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Gene Environment Interactions with a Novel Variable *Monoamine Oxidase A* Transcriptional Enhancer are Associated with Antisocial Personality Disorder

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Abstract

Monoamine Oxidase A (MAOA) is a critical enzyme in the catabolism of monoaminergic neurotransmitters. *MAOA* transcriptional activity is thought to be regulated by a well characterized 30 base pair (bp) variable nucleotide repeat (VNTR) that lies approximately ~1000 bp upstream of the transcriptional start site (TSS). However, clinical associations between this VNTR genotype and behavioral states have been inconsistent. Herein, we describe a second, 10 base pair VNTR that lies ~1500 bp upstream of the TSS. We provide *in vitro* and *in silico* evidence that this new VNTR region may be more influential in regulating *MAOA* transcription than the more proximal VNTR and that methylation of this CpG-rich VNTR is genotype dependent in females. Finally, we demonstrate that genotype at this new VNTR interacts significantly with history of child abuse to predict antisocial personality disorder (ASPD) in women and accounts for variance in addition to that explained by the prior VNTR.

Keywords

Monoamine Oxidase A; polymorphism; antisocial personality; methylation

INTRODUCTION

Monoamine Oxidase A (MAOA) is perhaps one of the best characterized genes in behavioral sciences. The gene consists of 15 exons that give rise to two splice variants of 2.1 and 5 kb that both code for a 527 amino acid protein (Billett, 2004; Chen et al., 1991). Transcription of *MAOA* is thought to be moderated by two regulatory motifs. The first is a 30 base pair (bp) variable nucleotide repeat (VNTR) whose biological activity has been extensively examined with the majority of studies concluding that 4 repeat (4R) allele is associated with greater transcriptional activation than the 3 repeat (3R) allele (Beach et al., 2010; Cirulli and

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Goldstein, 2007; Guo et al., 2008; Hotamisligil and Breakefield, 1991). The second regulatory motif is a set of two CpG islands flanking this VNTR (Philibert et al., 2008a).

Despite this understanding of transcriptional regulation at *MAOA* and extensive evidence that alterations in *MAOA* protein activity are associated with behavioral illness including smoking, depression and aggression (Berlin and Anthenelli, 2001; Brunner et al., 1993; Fowler et al., 1996; Shih et al., 1999), the association between genotype at this VNTR and any behavioral illness remains ambiguous (Craig and Halton, 2009; Fan et al., 2010; Li and He, 2008). The potential reasons for this ambiguity are numerous and include the possibility that difficulties in quantitating gene-environment interactions at this locus may be confounding attempts to directly link VNTR genotype to phenotype (Caspi et al., 2002; Kim-Cohen et al., 2006). However, another possibility is that previously unappreciated genetic variation may also be confounding our efforts to link VNTR genotype to phenotype.

This is particularly important for our studies of antisocial personality disorder (ASPD) in the Iowa Adoption Studies (IAS), the largest case and control adoption study of substance use and ASPD in the United States. In previous studies of this cohort, Cadoret and colleagues have shown strong gene-environment interactions (GxE) effects for ASPD and ASPD spectrum behavior (Cadoret et al., 2003; Cadoret et al., 1995; Riggins-Caspers et al., 2003). Spurred by the seminal findings of Caspi and colleagues who demonstrated significant GxE effects for ASPD at the previously described *MAOA* VNTR (Caspi et al., 2002), we recently examined our cohorts and found evidence supporting the original findings (Beach et al., 2010). These confirmatory findings using the IAS are particularly invigorating because the randomized adoption paradigm implemented by Dr. Cadoret ensures independence of genetic and environmental variables (Yates et al., 1998). However, the effect was weaker than expected given the richness of the IAS for the expected outcomes. In addition, although we were also able to confirm prior *in vitro* findings showing an effect of the VNTR variation on gene activation (Beach et al., 2010), the effects were rather modest and the association of methylation with genotype was not entirely consistent with our understanding of the role of methylation in the regulation of this gene (Philibert et al., 2010). Therefore, we began to look for alternative genetic variation.

Specifically, we hypothesized that there may be other genetic variation near the transcription start site of *MAOA* besides the previously described VNTR that could account for some of the discrepancies observed in the literature. During the course of this examination, we noted a CpG rich region near the previously described VNTR that had the hallmarks of a repetitive DNA element. In this communication, we report the discovery of this second VNTR, which we designate *MAOA* P2, approximately 1500 base pairs upstream of the previously described VNTR, which we designate *MAOA* P1. We present evidence that it is functional and describe its genotypic distribution and relationship to DNA methylation. Then using the IAS, we present evidence that GxE interplay at this locus may help improve our prediction of antisocial personality disorder (ASPD) in women.

