavior in males. Replications of this $G \times E$ interaction are now needed. Replication studies should use valid and reliable ascertainments of maltreatment history and should obtain multiple measures of antisocial outcomes, in large samples of males and females (*30*). If replicated, the findings have implications for research and clinical practice. With regard to research in psychiatric genetics, knowledge about environmental context might help gene-hunters refine their phenotypes. Genetic effects in the population may be diluted across all individuals in a given sample, if the effect is apparent only among individuals exposed to specific environmental risks. With regard to research on child health, knowledge about specific genetic risks may help to clarify risk processes. Numerous biological and psychological processes have been put forward to explain why and how experiences of maltreatment are converted into antisocial behavior toward others (*17*, *24*, *31*–*34*), but there is no conclusive evidence that any of these processes can account for the progression from childhood maltreatment to later criminal violence. Moreover, some youngsters make the progression, but others do not, and researchers have sought to understand why (*35*). The search has focused on social experiences that may protect some children, overlooking a potential protective role of genes. Genes are assumed to create vulnerability to disease, but from an evolutionary perspective they are equally likely to protect against environmental insult (*36*). Maltreatment studies may benefit from ascertaining genotypes associated with sensitivity to stress, and the known functional properties of *MAOA* may point toward hypotheses, based on neurotransmitter system development, about how stressful experiences are converted into antisocial behavior toward others in some, but not all, victims of maltreatment.

Until this study's findings are replicated, speculation about clinical implications is premature. Nonetheless, although individuals having the combination of low-activity *MAOA* genotype and maltreatment were only 12% of the male birth cohort, they accounted for 44% of the cohort's violent convictions, yielding an attributable risk fraction (11%) comparable to that of the major risk factors associated with cardiovascular disease (*37*). Moreover, 85% of cohort males having a low-activity *MAOA* genotype who were severely maltreated developed some form of antisocial behavior. Both attributable risk and predictive sensitivity indicate that these findings could inform the development of future pharmacological treatments.

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30. This study focused on males because their single X chromosome yields two straightforwardly characterized *MAOA* genotypes: high activity (63% in this sample) and low activity (37%). Females, having two copies of the X chromosome, fall into two homozygous groups, high-high (42% in this sample), low-low (12%), and a third heterozygous group, low-high (46%), that cannot be characterized with certainty because it is not possible to determine which of the two alleles is inactivated for each female participant. Given the rarity in females of both the low-low genotype (12%) and severe antisocial outcomes, such as violent conviction (2%), our cohort of 481 females, 11% of whom were severely maltreated, was too small to support all of the analyses reported here for males. However, adolescent conduct disorder could be analyzed, revealing that girls with the low–*MAOA* activity genotype were more likely to develop conduct disorder by a significant odds ratio of 5.5 (95% CI: 1.0 to 32.0) if they were maltreated. In contrast, among girls with high *MAOA* activity, maltreatment did not confer significant risk for conduct disorder (OR = 1.7, 95% CI: 0.75 to 4.2). This suggests that high *MAOA* activity exerts a protective influence against maltreatment for girls as well as boys, and raises the possibility that further research into X-linked genotypes may help to explain one of the least understood facts about serious antisocial behavior: the sex difference (*38*).
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Supporting Online Material
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Materials and Methods
Tables S1 and S2

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Cytomegalovirus Recruitment of Cellular Kinases to Dissolve the Nuclear Lamina

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The passage of large-sized herpesviral capsids through the nuclear lamina and the inner nuclear membrane to leave the nucleus requires a dissolution of the nuclear lamina. Here, we report on the functions of M50/p35, a β-herpesviral protein of murine cytomegalovirus. M50/p35 inserts into the inner nuclear membrane and is aggregated by a second viral protein, M53/p38, to form the capsid docking site. M50/p35 recruits the cellular protein kinase C for phosphorylation and dissolution of the nuclear lamina, suggesting that herpesviruses target a critical element of nuclear architecture.

Viral genes can target and abuse specific cellular functions for generating a favorable environment for virus maintenance and spread. Well-known examples are viral gene functions for immune evasion from host cell defense or capsid movement within host cells (*1, 2*). To identify new viral functions that target or divert cellular functions, we addressed an important step during morphogenesis: the exit of the newly formed virus capsid from the nucleus. Because the size of herpesvirus capsids (~100 nm) prevents their transport through the nuclear pore complex (NPC), egress requires the penetration of the nuclear envelope (NE) (*3*). Local duplications of the nuclear membrane and patches containing wrapped viral capsids have been observed in cells infected with cytomegalovirus (CMV), which belongs to the subgroup of β-herpesviruses (*4, 5*). However, the inner nuclear membrane (INM) is not easily accessible; it is lined and stabilized by the nuclear lamina (NL) layer, which constitutes an orthogonal filamentous protein meshwork 20 to 80 nm deep. This meshwork is a barrier to capsid budding, but is only dissolved during mitosis by phosphorylation with specific kinases. Here, we present evidence that a virus gene product with homology to resident INM proteins can breach this barrier by recruiting cellular kinases that act to dissolve the obstacle.

