

Nature and Nurture Predispose to Violent Behavior: Serotonergic Genes and Adverse Childhood Environment

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Aggressive behavior is influenced by variation in genes of the serotonergic circuitry and early-life experience alike. The present study aimed at investigating the contribution of polymorphisms shown to moderate transcription of two genes involved in serotonergic neurotransmission (serotonin transporter, *5HTT*, and monoamine oxidase A, *MAOA*) to the development of violence and to test for gene–environment interactions relating to adverse childhood environment. A cohort of 184 adult male volunteers referred for forensic assessment participated in the study. Each individual was assigned to either a violent or a nonviolent group. Logistic regression was performed and the best-fitting model, with a predictive power of 74%, revealed independent effects of adverse childhood environment and *MAOA* genotype. High environmental adversity during childhood was associated significantly with violent behavior. Forty-five percent of violent, but only 30% of nonviolent individuals carried the low-activity, short *MAOA* allele. Most interestingly, an interaction effect between childhood environment and *5HTT* genotype on violent behavior was found in that high adversity during childhood impacted only the later-life violence if the short promoter alleles were present. These findings indicate complex interactions between genetic variation of the serotonergic circuitry and environmental factors arguing against simplistic, mono-causal explanations of violent behavior. *Neuropsychopharmacology* (2007) **32**, 2375–2383; doi:10.1038/sj.npp.1301359; published online 7 March 2007

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INTRODUCTION

Aggression comprises heterogeneous neurobiological features and phenomenology. In consideration of apparently qualitative subtypes of aggression, a sensible construct was made in hypothesizing a dichotomy between an impulsive-reactive-hostile-affective subtype (defensive aggression) and a controlled-proactive-instrumental-predatory subtype (offensive aggression) (Vitiello and Stoff, 1997). Although most neurobiological studies of aggression and violence unfortunately do not differentiate between defensive and offensive aggression, this distinction may be relevant in understanding the neurobiological underpinnings of aggressive behaviors, even more so they are influenced by anatomically and functionally distinct neural circuits (Lesch and Merschdorf, 2000). It is believed that the propensity for

impulsive aggression, which is relatively unplanned and spontaneous but often culminates in physical violence, is associated with a low threshold for activating negative affect and with a failure to respond appropriately to the anticipated harmful consequences of behaving aggressively. Socio-psychological research underscores the relation between cognition, emotion, and aggression; negative affect such as fear and anxiety frequently precipitates, accentuates, and modulates aggressive behavior. Thus, it appears reasonable that neural circuitries that affect emotional states, like the central serotonergic system, also affect the predisposition towards aggressive behaviors.

Not only the genetic make-up, but environmental factors also influence human behavior. It is well known that early-childhood environment also influences the later-life predisposition toward violent behaviors. Longitudinal studies assessing the development of psychopathology have replicated the strong influence of adverse parental rearing on externalizing and aggressive behavior in children (Blanz *et al*, 1991; Deater-Deckard *et al*, 1998; Fergusson *et al*, 1996). Oppositional and aggressive behavior in childhood is predictive of according offensive, aggressive and antisocial behavior in adulthood (August *et al*, 1999; Barkley *et al*, 2004;

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Lahey *et al*, 2005) as are adverse family circumstances like childhood maltreatment (Fergusson and Lynskey, 1997).

Gene–environment interactions in the development of aggressive behavior were demonstrated for child maltreatment and the monoamine oxidase A (*MAOA*) gene (Caspi *et al*, 2002; Foley *et al*, 2004; Huang *et al*, 2004). *MAOA* degrades monoamine neurotransmitters thus terminating their availability. A repeat length polymorphism (*MAOA-uVNTR* (*MAOA*-variable number of tandem repeats)) in the promoter region consisting of a 30-bp repeat element with 3, 3.5, 4, 5 or (rare) 6 copies was described (Sabol *et al*, 1998). The 3-repeat allele results in significantly decreased expression of *MAOA* as compared to longer alleles (Deckert *et al*, 1999); the latter also resulting in higher cerebrospinal fluid (CSF) homovanillic acid levels, arguing for functional metabolic consequences of this polymorphism (Zalsman *et al*, 2005). For association studies, hence, *MAOA-uVNTR* has been dichotomized in short low-activity (3-allele) and long high-activity (3.5, 4, 5-allele) variants.

