

## ARTICLE

# The VNTR 2 repeat in MAOA and delinquent behavior in adolescence and young adulthood: associations and MAOA promoter activity

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Genetic studies of delinquent and criminal behavior are rare in spite of the wide recognition that individuals may differ in their propensity for delinquency and criminality. Using 2524 participants in Add Health in the United States, the present study demonstrates a link between the rare 2 repeat of the 30-bp VNTR in the MAOA gene and much higher levels of self-reported serious and violent delinquency. The evidence is based on a statistical association analysis and a functional analysis of MAOA promoter activity using two human brain-derived cell lines: neuroblastoma SH-SY5Y and human glioblastoma 1242-MG. The association analysis shows that men with a 2R report a level of serious delinquency and violent delinquency in adolescence and young adulthood that were about twice (CI: (0.21, 3.24),  $P=0.025$ ; and CI: (0.37, 2.5),  $P=0.008$  for serious and violent delinquency, respectively) as high as those for participants with the other variants. The results for women are similar, but weaker. In the functional analysis, the 2 repeat exhibits much lower levels of promoter activity than the 3 or 4 repeat.

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## Introduction

Studies that investigate the connections between genetic variants and delinquent and criminal behavior in humans have been rare in spite of the wide recognition that individuals may differ in the propensity to commit serious delinquent and criminal acts.<sup>1–3</sup>

The MAOA gene has been a focus in the investigation of aggression in animals and violent behavior in humans. Monoamine oxidase A (MAOA) is one major enzyme that

catalyzes the oxidative deamination of a number of biogenic amines in the brain, including dopamine. Because of its ability to catabolize neurotransmitters, MAOA is frequently a candidate gene in the study of psychiatric diseases and behavioral traits. Evidence that implicates the MAOA gene in aggressive behavior has come from knock-out mouse models and human data. Cases *et al*<sup>4</sup> and Shih and Thompson<sup>5</sup> developed a line of mice with a targeted disruption of the MAOA gene. They observed an increase in the brain levels of dopamine, serotonin, and norepinephrine, and an increase in manifested aggression among men. Brunner *et al*<sup>6</sup> reported mental retardation and impulsive aggression among eight men in an extended Dutch family with an uncommon sex-specific point mutation in the MAOA gene.

The MAOA genomic sequences and promoters<sup>7–9</sup> were studied extensively in search of polymorphisms that might

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be potentially associated with psychiatric disorders and behavioral traits. These basic studies led to a discovery of a 30-bp promoter region VNTR in MAOA, affecting level of transcriptional activity by Sabol *et al.*<sup>10</sup> The PCR product usually consists of five possible fragment sizes that include 2, 3, 3.5, 4, and 5 copies of the repeat sequence; the 3 and 4 repeats are much more common than the 2, 3.5, and 5 repeats in human populations. Sabol *et al.*<sup>10</sup> showed that alleles with 3.5 (3.5R) or 4 (4R) copies of the repeat sequence are transcribed more efficiently than alleles with 3 (3R) or 5 (5R) copies of the repeat. Sabol and colleagues did not examine the MAOA 2R.

Caspi *et al.*<sup>11</sup> did not find a main effect of MAOA variants, but reported that maltreated male children in New Zealand with the 3R or 5R of the VNTR in MAOA were more likely to engage in violent behavior than maltreated children with the 3.5R or 4R of the VNTR. Widom and Brzustowicz<sup>12</sup> reported a replication of these results. Haberstick *et al.*<sup>13</sup> failed to replicate the gene–environment interaction findings in this same Add Health data set used in this study. The present study focuses on the main effects of MAOA variants. Meyer-Lindenberg *et al.*<sup>14</sup> studied the impact of the MAOA VNTR on brain structure and function with MRI in a large sample of healthy human volunteers. The study showed that the low expression variants predict differences in the size of limbic structures such as the amygdala and that men with the low expression variants exhibited increased reactivity of the left amygdala and hippocampus during the recall of aversive information.

The objective of this study is twofold. The First, to investigate the association between the self-reported serious and violent delinquency and the 30-bp VNTR in the MAOA gene in a cohort of 2524 adolescents and young adults in the United States in the National Longitudinal Study of Adolescent Health (Add Health). The specific hypothesis is that the MAOA 2R is associated with higher levels of delinquency. Second, to perform a functional analysis that evaluates the promoter activity in an MAOA 1.3-kb promoter-luciferase construct containing 2-, 3-, and 4-repeat sequences of the 30-bp VNTR, using two human brain-derived cell lines: neuroblastoma SH-SY5Y and human glioblastoma 1242-MG.