METHODS

The clinical and genetic data described in this manuscript are derived from the Iowa Adoption Studies (IAS). All procedures and protocols for the IAS were approved by the University of Iowa Institutional Review Board.

The overall study design of the IAS has been described elsewhere (Philibert, 2006). Briefly, the IAS is a longitudinal case and control adoption study of common behavioral illness. The data used in this study are derived from two waves (1997–2003–2004–2008) of clinical interviews with the Semi-Structured Assessment for the Genetics of Alcoholism, Version II

(Bucholz et al., 1994). During the second wave, each subject was also phlebotomized in order to provide biomaterial for the current studies in a manner coordinated with the second clinical interview. The age of the individual that is reported is that of the subject at the time of the second interview. Antisocial personality symptoms were assessed using DSM-III-R, DSM-IV and Feighner criteria (American Psychiatric Association, 1987, 1994; Feighner et al., 1972).

Abuse variables were derived as previously described (Beach et al., 2010). Briefly, we focused on child maltreatment within the family involving 1) any injury sustained in the context of punishment by a parent 2) any childhood sexual contact with any family member, and 3) consistent use of harsh physical punishment by a parent. To assess childhood injury in the context of punishment, adult participants were asked “Did your Mother/Father ever physically punish you so hard that you hurt the next day or had to see a doctor?” To assess childhood sexual contact with family members, participants were asked “Before you were the age of 16 years old, were there any sexual contacts between you and any family members, like a parent or step-parent, grandparent, uncle, aunt, brother, sister, or cousin? “Was there sexual contact with a parent or grandparent?” They were also asked “What was the usual way in which your Mother/ Father punished you”, with harsh physical punishment being one option (in contrast to non-physical, mild physical, and no punishment). The Child Maltreatment Index (CMI) was incremented by one for all affirmative answers, so that larger scores indicated greater evidence of childhood maltreatment.

Sequence for the *MAOA* DNA region was obtained from the University of California Santa Clara (UCSC) website using human genome build 19 (HG19). Genotyping of the P1 VNTR was conducted as previously described (Beach et al., 2010). Amplification of the P2 VNTR for genotyping was accomplished using the following primers 5' Fam AGCGCCTCAGCTTGAAAAACC and AGAGTGGACTTAAGGAAGCAGA, standard PCR conditions supplemented by the addition of 7-deazaGTP and 5% DMSO using an annealing temperature of 54° C. Capillary electrophoresis and florescent detection of the amplified PCR products was accomplished using a 3730 Applied Biosystems (Foster City, CA) DNA analyzer in combination with Peak Scanner™ software at the University of Iowa DNA facility.

Sequencing of DNA regions and constructs was also performed by the University of Iowa DNA facility. Sequence for each P2 allele was determined using DNA from at least two individuals except for the 8R allele for which only one independent sequence was obtained. Prediction of enhancer regions was accomplished using ProScan version 1.7 software (Prestridge, 2000) hosted on the Bioinformatics and Molecular Analysis Section (BIMAS) website (<http://www-bimas.cit.nih.gov/molbio/proscan/>).

Luciferase transfection assays were conducted as previously described (Beach et al., 2010). Briefly, human NT2 cells were grown to 80 to 90% confluence. Using Lipofectamine 2000® (Invitrogen, Carlsbad, CA), cells were transfected with a 1:50 ratio of pRL-SV40 (Renilla) plasmid to pGL3 experimental construct. Luciferase activity was measured using a Dual-Glo® Luciferase Assay System (Promega, Madison, WI) according to the manufacturer's protocol and normalized using a Renilla internal control. The pGL3 vector contains sequence corresponding to the -63 bp to -1787 bp relative to the transcription start site (TSS). Each construct contained a constant background of the 3 repeat (3R) allele at the P1 (first) VNTR site and varied with respect to VNTR sequence at the P2 site (see Supplemental Figure 1). Sequence of each construct is contained in supplemental Figure 1. All constructs are available upon request to RAP.