First evidence for an involvement of *MAOA* in aggression came from two different lines of evidence: in a large kindred, a rare stop mutation of *MAOA* co-segregated with aggressive behaviors in affected males (Brunner *et al*, 1993). Likewise, mice with a targeted deletion of *MAOA* show significantly increased aggressive behavior in several paradigms (Cases *et al*, 1995). Following the discovery of functional *MAOA* variants, the impact of these polymorphisms on human aggression and related behaviors was investigated. Initial studies (Samochowiec *et al*, 1999; Schmidt *et al*, 2000) argued that antisocial personality disorder in alcoholism was connected to the low-expression genotype, although this was not replicated in two rather small studies (Koller *et al*, 2003; Lu *et al*, 2003). Convincing evidence, however, supports the notion that *MAOA-uVNTR* regulates aggression and impulsivity in a gene–environment-dependent manner. A pilot study on 110 healthy males demonstrated an association of short *MAOA-uVNTR* alleles with lower levels on a composite measure of aggression and impulsivity (Manuck *et al*, 2000). Data from the Dunedin longitudinal study (Caspi *et al*, 2002) suggested that childhood maltreatment is a risk factor for conduct disorder, antisocial personality disorder, and disposition toward as well as execution of violence. High *MAOA* activity, that is, long *MAOA-uVNTR* alleles, protected against this in the presence of childhood maltreatment or, conversely, short *MAOA-uVNTR* alleles predisposed toward violent behavior in the case of maltreatment only. Likewise, low-activity *MAOA* alleles could be found more often in conduct disorder exclusively in the presence of adverse childhood environment (Foley *et al*, 2004). A Swedish study (Nilsson *et al*, 2006) recently demonstrated that the short *MAOA-uVNTR* allele interacted with adverse psychosocial risk factors during adolescence to result in criminal behavior in general, but also specifically with violence. Conflicting with some of these findings, short *MAOA-uVNTR* alleles in turn appear to be associated with a history of childhood abuse and with higher impulsivity measures, but not with Brown-Goodwin Aggression Scale scores (Huang *et al*, 2004). A further study failed to replicate the Dunedin findings, although there was a nonsignificant trend (Haberstick *et al*, 2005). Analogous to humans, rhesus monkeys—which feature a polymorphic *VNTR* in their *MAOA* regulatory region as well (Wendland *et al*,

2006)—show higher aggression levels in the presence of low-activity alleles, however, this was significant only when monkeys were mother- and not peer-reared (Newman *et al*, 2005).

The serotonin transporter (5HTT) is the key molecule in regulating the availability of synaptic serotonin and thus crucially balances serotonergic tone. In subjects displaying impulsive, offensive aggression, 5HTT availability is significantly reduced in the anterior cingulate cortex arguing for a role of 5HTT in violent behaviors (Frankle *et al*, 2005). Most interestingly, mice lacking 5HTT feature lower levels of aggression, further underscoring this notion (Holmes *et al*, 2002). A length variation of a repeat in the transcriptional control region of the *5HTT* gene serotonin transporter repeat length polymorphism (5HTTLPR) regulates expression and serotonin (5HT) uptake, with short alleles (s, as opposed to long, l) resulting in lower transcriptional activity and consequently decreased re-uptake of the transmitter. The polymorphism has been shown to be associated with neuroticism (Lesch *et al*, 1996) especially in patients with cluster C personality disorders (Jacob *et al*, 2004) and, in a gene–environment-dependent manner, with depression (Caspi *et al*, 2003). Despite the vast literature on the connection between serotonin and aggression (Lesch and Merschedorf, 2000), studies investigating the role of 5HTTLPR genotype on offensive aggression remain sparse. In heroin addicts, the s/s genotype was associated with violent offence, but not Buss-Durkee Hostility Inventory scores (Gerra *et al*, 2004). Additional data also pointed toward an association of the s/s genotype with violent crime (Liao *et al*, 2004), and a study from our group demonstrated that the s allele was found significantly more often in violent-crime offenders especially when attention deficit/hyperactivity disorder (ADHD) psychopathology was present, explaining 5% of the genetic variance of violent behavior (Retz *et al*, 2004).