## Materials and methods

### Subject

The data source for our analysis is the sibling subsample of 2524 participants in the National Longitudinal Study of Adolescent Health (Add Health), which started as a nationally representative sample of more than 20 000 adolescents in grades 7–12 in 1994–1995 (Wave I) in the United States.<sup>15</sup> Add Health is longitudinal; the respondents have been followed by two additional in-home interviews in 1995–1996 (Wave II) and 2001–2002 (Wave III). Add Health was stratified by region, ethnic mix, size,

**Table 1** Mean (SD), proportion, or frequency of sample characteristics

|                             | Males       | Females     |
|-----------------------------|-------------|-------------|
| <i>Serious delinquency</i>  |             |             |
| Wave I                      | 2.42 (4.31) | 1.14 (2.60) |
| Wave II                     | 1.64 (3.42) | 0.75 (1.85) |
| Wave III                    | 1.20 (2.39) | 0.32 (1.15) |
| <i>Violent delinquency</i>  |             |             |
| Wave I                      | 1.67 (3.11) | 0.70 (1.75) |
| Wave II                     | 1.05 (2.35) | 0.43 (1.75) |
| Wave III                    | 0.71 (1.66) | 0.15 (0.64) |
| <i>Genotype</i>             |             |             |
| Any2R                       | 0.92%       | 2.39%       |
| Only 3R or 5R               | 42.4%       | 17.3%       |
| 3.5R or 4R                  | 56.7%       | 80.35%      |
| <i>Ethnicity</i>            |             |             |
| Caucasian                   | 56.7%       | 57.6%       |
| Non-Caucasian               | 43.2%       | 42.3%       |
| <i>Age (mean; in years)</i> |             |             |
| Wave I                      | 15.6        | 15.5        |
| Wave II                     | 16.57       | 16.42       |
| Wave III                    | 21.34       | 21.29       |
| <i>Sibling type</i>         |             |             |
| MZ pairs                    | 92          | 94          |
| DZ pairs (same sex)         | 184         | 164         |
| Full sib pairs (same sex)   | 97          | 102         |
| Singletons                  | 554         | 504         |
| Sample size                 | 1200        | 1324        |

urbanicity (urban/suburban/rural), and school type (public/private/parochial). Our analysis uses the sibling sample of Add Health because DNA measures collected at Wave III in 2002 are available only for this subset of the Add Health respondents. Table 1 provides the mean (SD), proportion, and frequency of the sample characteristics.

### Measures

We constructed a serious delinquency scale and a violent delinquency scale using the 12 questions asked to all the Add Health respondents at Waves I–III. The questions and scaling weights used to create the scales are given in Appendix 1. These two scales are variations of a widely used type of scales in contemporary research on delinquency and criminal behavior.<sup>16</sup> Our scales are closely related to the scales used by, for example, Hagan and Foster<sup>17</sup> and Haynie<sup>18,19</sup> in the analysis of Add Health data and by Hannon<sup>20</sup> in the analysis of data from the National Longitudinal Study of Youth. Our serious delinquency scale overlaps with the delinquency scale of Hagan and Foster<sup>17</sup> to a substantial extent. As the descriptor suggests, our violent delinquency scale focuses on an array of violent delinquent behavior that could potentially be classified as violent offenses by the criminal justice system.

Following the delinquency literature,<sup>17–19</sup> we divided the 12 items into the nonviolent and violent types. The nonviolent delinquency includes stealing amounts larger or smaller than \$50, breaking and entering, and drug selling. Violent delinquency includes serious physical fighting that resulted in injuries needing medical treatment, use of weapons to get something from someone, involvement of physical fighting between groups, shooting or stabbing someone, deliberately damaging property, and pulling a knife or gun on someone. The serious delinquency scale (nonviolent and violent) is based on the entire 12 items and the violent scale is based on a subset (8) of the 12 items. The Cronbach's  $\alpha$ -values for the serious delinquency scale for Waves I, II, and III are 0.81, 0.79, and 0.73, respectively. For Waves I, II, and III, the Cronbach's  $\alpha$ -values for the violent delinquency scale are 0.75, 0.74, and 0.66, respectively.

Measuring delinquency and crime is challenging. Official measures based on the police reports and the justice system have been long known to substantially underestimate delinquency and crime,<sup>16,21–23</sup> because official measures reflect not only the behavior of offenders, but also the decisions made by the justice system. For these reasons, many criminologists have turned to self-reports in recent decades.<sup>24,25</sup> Self-reports are now a fundamental method of measuring criminality and seem capable of yielding reliable and valid data.<sup>16</sup>

As with any survey of sensitive private information, reporting accuracy is a concern. To protect confidentiality, reduce nonresponses, and increase reporting accuracy, this section of the interview in Add Health was self-administered by audio-CASI (computer-assisted self-interview). The sensitive question was read to respondents by means of audio headphones. Respondents were given instructions by the computer on how to complete their answers. Self-reported rates of illegal and embarrassing behavior are higher when computer-assisted techniques, particularly self-administered techniques, are used.<sup>26,27</sup>