DNA methylation measurements were conducted as previously described (Philibert et al., 2007; Philibert et al., 2008b). Briefly, using biomaterial contributed by each of the subjects during the last wave (2004–2008) of the study, lymphoblast cell lines were prepared using standard EBV transformation techniques (Caputo et al., 1991). After transformation, cell lines were grown using standard bovine serum-based growth media supplemented with l-glutamine and antibiotics. The media was changed for each of the cell lines 24 hours prior to cell harvesting. DNA was then prepared from lymphoblasts using the method of Lahiri (Lahiri and Schnabel, 1993). The methylation status of the 71 CpG of the 81 CpG residues present in the 799 bp CpG island was determined as described previously in detail under contract by Sequenom Inc. (San Diego, USA) (Philibert et al., 2010; Philibert et al., 2008a). Briefly, coded aliquots of DNA were bisulfite treated under basic conditions to convert unmethylated cytosines to uracils (Thomassin et al., 1999). The region of DNA corresponding to the CpG island was then PCR amplified using two separate amplicons, methylation sensitive primers and touchdown PCR procedures (Philibert et al., 2008b). The first contig was amplified with the primers 5'GGGTTTTTATATG-GTTTGATTTTATAGATAG and 5' CCTACTCCTTTATACAACCTCCCCC. The second contig was amplified with the primers: 5' GGTTATTTAGAGATTAGATTATGTGAGGGT and 5'CCTACAACAATAAACAAAAA-AACCCC. After inspection of each product for complete bisulfite conversion, methylation ratios (Methyl CpG/total CpG) for each of the 74 CpG residues described in this study were then determined using quantitative mass spectroscopy coupled with proprietary peak picking and spectral interpretation tools (Ehrich et al., 2005; Ehrich et al., 2007). As per our standard protocols, in order to stabilize variance estimates (Siegmund and Laird, 2002), all methylation data was Z-transformed prior to formal statistical testing and used as a continuous variable.

Statistical Analyses

Genetic and luciferase transfection data were analyzed using the ANOVA, Chi Square and t-test subroutine in the JMP Genomics analytics suite (SAS Institute, Cary, NC) as indicated in the text. All reported p-values are two tailed unless noted otherwise.

Model fitting was conducted using Mplus version 6 (Muthen & Muthen, 2010) with manifest indicators. The fit function used was maximum likelihood. Based on zero-order relationships in this data set as well as prior research, we set the direct effect of each promoter region to be zero. Because males and females have differing numbers of X-chromosomes and the clinical phenomenology of ASPD differs between males and females in this population, male and female data were analyzed separately. We freely estimated other paths in the hypothesized models, and standardized parameter estimates and their significance are reported. We examined the fit of the hypothesized model using the overall chi-square test in each case. To explicate direction of interaction effects, simple correlations between CMI and ASPD were computed within activity level of MAOA P2, contrasting those with at least one low-activity allele to those who had no low-activity alleles.

RESULTS

The sequence for the *MAOA* P2 VNTR is given in Figure 1. The sequence contained within the GenBank reference represents the consensus sequence for the 10 repeat (10R) P2 allele. The repetitive region consists of two decamer repeats, CCCCTCCCCG (A Repeat) and CTCCTCCCCG (B Repeat). The sequence of the first 60 base pairs (6 repeat units) of the element is invariant in all subjects examined with the exception of a C to T polymorphism present at bp 6365508 (see Figure 1). Variation in the enhancer region's length results from a variable number of "A" repeats after the first 60 bp. For example, the 7 repeat (7R) has a structure of ABABABA, the 8R allele has a structure of ABABABAA, while the 10R allele has a structure of ABABABAAAA.

The region was genotyped in the Iowa Adoption Studies (IAS) population as described in the methods for both the *MAOA* P1 and P2 VNTRs. Table I gives the clinical characteristics of the cohort while Table II gives the distribution of the alleles for both the *MAOA* P1 and P2 alleles. As Table I illustrates, the adoptees are largely a largely White population who at the time of phlebotomy during the 2004–2008 wave, were in their late 40's. Consistent with the intentional loading of the cohort for the biological diathesis for ASPD, the participants tend to have elevated ASPD symptom counts. As shown in Table II, in this population, the 9R and 10R alleles are far and away the most common at the P2 site. Distribution of genotypes at both the P1 and the P2 VNTR were consistent with Hardy-Weinberg equilibrium.