The dopamine transporter (DAT) regulates dopaminergic tone and is another candidate gene for neuropsychiatric disorders. A *VNTR* in the 3'-untranslated region of *DAT* has been suggested to influence *DAT* mRNA stability (Michelaugh *et al*, 2001), expression (Fuke *et al*, 2001), as well as transporter availability as assessed by single photon emission computed tomography (SPECT) imaging (van Dyck *et al*, 2005). No study on the interaction of this genetic variant with adverse family environment on aggressive and violent behavior in adulthood has been carried out to date. As *MAOA* and *5HTT* have repeatedly been associated with aggressive behaviors, we sought to examine whether *MAOA-uVNTR*, 5HTTLPR, and *DAT* alone or in combination are associated with violent criminality in imprisoned subjects. Furthermore, the impact of adverse childhood environment on later violent criminality and a possible interaction with these genotypes was investigated as it was previously suggested that *MAOA* genotype exerts its effect on violent or antisocial behavior only following childhood maltreatment.

MATERIALS AND METHODS

Subjects

A sample of 184 adult male, caucasian volunteers, referred for a forensic examination to the Institute of Forensic

Psychiatry of the University of the Saarland by legal authorities for evaluation of legal responsibility or risk assessment, entered the study. Only subjects who gave written informed consent after explanation about scope and aim of the investigation were enrolled. Mean age was 34.1 years (SD, 11.7 years). Subjects with a diagnosis of current substance dependence, acute schizophrenia, major depression/bipolar disorder, or any other severe Axis-I diagnosis according to DSM-IV as well as mentally retarded subjects (IQ < 70) were not included. Psychiatric lifetime diagnoses according to DSM-IV criteria comprised substance-use disorders (46.2%), psychotic disorders (substance-induced or because of general medical conditions; under stable remission) (8.2%), paraphilias (4.5%), affective disorders (2.2%), neurotic disorders and disorders of impulse control (3.8%). Cluster B personality disorders were present in 27.2%, personality disorders of Clusters A and C in 7.6% of the sample. As expected in an offender population, the educational and professional status of most of the subjects was low (special education, school dropout 13.4%, junior high school/<8 classes 70.1%, high school/>8 classes 16.5%, completed professional training 55.5%, no professional training 44.5%) and the rate of unemployment was high (employed 39.3%, unemployed 60.8%). In all, 20.1% were married or lived in stable partnerships, 23.8% were divorced, and 56.1% never married.

All subjects underwent a semi-structured psychiatric interview according to the assessment and documentation in physical psychopathology (AMDP) system (AMDP, 2000) by well-trained psychiatrists, and a neurological examination. Childhood symptoms of ADHD were assessed using the German short version of the Wender Utah Rating Scale (WURS-k), current ADHD symptoms by the ADHD-SB (Retz-Junginger *et al*, 2003; Rösler *et al*, 2004). Each subject was assigned to a violent and a nonviolent group according to his policy record and current crimes based on a careful evaluation of all available documents. Violence, as an outcome variable of predisposition toward offensive aggression, was defined as an abiding 'overt and intentional physically aggressive behavior against another person' as suggested by Volavka (1999) and adopted by an expert consensus (Filly *et al*, 2001). By this means, only the subjects with habitual aggressive and violent behavior were included in the violent group, whereas offenders with a history of only nonviolent crimes were assigned to the nonviolent group. Subjects with no explicit preponderance of either criterion (eg, one severe violent crime) were not recruited for this study. Reliability of phenotype assessment (violent or nonviolent) between different raters (MR, WR) was tested in a subset of 25 subjects. Inter-rater reliability was 0.92 (assessed by Cohen's Kappa). Criminal offences of the participants of the study comprised homicide, physical injury, and robbery ($n=75$), property offenses and fraud ($n=41$), sexual offenses ($n=33$), drug offenses ($n=12$), and others ($n=67$). Furthermore, DNA samples were collected from 150 male control subjects (mean age 34.0 ± 11.4 years) consisting of healthy blood donors from Germany; the sample was not screened for a history of psychiatric disorders, ADHD, or violent behavior and constitutes a population-based control sample. All control subjects, however, were free of any regular medication, and the study was explained to them. The study was approved

by the Ethics Committee of the University of the Saarland and the University of Würzburg.