### DNA preparation and genotyping

At Wave III, in collaboration with the Institute for Behavioral Genetics in Boulder (CO, USA), Add Health collected, extracted, and quantified DNA samples from the sibling subsample. Genomic DNA was isolated from buccal cells using a modification of published methods.<sup>28–31</sup> All of the methods employed Applied Biosystems instruments and reagents. Microsatellite and VNTR polymorphisms were performed using fluorescent primers that were analyzed on an ABI capillary electrophoresis instrument. To reduce errors, two individuals independently scored all genotyping. The additional details on DNA collection and genotyping can be found at Add Health website (Smolen and Hewitt, <http://www.cpc.unc.edu/projects/addhealth/>).

The MAOA-uVNTR polymorphism was assayed by a modified method.<sup>10,13</sup> The primer sequences for the

30-bp VNTR in the promoter region of the MAOA open reading frame were: forward, 5'ACAGCCTGACCGTGGAGAAG-3' (fluorescently labeled); and reverse, 5'-GAACGTGACGCTCCATTCCGGA-3'.<sup>10</sup> The reaction yielded five fragment sizes that included 291, 321, 336, 351, and 381 bp (2, 3, 3.5, 4, and 5 repeats, respectively). This analysis focuses on the effect of 2R (291) vs all the other alleles. Only 11 men possess a 2R. These 11 individuals are from 11 families with six from six pairs of DZ twins and five from another five families of full siblings. Of 31 women who possess one or two 2R alleles, six are from three pairs of MZ twins, three from three pairs of DZ twins, and 22 from 19 families of full siblings. We performed a Hardy–Weinberg equilibrium test for the 2R allele among women and obtained a  $\chi^2$ -value of 0.012 for one degree of freedom indicating that the equilibrium is not violated.

### Analytical strategies

To test the associations of the MAOA 30-bp VNTR polymorphisms with serious and violent delinquency, we followed a three-step analytical strategy. The first step is a contingency table analysis in which the mean scores of serious delinquency and violent delinquency across genotypes were compared within each Add Health Wave and gender (Add Health Waves refer to the initial Add Health study in 1994 and two follow-up studies in 1995–1996 and 2002).

The second step is regression analysis. Our sample consists of twins and siblings as well as the repeated observations of the same individual over different Add Health Waves; these observations are not independent. The mixed model has long been established in the statistical literature for the analysis of data that are not independent.<sup>32,33</sup> The following equation describes the basic structure of the mixed models used in our analysis

$$\begin{aligned} \text{Delinquency}_{jit(s)} &= \beta_0 + \beta_1 \text{genotype}_{ji} + \beta_2 \text{age}_{jit} + \beta_3 \text{age}_{jit}^2 + \beta_4 \text{gender}_{ji} \\ &+ \beta_5 \text{ethnicity}_{ji} + u_{j0(s)} + v_{ji} + e_{jit(s)} \end{aligned} \quad (1)$$

where  $j$ ,  $i$ , and  $t$  index sibling pair or cluster, individual, and Add Health Waves, respectively;  $s = m, d$ , or  $f$  indicates whether the sibling cluster or pair are MZ twins, DZ twins, or full biological siblings. The basic trajectory of serious and violent delinquency is described by age and age<sup>2</sup>, and their parameters. The model allows the random effect at the sibling cluster level and the level of observations to vary by type of sibling cluster because the strength of the correlation in these types of sibling clusters varies considerably. Conditional on the three random intercepts at the level of sibling clusters and one random intercept at the individual level, the siblings and repeated measures are assumed to be independent. The models are estimated by SAS.

In our third step, we used two strategies to address the potential impact of population structure. First, we adjusted for self-reported race/ethnicity in all regression analysis. Tang *et al*<sup>34</sup> showed a near-perfect correspondence between the four self-reported ethnic categories (European Americans, African Americans, East Asians, and Hispanics) and the categories determined by 326 microsatellite markers.