The haplotype distribution of the alleles in White male subjects is given in Table III. As the Table illustrates, in these subjects, the 4R repeat at P1 is in complete linkage disequilibrium with the 9R repeat at *MAOA* P2. However, the situation with respect to the 3R allele at P1 is more complex with the 3R P1 allele being found in association with the 7R, 8R, 9R, 10R and 11R P2 genotypes. Accordingly some individuals who would be characterized as low-activity based on their P1 allele might not be characterized as low-activity based on their P2 allele, raising the potential for characterization at P2 to account for additional variance in outcomes.

Because of the region's proximity to the transcription start site (TSS) of *MAOA* and enriched GC content, the sequence of each of the P2 alleles was analyzed for possible enhancer activity using Proscan Version 1.7. As the Table IV indicates, significant promoter activity (cutoff score for no significant activity is 53) is predicted in both strands with score in the reverse strand remaining constant but with the score in the forward strand varying with the number of repeats. The 9R and 10R alleles are predicted to have higher activity as compared to the 8R and the 11R alleles. Interestingly, according to the ProScan results, the region containing the P1 VNTR does not contain Pol II promoter activity (data not shown).

In order to further examine and validate these predictions, we constructed luciferase expression vectors to examine the relationship between sequence variation at the P2 VNTR and transcriptional activation. The complete sequence of the insert is given in supplemental Figure 1. Briefly, at the P1 locus each ~1.7 kb construct contains the sequence for the 3R P1 allele with constructs varying at the P2 site (i.e. having the 8R, 9R, 10R or 11R sequence). The results of the transfections are given in Figure 2. As the figure demonstrates, the 9R repeat has the greatest amount of luciferase activity while the 10R repeat, which had the least amount of activity (9R vs 10R, $p < 0.001$). The 8R and 11R had an intermediate levels of luciferase activation both of which were significantly less than that of the 9R allele ($p < 0.02$ and $p < 0.03$, respectively).

In prior examinations, we have demonstrated that the amount of methylation of the two promoter associated CpG islands at this locus was dependent on *MAOA* P1 genotype in female subjects (Philibert et al., 2009; Philibert et al., 2008a). Using the same methylation data, we examined the relationship of methylation with respect to the P2 VNTR. As Figure 3 demonstrates, those female subjects homozygous for the 9R allele ($n=97$) had significantly less methylation than those either homozygous or heterozygous for the 10R allele ($n=46$; $p < 0.03$). There was no significant effect of P2 genotype on *MAOA* methylation in the male subjects ($p < 0.92$).

Building upon prior work with this sample that reported significant interactions between history of child abuse and *MAOA* genotype in the prediction of adult symptoms of ASPD (see Beach et al., 2010), we wondered whether variation in the newly described promoter might enhance the prediction of ASPD. As before, we used data from the Semi-Structured

Assessment for the Genetics of Alcoholism (SSAGA-II) to assess maximum lifetime symptom levels for ASPD. The SSAGA is a polydiagnostic instrument that assesses depression and antisocial personality disorder, among others, in a manner consistent with DSM-III-R, DSM-IV, and Feighner RDC (Research Diagnostic Criteria). Sample alpha coefficient was .71 for ASPD lifetime symptom scales respectively. The correlation of lifetime ASPD symptoms between the two waves of assessments was .72, providing the average ASPD symptom scale used in the current research with an effective reliability of .84 (Rosenthal & Rosnow, 1991).

In keeping with the luciferase expression results we treated the 10 allele at the P2 promoter as the low activity allele and contrasted those who had a 10 allele with others. We examined the predictive effect of the two promoters considered simultaneously to see whether unique variance in outcomes was predicted by the interaction of child abuse with variation in each promoter region. Follow-up analyses of significant effects were conducted to explicate direction of effects. In all cases analyses were conducted separately by sex to allow characterization of the impact of the genotype variable as a function of sex.

We examined the two regions (P1 vs. P2) simultaneously for those participants with complete data ($N = 518$). Setting direct effects of the VNTRs to zero and freely estimating both interaction terms simultaneously, we found that for males ($N = 234$), symptoms of ASPD were not significantly uniquely associated with the interaction term for either locus. For females ($N = 284$) we found that the interaction of P2 with history of child abuse accounted for significant unique variance in symptoms of ASPD ($P2: \beta = -.229, p = .007$, two-tailed) whereas the interaction of child abuse with variation at P1 did not account for unique variance ($P1: \beta = .073$, NS). Fit of the overall model was good ($P2: \chi^2(2) = 2.88$, NS). Controlling for age or ethnicity produced no change in the pattern of significant results.