Life History

All forensic participants of the study were rated by an independent investigator (DW), who was blinded to the diagnostic status and genotype of the probands, with regard to the following criteria toward childhood (defined as 0–10 years) environment: social status, family structure, emotional family climate, social integration, and school education. The items were graded from 0 to 2 points, with higher ratings indicating worse environment conditions (Table 1). A mean score of adverse childhood environment (childhood adverse environment index, CAEI) was calculated for each subject, giving a relative total adverse environment index of 0 (optimal childhood environment) to 2 (most adverse childhood environment).

Genotyping

Venous blood was drawn and DNA was extracted using a standard method. MAOA-uVNTR and the 5-HTTLPR were genotyped using previously published protocols (Deckert *et al*, 1999; Lesch *et al*, 1996). PCR of the DAT VNTR was carried out in a 25 μ l volume containing 20 ng genomic DNA and 0.4 μ M primers. The amplification protocol consisted of 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, 67.5°C for 45 s and 72°C for 45 s. PCR products were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide and visualized by UV transillumination. Primers as well as detailed PCR conditions are available on request.

Statistical Analysis

Owing to their proposed functionality and analogous to previous studies, MAOA-uVNTR, DAT VNTR, and 5HTTLPR were assessed as dichotomized alleles: MAOA short alleles (ie 3 repeats) were compared to MAOA long alleles (ie 4 or 5 repeats). For 5HTTLPR, genotypes s/s and s/l were grouped together (group S; Lesch *et al*, 1996) and compared to the l/l genotype (group L) owing to their functionality. For the DAT VNTR, genotypes 9/9, 9/10, and 9/11 were compared with genotypes 10/10 and 10/11. All polymorphisms were in Hardy–Weinberg equilibrium. Forensic subjects were compared with regard to the presence or absence of violent behavior as described above. Univariate, exploratory assessment of possible confounding variables on the presence of violence (age, history of personality disorder, history of drug addiction, ADHD symptoms in childhood and at present) was performed by χ^2 -, *t*- or Wilcoxon rank-sum tests. A *p*-value < 0.10 was set as a threshold for association of violence with possible confounding variables. These confounding variables were controlled for in the stepwise logistic regression procedure performed to analyze the impact of genotypes and adverse childhood experience on violent behavior. Violent behavior was the dependent, and the dichotomized genotypes (MAO-A, DAT, 5-HTTLPR), adverse childhood experience (continuous variable) and interaction terms of genotypes as well as adverse childhood environment the independent

Table 1 Standardized Assessment of Life History, of which the CAEI Score (Ranging from 0 to 2) is Derived

				Possible	Definite	Unknown
F1. Social status, life standard	High	□□	Low	□□	□□	□□
	No financial problems, parents employed, secured living	0	Low family income, unemployment, welfare, single parenthood etc.	1	2	?
F2. Family structure	Well-ordered	□□	Labile	□□	□□	□□
	Grown up in reliable family structure, constant attachment figures, together with siblings	0	Early separation (nursing home, foster parents), frequently changing attachment figures, parents divorced / frequent change of partner	1	2	?
F3. Emotional family climate	No violence	□□	Exposure towards violence	□□	□□	□□
		0	Being violently assaulted, violence within the family, family members delinquent	1	2	?
F4. Social integration	Good	□□	Bad	□□	□□	□□
	No problems in kindergarten and school, in clubs, etc.	0	Only child, little contact to other children, rounder	1	2	?
F5. School education	Regular	□□	Irregular	□□	□□	□□
	Regular school education	0	Frequent change of schools, repeater, school termination	1	2	?

variables of interest. If an interaction term did not show an influence on the dependent variable violent behavior, it was successively taken off the model. Possible confounding variables (continuous variable age; dichotomized variables, ie presence or absence of history of personality disorder and history of drug addiction) remained in the model. The results of the final model are reported. From this model, mean cross-validation probability of violent behavior was calculated for different combinations of risk factors (dichotomized MAOA uVNTR, 5HTTLPR, and CAEI), which were compared in *post hoc* analyses by χ^2 - and Wilcoxon rank-sum tests. Univariate tests were performed similarly. No adjustment of multiple testing was made as, initially, all variables were assessed in one model, and *post hoc* analyses were performed only on variables which had shown an effect in the logistic regression model. Statistical analyses were performed by the SAS statistical package (SAS/STAT version 8.2. SAS Institute Inc., Cary, NC, USA, 1999–2001).