As a second strategy, we applied the procedure by Allison *et al*<sup>35</sup> to test for possible population stratification. Following the idea used in the development of sibship tests of linkage and association,<sup>36–38</sup> Allison *et al* reasoned that the probabilities of genotypes of siblings depended entirely on parental genotypes and that controlling for the effects of sibship would be equivalent to controlling for parental genotypes. Indexing sibships by *j*, individuals by *k*, and genotypes by *i*, they proposed a procedure that can be written as a mixed model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk} \quad (2)$$

where  $\alpha_i$ , or the effect of genotype *i*, is assumed to be fixed;  $\beta_j$ , or the effect of sibship *j*, is assumed to be random; and  $(\alpha\beta)_{ij}$  is an interaction term specifying the dependence of the random effect of sibship on genotype. This model is a special case of the mixed model.<sup>32,33</sup>

### Functional analysis of promoter activity

The analysis of promoter activity was based upon two human brain-derived cell lines: neuroblastoma SH-SY5Y and human glioblastoma 1242-MG. The MAOA 1.3-kb promoter-luciferase construct was generated by PCR using an MAOA 2-kb luciferase reporter gene construct (containing 4.5 repeat) as a template. The PCR product (*MluI/HindIII*) of MAOA promoter fragment (–1336/–64 bp) was cloned into the polylinker site (*MluI/HindIII*) upstream of the luciferase gene in the pGL2-Basic vector. Site-directed mutagenesis was utilized to generate 4, 3, and 2 repeats, respectively, using the MAOA 1.3-kb promoter-luciferase construct as a template (Figure 1). The three primers used for mutagenesis of VNTR sites were the following (deleted

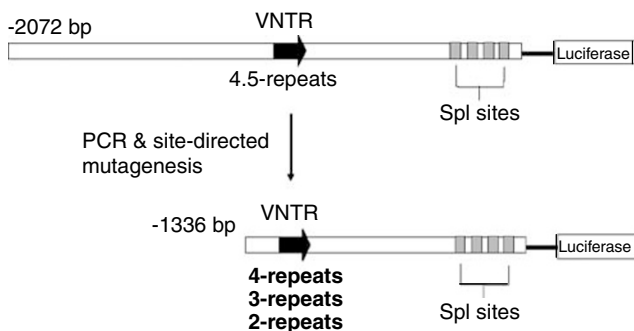
nucleotides are underlined and in lowercase): 5'- GCACCA GTACCCGCACCAGTaccggcaccggcaccGAGCGCAAGGCGG AGGGCCCCGCC-3' (–1117 to –1113; for generating 4-repeat sequence; deleted 15-bp nucleotides using MAOA 1.3-kb promoter-luciferase construct containing 4.5-repeat sequence as a template); 5'-GCACCAGTACCCGCACCAG TaccggcaccggcaccagtaccggcaccagtGAGCGCAAGGCGGAGG GCCCGCC-3' (–1199 to –1113 bp; for generating 3-repeat sequence; deleted a 30-bp nucleotides using MAOA 1.3-kb promoter-luciferase construct containing 4-repeat sequence as a template); and 5'-GCACCAGTACCCGCACCA GTaccggcaccggcaccagtaccggcaccagtGAGCGCAAGGCGGAG GGCCCCGCC-3' (–1231 to –1113 bp; for generating 2-repeat sequence; deleted another 30-bp nucleotides using MAOA 1.3-kb promoter-luciferase construct containing 3-repeat sequence as a template).

The mutated nucleotide sequences of all mutant constructs were confirmed by DNA sequencing. Transfections in SH-SY5Y and 1242-MG cells were performed using Lipofectamine 2000 (Invitrogen). Cells were plated at a density of  $5 \times 10^5$  cells/well in 6-well plates. In the following day, 0.5  $\mu$ g MAOA promoter-luciferase construct (for one well) was co-transfected with 20 ng of plasmid pRL-TK (the herpes simplex virus thymidine kinase promoter fused upstream to the *Renilla* luciferase gene, which is used as an internal control; Promega) into the cells as described previously. Controls were the pGL2-basic luciferase reporter gene vector instead of MAOA promoter-luciferase construct. After 24 h, cells were harvested with luciferase assay lysis buffer (Promega). The cell lysates were assayed for luciferase activity using the Promega Dual Luciferase Assay system.

## Results

### Contingency table analysis

Table 2 compares the mean score of serious and violent delinquency across genotypes within each gender and each of the three Add Health Waves. The number of observations and the standard deviation are also given for each mean score in parentheses. The declining trend of both serious and violent delinquency over the Waves is a reflection of the well-known age pattern of delinquent and criminal behavior. For men, the genetic variants on the single X chromosome are grouped into three categories based on the finding by Sabol *et al* (1998): 2R; 3R or 5R; and 3.5R or 4R. The most striking result for men is the much higher scores of those with a 2R allele than those in the other two categories. The much higher score for 2R holds for both serious and violent delinquency and is present at all Waves of Add Health. In contrast, the delinquency scores of those with 3R or 5R do not seem to differ systematically from individuals with the 3.5R or 4R. The delinquency score for 2R tends to be twice as high as those in the other two genotype categories. The basic



**Figure 1** The human MAOA promoter-luciferase construct. The MAOA 1.3-kb promoter containing 2, 3, and 4 repeats were generated by PCR and site-directed mutagenesis using MAOA 2-kb promoter luciferase reporter gene vector as a template.