We next examined the model dropping the non-significant interaction of P1 with CMI. Symptoms of ASPD were again significantly associated with the interaction term of MAOA P2 X CMI ($\beta = -.177, p = .002$, two-tailed). Fit of the overall model was good ($P2: \chi^2(1) = 2.28$, NS). To explicate direction of effects at P2, we examined the correlation of CMI with ASPD within low MAOA P2 activity level. For the low activity allele females (i.e. those females who had at least one 10R allele at P2) the correlation of CMI to APSP was $r(97) = .459, p < .001$. Conversely among those with no low activity alleles, the correlation of CMI with ASPD symptoms in adulthood was $r(187) = .162, p = .026$. Thus, presence of the low activity allele at P2 was associated with significantly greater vulnerability to the disruptive effects of CMI for females.

To explicate the impact of variability at P2 beyond any shared variance with the P1 promoter region, we also examined the subset of females with a low expressing variant of the P1 promoter (2R or 3R). In this subsample of $N = 159$ females with a 2R or 3R allele at P1, we found that those who also had a low activity allele at P2 had a correlation of CMI to APSP of $r(96) = .458, p < .001$. Conversely those with a 2R or 3R allele at P1 who did not have a low activity allele at P2 were found to have a correlation of CMI with ASPD symptoms of only $r(63) = .23, p = .06$.

DISCUSSION

In summary, we describe a previously unknown enhancer element in the promoter region of MAOA, demonstrate its functionality, delineate its relation to the better known P1 VNTR, show that methylation at this locus is also a function of P2 genotype, that clinical characteristics ascribed to P1 VNTR variation also are associated with P2 variation and that

genotype at the P2 may help account for additional variance in outcomes traditionally associated with the interaction between P1 and child abuse experiences.

The current findings suggest that the genetic regulation of *MAOA* activity may be more complex than previously appreciated and that the strength of previous findings at this locus may be enhanced by consideration of variation at the newly discovered promoter. In the current analyses, the inclusion of P2 genotype into our analyses of ASPD had relatively minor effects on the strength of the association and we would have obtained significant results using either P1 or P2 genotype. However, this may not be true for other studies, in particularly small studies, performed by others. Therefore, given the key role of monoaminergic neurotransmitters such as dopamine, serotonin and norepinephrine, in human behavior, it may well be that the increased power afforded by genotyping this locus may uncover previously undetected effects and that many conditions thought not to be affected by *MAOA* a genetic variation may now be shown to be regulated by variation at this locus.

It is likely that other significant transcriptional regulatory elements in this gene remain to be discovered. We only uncovered this element in the course of refining our analyses of this gene region (Philibert et al., 2010; Philibert et al., 2008a). Since regulatory elements for genes can occur at a considerable distance from the TSS and this gene codes for at least two splice variants, we would encourage others to further analyze this locus to delineate further regulatory factors.

The results of our *in silico* analyses are in slight contrast with the experimental results. To be specific, the *in silico* analysis predicts that the 10R variant would have equal enhancer properties as the 9R variant. Instead, the 10R variant had the least activity. Interestingly, in contrast to the other three alleles, the 10R repeat contains the T allele at the bp 6365508^{C→T} polymorphism. Like the hypothesized effect of the Long “G” variant at the 5HTTLPR (Hu et al., 2006), it very well may be that this variation disrupts a binding site for a transcription factor thus accounting for the lower enhancer properties.

Similar to our findings with respect to the P1 VNTR (Philibert et al., 2010; Philibert et al., 2008a), P2 genotype is also associated with differential methylation of lymphoblast DNA derived from female subjects. This is important because in contrast to the repeat variation seen at the P1 VNTR, each of the A and B repeat motifs at the P2 locus contains a CpG residue. Since DNA methylation of CpG residues is normally associated with decreased transcription from the region containing these residues, it will be important to understand how the enhancer activities afforded by varying numbers of decamer repeat units interacts with methylation at this site to affect *MAOA* mRNA transcription. However, because the methylation was measured in lymphoblast DNA, not actual brain DNA itself, and some studies suggest that lymphoblasts may not precisely reflect the methylation status of the cognate lymphocytes from which they are derived (Brennan et al., 2009; Grafodatskaya et al., 2009; Nguyen et al., 2010), it will be important to repeat these methylation studies using fresh lymphocyte DNA and if possible, examine the methylation status in areas of the brain expressing *MAOA* activity.