RESULTS

Of the 184 subjects undergoing forensic assessment, 72 (39.1%) had a history of recurrent violent behavior. Violent behavior was somewhat less common in older subjects (mean age violent behavior: 32.1 ± 12.1 years; nonviolent behavior: 35.4 ± 11.3 years; $t = -1.8$, $p = 0.07$). Individuals with a history of drug abuse were more violent (54.2%) compared with those without a history of drug abuse (37.5%, $\chi^2 = 4.9$, $p = 0.03$). Genotype frequency, however, did not differ in individuals with history of substance

abuse compared with individuals without (MAOA-uVNTR: $\chi^2 = 0.4$, $p = 0.516$; DAT: $\chi^2 = 0.0$, $p = 0.850$; 5-HTTLPR: $\chi^2 = 1.7$, 1DF, $p = 0.187$). Personality disorders were more common in violent (43.0%) than in nonviolent individuals (29.5%; $\chi^2 = 3.6$, $p = 0.06$). Genotype frequency, again, did not differ in individuals with history of personality disorder compared with the individuals without (MAOA-uVNTR: $\chi^2 = 0.1$, $p = 0.723$; DAT: $\chi^2 = 0.0$, $p = 0.851$; 5-HTTLPR: $\chi^2 = 0.8$, $p = 0.383$). No difference in self-reported childhood or current ADHD symptoms was found between violent and nonviolent subjects. Therefore, age, history of substance abuse, and history of personality disorder were controlled for in further analyses. In 169 of the 184 individuals, complete datasets on environmental risk factors and genotypes were available.

The best-fitting model did show a predictive power of 74% and is shown in Table 2. With regard to nongenetic factors, adverse childhood environment, as assessed by the CAEI, was significantly associated with later-life violent offence. DAT did neither show an influence on violent behavior nor did it interact with MAOA regarding violent behavior (interaction term 'MAO-A*DAT' not shown). The short alleles of MAOA were associated with violent behavior (OR = 2.3). No independent association of 5HTTLPR, genotypes with violent behavior was found, however, a strong interaction of this polymorphism with childhood environmental risk factors was elicited (Wald $\chi^2 = 6.5$, $p = 0.011$). The interaction between MAOA-uVNTR, 5HTTLPR, and childhood environmental factors are depicted and explained in Figure 1, Tables 2 and 3. Childhood environmental risk factors did only show an influence on violent behavior in the presence of the ss and sl genotypes,

Table 2 Association of Childhood Environment and Genetic Polymorphisms Controlled for Age, Substance Use, and Personality Disorders

Risk factor	β -Estimate (SE)	Wald χ^2 (1 DF)	p-Value	Odds ratio (95% CI)
Childhood environment	1.42 (0.45)	10.2	p = 0.001	4.1 (2.6–6.5)
MAO-A uVNTR short allele vs long alleles	0.82 (0.37)	4.9	p = 0.027	2.3 (1.1–4.7)
5HTTLPR ll vs sl+ss	0.01 (0.50)	0.0	p = 0.980	1.0 (0.6–1.6)
5HTTLPR*childhood environment	-1.65 (0.65)	6.5	p = 0.011	0.2 (0.1–0.4)
DAT short vs long alleles	0.54 (0.35)	2.3	p = 0.127	1.7 (0.9–3.4)
No vs history of substance abuse	-0.76 (0.36)	4.4	p = 0.036	0.5 (0.2–1.0)
NO vs history of personality disorder	-0.11 (0.38)	0.1	p = 0.778	0.9 (0.4–1.9)
Age	-0.01 (0.02)	0.6	p = 0.436	1.0 (1.0–1.0)
Intercept	-0.9 (0.48)	3.6	p = 0.056	

CI, confidence interval; DF, degree of freedom.

Test of global null hypothesis: Wald $\chi^2 = 24.9$, 8 DF, $p = 0.002$.