**Table 2** Mean score of serious and violent delinquency scales, number of observations, SD by MAOA genotype, Add Health Wave (age), and gender

| Genotype          | Serious delinquency (n; SD) |                        |                        | Violent delinquency (n; SD) |                        |                        |
|-------------------|-----------------------------|------------------------|------------------------|-----------------------------|------------------------|------------------------|
|                   | Wave I                      | Wave II                | Wave III               | Wave I                      | Wave II                | Wave III               |
| Age (in years)    | 12–18                       | 13–19                  | 19–23                  | 12–18                       | 13–19                  | 19–23                  |
| <i>Males</i>      |                             |                        |                        |                             |                        |                        |
| 2R                | <b>5.63 (11; 9.05)</b>      | <b>3.18 (10; 6.08)</b> | <b>1.59 (10; 2.65)</b> | <b>3.95 (11; 7.04)</b>      | <b>2.45 (10; 5.03)</b> | <b>1.36 (10; 2.26)</b> |
| 3R or 5R          | 2.33 (508; 4.27)            | 1.61 (478; 3.24)       | 1.55 (379; 2.45)       | 1.13 (508; 3.15)            | 1.18 (478; 2.25)       | 0.74 (397; 1.84)       |
| 3.5R or 4R        | 2.41 (681; 4.21)            | 1.66 (625; 3.49)       | 1.19 (580; 2.33)       | 1.64 (681; 2.95)            | 1.04 (625; 2.36)       | 0.66 (520; 1.51)       |
| <i>Females</i>    |                             |                        |                        |                             |                        |                        |
| Any2R             | <b>1.16 (31; 2.18)</b>      | <b>1.96 (28; 3.56)</b> | <b>1.03 (27; 3.76)</b> | <b>0.77 (31; 1.75)</b>      | <b>1.46 (28; 3.12)</b> | <b>0.68 (27; 2.05)</b> |
| Only 3R or 5R     | 1.17 (227; 3.10)            | 0.75 (212; 1.62)       | 0.43 (189; 1.10)       | 0.78 (227; 2.16)            | 0.50 (212; 1.17)       | 0.16 (189; 0.55)       |
| Any3.5R and Any4R | 1.17 (1066; 2.50)           | 0.75 (988; 1.82)       | 0.33 (853; 0.96)       | 0.68 (1066; 1.65)           | 0.42 (988; 1.13)       | 0.15 (853; 0.56)       |

**Table 3** Estimated association of the genetic variants in the VNTR of MAOA with serious and violent delinquency among adolescents and young adults<sup>a</sup>

|                        | Serious delinquency |                     |             |                       | Violent delinquency |                    |             |                       |
|------------------------|---------------------|---------------------|-------------|-----------------------|---------------------|--------------------|-------------|-----------------------|
|                        | Males               |                     | Females     |                       | Males               |                    | Females     |                       |
|                        | Model 1             | Model 2             | Model 3     | Model 4               | Model 1             | Model 2            | Model 3     | Model 4               |
|                        | $\beta^a$           | 95% CI              | $\beta$     | 95% CI                | $\beta$             | 95% CI             | $\beta$     | 95% CI                |
| Intercept              | -3.65               | (-7.81, 0.51)       | 5.88        | (3.59, 8.19)          | -1.35               | (-4.30, 1.59)      | 4.65        | (3.14, 6.18)          |
| Age (in years)         | 0.73                | (0.26, 1.20)        | -0.45       | (-0.714, -0.201)      | 0.37                | (0.048, 0.70)      | -0.40       | (-0.570, -0.229)      |
| Age squared (in years) | -0.024              | (-0.035, -0.012)    | 0.009       | (0.002, 0.016)        | -0.013              | (-0.022, -0.003)   | 0.008       | (0.004, 0.014)        |
| European American      | —                   | —                   | —           | —                     | —                   | —                  | —           | —                     |
| African American       | 0.38                | (-0.054, 0.82)      | 0.072       | (-0.15, 0.29)         | 0.34                | (0.038, 0.65)      | 0.24        | (0.099, 0.383)        |
| Hispanic               | 0.49                | (0.031, 0.96)       | 0.16        | (-0.080, 0.41)        | 0.38                | (0.055, 0.71)      | 0.17        | (0.013, 0.322)        |
| Asian                  | 0.26                | (-0.33, 0.85)       | -0.12       | (-0.46, 0.21)         | 0.12                | (-0.52, 0.29)      | -0.050      | (-0.259, 0.160)       |
| Others <sup>b</sup>    | —                   | —                   | —           | —                     | —                   | —                  | —           | —                     |
| 2R                     | <b>1.72</b>         | <b>(0.21, 3.24)</b> | —           | —                     | <b>1.46</b>         | <b>(0.37, 2.5)</b> | —           | —                     |
| Others <sup>c</sup>    | —                   | —                   | —           | —                     | —                   | —                  | —           | —                     |
| Any2R                  | —                   | —                   | <b>0.48</b> | <b>(-0.087, 1.04)</b> | —                   | —                  | <b>0.29</b> | <b>(-0.065, 0.65)</b> |
| -2 log L               | —                   | 16 844.0            | —           | 14 893.5              | —                   | 14 626.6           | —           | 11 896.4              |
| No. of persons         | —                   | 1198                | —           | 1314                  | —                   | 1198               | —           | 1314                  |
| No. of measures        | —                   | 3221                | —           | 3592                  | —                   | 3221               | —           | 3592                  |