In CMV-infected cells, modifications of the NE can be detected by an irregular, sometimes ruffled staining for lamins and a non-uniform lamin-associated polypeptide 2β (LAP2β) staining, as well as by deformation of the nucleus (*4, 5*). To identify the viral gene(s) responsible for this phenotype, we subcloned a series of candidate open reading frames (ORFs) of murine CMV (MCMV) into a eukaryotic expression vector and then screened the ORFs for this phenotype in transient assays. This approach led to the identification of M50, which is predicted to be a type II transmembrane protein 35 kD in size (p35) (Fig. 1A). M50/p35 showed a rim staining, typical for proteins of the INM such as lamins and LAP2β (Fig. 1, B to E, I, J, N, and O). Moreover, in both transfected and CMV-infected cells, M50/p35 was localized in membranous cytosolic structures occasionally containing lamin B. Remarkably, cells expressing only M50/p35 in the absence of any additional viral protein showed substantial alterations of the NE, characterized by exclusion of lamins A, B, and C as well as LAP2β in areas of the INM where M50/p35 accumulated. A similar phenotype was observed after expressing UL50, the homologous protein encoded by human CMV (HCMV) (*6*). Cells were costained for M50/p35 and propidium iodide to test whether the induced phenotype represented a proapoptotic event. In M50/p35-positive cells, no chromatin condensation (Fig. 1, K to M) and no staining for terminal deoxynucleotidyl transferase–mediated nick-end labeling (TUNEL) (*6*) were detected 24 hours after transfection, excluding apoptosis. Nonetheless, the M50/p35-induced phenotype is probably incompatible with normal cellular functions, as we failed to isolate stable transfectants. Remarkably, the extent of the M50/p35-induced alterations of the NE differed from those seen in CMV-infected cells (Fig. 1, F to H).

In infected cells, M50/p35 was concentrated in distinct, smaller aggregates associated with the NE. In addition, cytosolic M50/p35 positive structures were less frequent, smaller, and more uniform in size. We therefore tested whether additional viral proteins modulate the M50/p35 distribution to induce the infection phenotype. This led to the identification of M53/p38. Whereas M53/p38 is diffusely distributed within the nucleus if expressed alone (Fig. 2, D to F), in cells cotransfected with M50/p35 and M53/p38 the localization of both viral proteins was changed, resulting in a phenotype indistinguishable from infection (Fig. 2, G to I). The direct interaction of the two proteins was confirmed by coprecipitation (Fig. 2J). Thus, we propose that M50/p35 strongly modifies the NL, whereas M53/p38 qualitatively and quantitatively modulates this effect.

The morphological stability of the NE critically depends on the integrity of the NL. Depolymerization of the NL preceded by a phosphorylation of lamins is required for the major architectural changes of the NE during mitosis (*7*). Phosphorylation and dephosphorylation of lamins and proteins of the INM by different kinases at distinct sites regulate the dynamic properties of the NL during interphase and mitosis (*8–11*). In a cell cycle–dependent manner, NL dissolution during mitosis requires the sequential phosphorylation by protein kinase C (PKC) during the interphase and by $p34^{cdc2}$ during mitosis (*12*). We thus tested whether kinases are also involved in NL dissolution during MCMV infection. By immunofluorescence we examined different kinases for a redistribution to the NL in MCMV-infected cells. Among the kinases tested, only Ca-dependent PKCs were found to be redistributed to the NE by viral proteins (*13*). Because the distribution of Ca-dependent PKC during MCMV infection correlated well with the localization of M50/p35, we hypothesized that M50/p35 recruits Ca-dependent PKCs to the INM. In cells transiently transfected with M50/p35 and M53/p38, Ca-dependent PKCs were recruited to the NE (Fig. 3, A to C). In MCMV-infected cells, M50/p35 colocalized with M53/p38 and Ca-dependent PKC (Fig. 3, G to I) but

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Role of Genotype in the Cycle of Violence in Maltreated Children

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