Bold values indicate significant results.

but not in the presence of ll, that is, the L genotype was protective against the influence of childhood adversity with respect to later-life violent behavior. For *post hoc* analyses, the CAEI was dichotomised at the cutoff of 0.4, which represents the median value of the full sample. High CAEI, therefore, has been assumed for a CAEI > 0.4, and low CAEI has been assumed for CAEI ≤ 0.4. Mean CAEI was 0.7 ± 0.6 in violent, and 0.4 ± 0.6 in nonviolent individuals, which was significant in *post hoc* univariate analyses ($Z = 3.0$, $p = 0.003$). This also revealed that MAO-A short alleles ($n = 31$ in 72 violent individuals vs $n = 32$ in 112 nonviolent individuals) were significantly associated with violent behaviors ($\chi^2 = 4.1$, $p = 0.043$). When mean cross-validation probabilities were computed, carrying the short MAOA and 5HTT alleles and having been exposed to childhood environment, most strongly predicted violent behavior (Table 3).

MAOA-uVNTR, 5HTTLPR, and DAT genotypes have furthermore been determined in a population-based control sample with unknown childhood environment; all genotypes were not significantly different from the forensic sample as a whole (MAOA-uVNTR, short alleles 33%; 5HTTLPR L group 33%; DAT 9/9 + 9/10 alleles 49%; all $p > 0.1$) arguing that the forensic sample as a whole is genetically homogenous to its provenance population.

DISCUSSION

The major findings of the present study are (a) MAOA genotype and adverse childhood environment independently of each other increased the risk for later-life violent behavior, (b) 5HTT polymorphism was associated with violence in a gene–environment-dependent manner, ie the ll/l genotype was protective against childhood adversity, and (c) a DAT variation does not seem to play a role in the disposition toward violence.

MAOA-uVNTR has repeatedly been suggested to be involved in the regulation of impulsivity and aggression, both in primates and in humans. This regulatory polymorphism is thought to be an example of balanced selection, that is, it shifts the predisposition of its carrier either toward offensive, risk-taking behavior or toward

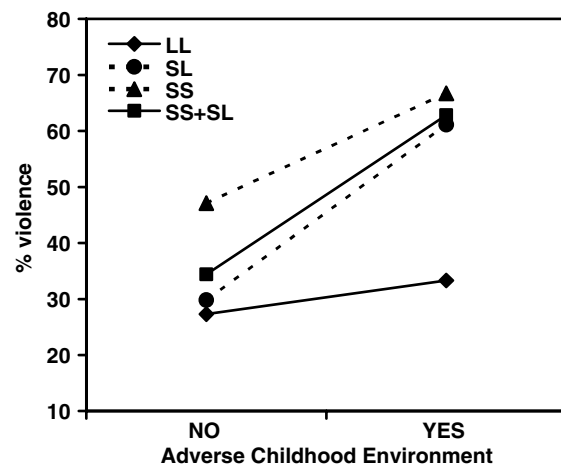


Figure 1 A 5HTT polymorphism is associated with violence in a gene–environment interaction manner. Subjects were stratified for the presence or absence of adverse childhood environment; the cutoff was 0.4 according to the median adverse environment score. Probands which were homozygous for long alleles were less prone to commit violent crime as opposed to heterozygous and homozygous short-allele carriers. In homozygous long allele carriers, adverse childhood environment did not increase violent behavior, whereas in homozygous and even more so in heterozygous short-allele carriers, adverse childhood environment did significantly increase the rate of violent behavior indicating a gene–environment interaction of 5HTTLPR.

defensive subordination. Obviously, both of these opposites have evolutionary advantages as well as disadvantages leading to the preservation of respective ‘risk’ alleles. Given that the effects of MAOA-uVNTR rely on this polymorphism and not other genetic variations, it appears to be a forceful moderator of aggressive behaviors. This influence was suggested to depend on environmental factors: especially when adverse childhood conditions exist, short MAOA-uVNTR alleles may increase the risk for higher childhood (Foley et al, 2004) as well as later-life (Caspi et al, 2002; Huang et al, 2004) aggression and impulsivity. Contrasting these investigations, we found that MAOA genotype independently of childhood experiences, increased the risk to commit violent crime. Likewise, Haberstick et al (2005) found no interaction of MAOA and