<sup>a</sup>Estimated regression coefficient.<sup>b</sup>Reference category for 2R for men: 3.5R, 4R, 3R, or 5R.<sup>c</sup>Reference category for Any2R for women: Any3.5R, Any4R, Any3R, or Any5R. Random parameters are not presented in Table 3.

findings remain the same whether 3.5R and 5R are included or not, because 3.5R and 5R each account for only about 1% of the samples in the data of Sabol *et al* as well as ours.

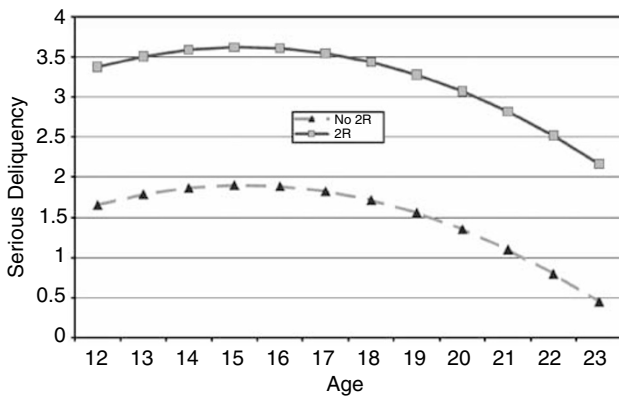
Grouping women into genotype categories is less straightforward because each woman possesses two X chromosomes and it is unknown which of the two alleles is inactivated. The female participants were classified into three genotype categories: Any2R, those with only 3R or 5R, and those with 3.5R or 4R. For women in Table 2, the 2R stands out being associated with much higher scores for both serious and violent delinquency at Waves II and III. The serious and violent delinquency scores for the other two genotype categories do not seem to differ from one another.

Because of the sibling clustering in the data, standard significance tests are not valid for these comparisons. The next section presents significance tests for the genotype effects obtained from the mixed regression models that take the correlations into consideration. These exploratory results suggest that the genotype effects may be relatively constant over ages in adolescence and young adulthood, or the trajectories of delinquency across genotypes appear to be parallel over the age range.

### Regression analysis

The regression analysis compares serious and violent delinquency scores between the 2R genotype and all the other genotypes within each gender after adjusting for the effects of age and self-reported race/ethnicity (Table 3). The

regression analysis has yielded findings that are consistent with those from the contingency table analysis (Table 2). The regression coefficients in mixed models can be interpreted exactly as those in the ordinary least square linear regression. For men, the 2R genotype scored 1.72 ( $P=0.025$ ) points higher than the other genotypes on the serious delinquency scale. For the violent delinquency scale, men with the 2R genotype scored 1.45 ( $P=0.008$ ) points higher than the other genotypes. Figure 2 plots the model-predicted serious delinquency over age for those with the 2R and those with 3R, 3.5R, 4R, or 5R. The 2Rs on average scored about twice as high as the non-2Rs. The effects of 2R for violent delinquency are similar to those in Figure 1 and the data not presented. Using 3.5R or 4R as the reference category in a reestimated regression model shows that the 3R or 5R genotype does not differ from the 3.5R or 4R genotype.



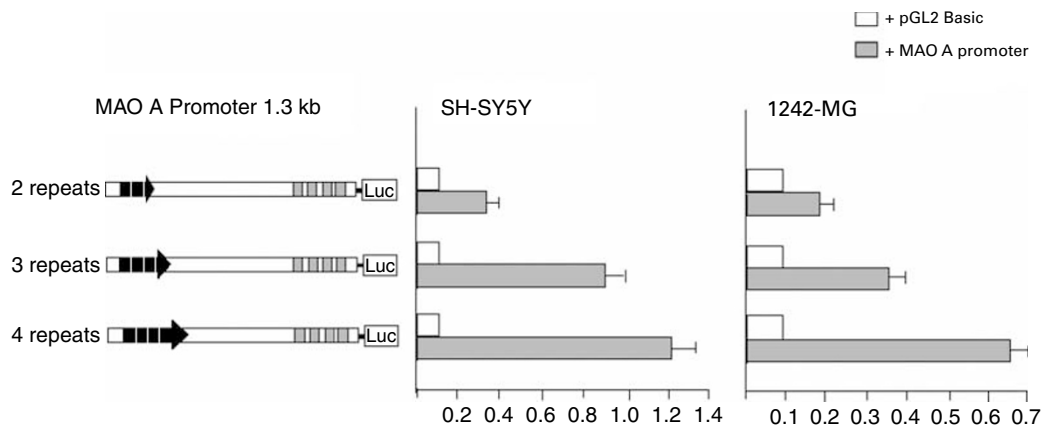
**Figure 2** Projected MAOA 2R and non-2R serious delinquency by age.

The female effect of Any2R genotype is similar to the male effect. Women with the Any2R genotype scored 0.47 ( $P=0.097$ ) and 0.29 ( $P=0.108$ ) points higher than the other genotypes on the serious and violent delinquency scales, respectively. These regression coefficients represent a large increase in the serious and violent delinquency scores among women.

To address potential bias from population stratification, we controlled for self-reported race/ethnicity in all regression models presented in Table 3. In addition, we performed the procedure of Allison *et al*<sup>4</sup> (Equation (2)) The model includes one random effect ( $\beta_j$ ) at the sibling-cluster level, a second random effect at the individual level ( $e_{ijk}$ ), and the key interaction term  $[(\alpha\beta)_{ij}]$  between genotype and the random effect at the sibling-cluster level. In all Allison's models we have estimated, the random interaction term is not significant indicating that within-family effects may not be sufficiently influential to generate population stratification.

**Promoter activity**

To test whether the statistical link between the MAOA VNTR 2R and delinquency has a biochemical basis, we carried out an analysis of MAOA promoter activity by genotype. As shown in Figure 3, the transient transfection and luciferase assay reveals three levels of promoter activity for the three 30-bp nucleotide repeat sequences in MAOA in SH-SY5Y and 1242-MG cells. The 4R sequence exhibited a higher level of promoter activity than the 3R sequence. Alleles with the 3.5R or 4R sequence had been previously shown to transcribe more efficiently than those with the 3R or 5R sequence.<sup>10</sup> Our finding is that the 2R sequence of the MAOA promoter displayed the lowest level of promoter



**Figure 3** Relative luciferase activity. The effect of the 30-bp nucleotide repeat sequence on the human MAOA promoter activity in SH-SY5Y and 1242-MG cells. The MAOA promoter 1.3-kb luciferase constructs were transfected into either SH-SY5Y or 1242-MG cells for 24 h. Then cells were harvested and luciferase activity was determined. Controls were pGL2-Basic vector as indicated. Please note that 2-repeat sequence of MAOA promoter shows the lowest activity and 4-repeat sequence of MAOA promoter shows the highest activity. Data were the mean  $\pm$  SD from three independent experiments with triplicates for each experiment. The six *t*-tests (three for each cell) between 2 and 3 repeats, 2 and 4 repeats, and 3 and 4 repeats are all highly significant ( $t>6$ ).

activity. In both SH-SY5Y and 1242-MG cells, the level of promoter activity for the 2R sequence is substantially lower than that of the 3R and only a fraction of that for 4R. The extremely low level of promoter activity in the 2R sequence corresponds to the much heightened level of serious and violent delinquency for the 2R genotype.

We performed a series of *t*-tests between the 2R, 3R, 4R, and the controls (pGL2-Basic) and among 2R, 3R, and 4R (3R vs 2R, 4R vs 2R, and 3R vs 4R). All the *t*-tests are highly significant, with *t*-ratios reaching 10 or above in most cases. The smallest *t*-ratio is 6.

## Discussion

Genetic studies of serious delinquent and criminal behavior in non-patient or general human populations are rare. Caspi *et al*<sup>11</sup> reported an interaction between maltreatment in childhood and level of MAOA activity for violent behavioral problems; they did not find a main effect of the MAOA gene. Chen *et al*<sup>39</sup> hypothesized the association of aggressive behavior in adolescents with both the dopamine D2 receptor gene and the dopamine transporter gene. They provided suggestive evidence from a small study of 11 adolescents diagnosed to have impulsive-aggressive violent behavior. Guo *et al*<sup>40</sup> reported the main effects of the *TaqI* polymorphism in the *DRD2* gene and the 40-bp VNTR in the *DAT1* gene on serious and violent delinquency among men in the same Add Health data.