Table 3 Mean Probabilities for Presence of Violence According to Logistic Regression Model in Eight Different Risk Groups

No.	Risk groups	Mean cross-validation probability for presence of violence	SD	Number of individuals in risk group	Difference to groups ^a (group numbers)
1	MAO-A = short allele 5-HTTLPR = ss or sl CAEI > 0.4	0.78	0.11	14	2,3,4,5,6,7,8
2	MAO-A = short allele 5-HTTLPR = ss or sl CAEI ≤ 0.4	0.43	0.12	26	5,6,7,8
3	MAO-A = short allele 5-HTTLPR = ll CAEI > 0.4	0.46	0.12	5	5,6,7,8
4	MAO-A = short allele 5-HTTLPR = ll CAEI ≤ 0.4	0.44	0.17	11	5,6,7,8
5	MAO-A = long allele 5-HTTLPR = ss or sl CAEI > 0.4	0.58	0.16	36	1,2,3,4,6,7,8
6	MAO-A = long allele 5-HTTLPR = ss or sl CAEI ≤ 0.4	0.29	0.12	38	2,3,4,5
7	MAO-A = long allele 5-HTTLPR = ll CAEI > 0.4	0.22	0.07	18	2,3,4,5
8	MAO-A = long allele 5-HTTLPR = ll CAEI ≤ 0.4	0.24	0.10	21	2,3,4,5
	Statistic across groups	Kruskal–Wallis- $\chi^2 = 103.2$ (7 DF) p -value < 0.0001			

CAEI, childhood adverse environment index; SD, standard deviation of the mean.

A cross validation probability of 1 would fully predict violent behavior; whereas a probability of 0 would fully predict no violent behavior.

^aDifference assessed by Wilcoxon two-sample test; criterium $p < 0.05$.

childhood maltreatment, but also no effect of *MAOA* genotype alone on a composite index of antisocial behavior. Explanations for these discrepant findings are manifold: first, our main outcome variable is habitual violent behavior. Antisocial behavior or conduct disorder include these but also a number of other behaviors, thus being a much broader defined measure with a corresponding loss of discriminatory power. Likewise, impulsivity scores and related questionnaires differ from our categorical division. Moreover, impulsivity and aggression are distinct behaviors which share neurobiological mechanisms only partially. A further difference is the assessment of childhood environment. Our study has the disadvantage of retrospective data collection and bases presumably on self-report rather than on established adversity measures. However, the finding that our adversity score was associated with violent crime—in accordance with most other studies related to this topic—argues for the construct validity of this score.

Another study (Nilsson *et al*, 2006) also demonstrated that both criminal behavior in general as well as violence were influenced by *MAOA* genotype only in the presence of psychosocial risk factors (maltreatment and living in a multi-family house). Comparing this to our study, we

investigated a wider range of risk factors; furthermore, the number of subjects in each class was relatively small in the Swedish study, which dealt exclusively with adolescents. Violent behavior observed during puberty might constitute something different than abiding violent behavior in adulthood; furthermore, it is well known that genetic influences become more important with increasing age which may, in part, explain the contrasting findings.

Apart from these methodological differences, data suggesting an interaction of *MAOA* genotype with environment is not so consistent as it seems at first. The study by Huang *et al* (2004) demonstrated an association of *MAOA-uVNTR* with the experience of childhood abuse (not evident in other studies (Caspi *et al*, 2002; Haberstick *et al*, 2005)) relying on a small number of subjects and, likewise, their finding on increased BIS scores in maltreated *MAOA* short allele carriers is of borderline significance and based on few subjects ($n = 26$ and 18 , respectively). Similarly, Foley *et al* (2004) reported that short *MAOA-uVNTR* alleles result in conduct disorder in interaction with exposure to lifetime adversities. This finding derived mainly from those subjects having three or four lifetime adversities, comprising 20 subjects, six of which having the short *MAOA* allele, and

four of which were suffering from conduct disorder. At this extreme end of the scale, gene–environment interaction may be present although not evident in our sample. In a sub-analysis of the study by Caspi *et al* (2002), conviction for violence was examined allowing comparison with our findings; again, the finding on a gene–environment interaction resulted from approximately 15 subjects convicted in the short MAOA allele group with childhood maltreatment (from a total of 55 in this group). Thus, owing to this relatively small sample size, we agree with the authors that these findings need to be considered as preliminary.