The present study demonstrated a link between the 2 repeat of the 30-bp VNTR in the MAOA gene and much higher levels of self-reported serious and violent delinquency. The finding is supported by a statistical association analysis and a functional analysis of MAOA promoter activity. The association analysis showed that men with a 2R reported a level of serious delinquency in adolescence and young adulthood that were at least twice as high as that for those with the other variants in the VNTR. A very similar finding was obtained for violent delinquency for men. Women with Any2R also reported much higher levels of serious and violent delinquency than those with other repeats, although the *P*-values associated with these estimates are 0.097 and 0.108, respectively.

In the functional analysis, the 2 repeat exhibited the lowest level of promoter activity, that is, 25–30% of the promoter activity exhibited by the 4 repeat. The level of promoter activity for the 3 repeat is located between the 2 and 4 repeats, which is consistent with the previous report.<sup>10</sup> The excessively low promoter activity of the 2R suggests a biochemical basis for the excessively high levels of serious and violent delinquency.

Although the promoter activity differs significantly between 3R and 4R, we did not find a significant difference in serious and violent delinquency between those possessing 3R and those possessing 4R. This result is not

inconsistent with the findings reported by Caspi *et al*,<sup>11</sup> who found a higher level of violent behavior for 3R (and 5R) than 4R (and 3.5R) only among men who were maltreated in childhood. Kim-Cohen *et al*<sup>41</sup> reported a main effect of 3R against 4R as well as an interaction effect in a sample of 7-year old Caucasian boys born in England and Wales.

Although our sample is large, the 2 repeat is rare. Though the findings concerning 2R for men have passed the standard tests of significance in spite of the small category of 2R, it is possible that some of our findings could be attributable to chance. For this reason, it is important that these findings are replicated in a much larger population-based study. Future replications may prove the importance of the 2R allele, but the allele cannot possibly be involved in most delinquent behavior because of its rarity, just like the rare mutation in the MAOA gene the Dutch family<sup>6</sup> cannot explain most of the delinquency.

The weaker results for women could be due to the ambiguity in defining the X-linked MAOA VNTR genotypes for women, which is equivalent to measurement errors. The Any2R category used in the present study may include those whose 2 repeat is not fully active. A test of this hypothesis requires a much larger sample that contains a sufficient number of participants homozygous for the 2 repeat.

Our measures of serious and violent delinquency are constructed in the tradition of research on delinquency and crime. Serious delinquency measures the overall delinquency including violent delinquency, but it does not include acts more typically viewed as common adolescent deviance such as lying to parents/guardians about where they had been, minor vandalism, being loud in a public place, and driving a car without its owner's permission. Violent delinquency measures violent behavior that is typically treated as violent offense by the criminal justice system. Our findings suggest that the MAOA\*2R may be more predictive of violent delinquency than nonviolent delinquency. Future work should test this hypothesis explicitly using nonviolent and violent delinquency measures.

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## Appendix 1

## The serious and the violent delinquency scales

- In the past 12 months, how often did you hurt someone badly enough to need bandages or care from a doctor or nurse?<sup>a</sup>
- In the past 12 months, how often did someone hurt you badly enough to need bandages or care from a doctor or nurse?<sup>a</sup>
- In the past 12 months, how often did you use or threaten to use a weapon to get something from someone?<sup>a</sup>



4. In the past 12 months, how often did you take part in a fight where a group of your friends was against another group?<sup>a</sup>
5. In the last 12 months, how often did you deliberately damage property that did not belong to you?<sup>a</sup>
6. In the past 12 months, how often did you carry a handgun to school or work?<sup>a</sup>
7. In the past 12 months, how often did you steal something worth more than \$50?<sup>a</sup>
8. In the past 12 months, how often did you steal something worth less than \$50?<sup>a</sup>
9. In the past 12 months, how often did you go into a house or building to steal something?<sup>a</sup>
10. In the past 12 months, how often did you sell marijuana or other drugs?<sup>a</sup>
11. In the past 12 months, have you shot or stabbed someone?<sup>b</sup>
12. In the past 12 months, have you pulled a knife or gun on someone?<sup>b</sup>

<sup>a</sup>For this question, the score value on the scale is determined in the following manner: the score is coded as zero if the event did not occur in the past 12 months; the score is coded as one if the event occurred once or twice in the past 12 months; the score is coded as two if the event occurred three or four times in the past 12 months; the score is coded as three if the event occurred five or more times in the past 12 months.

<sup>b</sup>For this question, the score value on the scale is determined in the following manner: the score is coded as zero if the event did not occur in the past 12 months; the score is coded as three if the event did occur once or more during the past 12 months.

In the construction of the serious delinquency scale, individuals with more than two missing responses were excluded from analysis. In the construction of the violent delinquency scale, individuals with more than one missing response were excluded from analysis.

The violent scale is based upon 8 of the 12 items and they are items 1–6, 11, and 12.