Despite the limitations of the present study, that is, retrospective assessment of childhood environment, its major advance is that a higher number of subjects of the main outcome variable, that is, violence ($n=72$), was examined as compared to previous studies, increasing the power to detect gene–environment interactions. In our sample, presence of the short MAOA allele neither predisposed to a more adverse childhood environment, nor did it exert its influence only under certain and given environmental conditions. Thus, carrying a short allele of the MAOA-uVNTR acted as a true and independent risk factor for later-life aggression, further adding to the risk conveyed by childhood maltreatment. Work from our group has previously shown that not only aggression, but also other domains of disruptive, outward-bound behavioral traits seem to be under partial genetic influence of MAOA-uVNTR (Jacob *et al*, 2005). Individuals with cluster B, but not cluster C personality disorders carried short alleles more frequently, that is, MAO-A seems to play a regulatory role in the control of socially ‘inappropriate’ out-acting behavior.

A recent fMRI study shed some light on possible neurobiological underpinnings of MAOA genotype on aggressive behavior, demonstrating that the at-risk genotype results in amygdalar hyperreactivity in response to emotional arousal, paralleled by impaired response of prefrontal brain regions including the anterior cingulate cortex (Meyer-Lindenberg *et al*, 2006). Considering the data on reduced 5HTT availability in this structure in aggressive subjects (Frankle *et al*, 2005), serotonin metabolism in the cingulate gyrus seems to be a crucial determinant of disposition toward violent behavior. Accordingly, 5HTT constitutes another candidate gene for violent behavior. A small, yet increasing number of studies also investigated the implications of 5HTT genotype on aggression and demonstrated an association between short 5HTT alleles and violent behavior (Gerra *et al*, 2004; Retz *et al*, 2004; Liao *et al*, 2004), although the latter study conveyed several problems as the polymorphism was not in Hardy–Weinberg equilibrium. Gene–environment interactions have not yet been examined in humans. Here we show that homozygotes for the long allele are generally less likely to develop later-life aggressive behavior, whereas short allele homozygotes behave the opposite way. Most interestingly, only heterozygotes were influenced by environmental factors, that is, this polymorphism might have a role in balancing aggressive behaviors in differing societies. Corresponding to this finding, in rhesus monkeys adverse early environment interacted with the monkey analogue of the 5HTTLPR in exactly the same manner as in the present study (Barr *et al*, 2003), arguing further for the notion that this

polymorphism modulates the response toward adverse environments.

As behavioral traits are quantitative, they are influenced not only by one, but many interacting genes. A sensible rationale to search for epistasis is to investigate gene networks, where several genes interact functionally. Thus, we tested for but failed to detect an interaction of a presumably regulatory DAT polymorphism with MAOA-uVNTR—neither with respect to violence, delinquency, nor ADHD. Also considered independently, the DAT polymorphism did not seem to convey any risk toward any of these traits. Taken together, this suggests that serotonin degradation by MAO-A is the major moderator pathway influencing violent behavior, but not dopamine metabolism. With regard to aggression, DAT genotype has not previously been tested. However, our findings are in line with work in nonhuman primates (Wendland *et al*, 2005) showing that MAOA as well as 5HTT, but not DAT polymorphisms influence predisposition toward aggressive behavior.

No matter whether the genetic association of MAOA-uVNTR and 5HTTLPR with violent crime proves true in replication attempts or not, this does not make a case for an individual being exculpated by his genetic make-up. As opposed to rare cases of deleterious mutations resulting in severe mental disorder, common polymorphisms such as MAOA-uVNTR convey only a statistical predisposition toward a certain behavior. This is proven by the fact that ca. 30% of all males carry short MAOA-uVNTR alleles and are not acting violently. Although many lines of evidence indicate that common polymorphisms act probabilistic rather than deterministic (see also Kendler, 2006 for a thoughtful review on this topic), molecular genetic studies on the neurobiology of aggression and violence are, nevertheless, interesting not only for neuroscientists but also for policymakers and legislators in order to acknowledge gene–environment effects and to create environments protecting against violent behaviors.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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