The greater strength of the findings with respect to ASPD using the P2 data than with the P1 data also supports the assertion that the P2 motif may be more critical to *MAOA* transcriptional activity than the P1 repeat motif. However, it is important to realize that both elements are in tight linkage disequilibrium and the slightly stronger findings may be secondary to chance. Indeed, both elements probably have some regulatory activity and it very well may be that re-analysis of existing data sets using genetic information from the P2 locus may lead to additional findings.

Further studies of this allele variation in other conditions and in other populations are clearly needed. It is likely that other variants of this polymorphism will be discovered. Studies of these variants in other populations informative for other conditions linked to this locus, including aggression, substance use and depression (Berlin and Anthenelli, 2001; Brunner et al., 1993; Fowler et al., 1996; Shih et al., 1999), may lead to a better understanding of *MAOA* activity in human behavior. Furthermore, because this locus is an important therapeutic target (George and Weinberger, 2007), it may well be that a better understanding of genetic regulation at this locus could lead to more personalized medication management.

In summary, we report a variable new enhancer motif in the promoter region of *MAOA*. We suggest that by genotyping this new VNTR, investigators can gain increased power to detect genetic effects for traits known to be moderated by dopaminergic activity and that further studies of the role of this variant in regulating *MAOA* transcription and in human behavior are indicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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6365325 AGTTATTTTTCTTGCCTACTTGCTTCGATTTGGGTTAAGCGCCTCAGCTT
 6365375 GAAAAACCCACAGAGAACCAACAATTGCCTGGTCTCCCCAAGTGACGG
 6365425 TTCTCGCCCCGCCCCGTCCCGTCTCGCCTCGCCCCGCCCCCTCCCCGCTCC
 6365475 TCCCCGCCCCCTCCCCGCTCCTCCCCGCCCCCTCCGCTCCTCCCCGCCCC
 6365525 TCCCCGCCCCCTCCCCGCCCCCTCCCCGCCCCCTCCCCGCCCCCGCCCTCAACC
 6365575 TAGTGAGGGCTGGAGGCTGCGCAGACCTCGACGGGCCCTACATGACGTCA
 6365625 CAAAGGGGCCAGACCAAGTGGGGCAGCACCTGCGACCCTGCGATCCTGC
 6365675 CTGGCTCAGCCGCCTTCATATATCTGCTTCCTTAAGTCCACTCTTGCCCA

Figure 1.

The sequence and structure of the *MAOA* P2 VNTR region. Sequence numbering is from the GRCh37 reference assembly. Two decamer repeat units are found in the region:

CTCCTCCCCG (red) and CCCCTCCCCG (yellow). In areas with consecutive repetitive repeats, the boundary between the repeated domains is illustrated by alternating single and double underlining. The VNTR region is relatively enriched in CpG residues which are illustrated in blue. The position of the primers used to amplify the repeat are double underlined. The position of a C to T polymorphism (the T allele is present in the 10R allele only) is denoted by the box at bp 6365508.

Figure 2.

The Luciferase transfection assessment of enhancer activity. On the Y axis, the normalized luciferase activity (construct luciferase activity/cotransfected control plasmid activity) is given for each observation ($n=4$ for each group). The 9R repeat construct had the greatest activity the 8R (9Rvs 8R; $p<0.03$) and 11R (9Rvs 11R; $p<0.02$) repeat constructs had intermediate activity and the 10R repeat construct had the lowest activity (9Rvs 10R; $p<0.001$).

Figure 3.

Average *MAOA* promoter associated CpG island methylation for the 9R and 10R alleles for male and female subjects. On the Y axis, the average methylation Z score is indicated. Females with at least one 10R repeat (n=46) had significantly more methylation than those who were homozygous for the 9R allele (n=97; 9R vs 10R; $p<0.03$). There was no difference in methylation for the male subjects.

Table I**Clinical Characteristics of the Iowa Adoptions Studies**

Age		
Male	46 ± 8	(N=259)
Female	45 ± 7	(N=312)
Ethnicity		
African American	14	
White	535	
Hispanic	14	
American Indian	2	
Asian	0	
Unknown/other	6	
Lifetime DSM-IVASPD Symptom Count		
Count	Males	Females
0	37	107
1	75	110
2	55	40
3	29	20
4	25	16
5	24	8
6	11	11
7	3	0

Table II

MAOA P1 and P2 Distribution in the Iowa Adoption Studies

P1 VNTR		
Genotype	Female	Male
2, 2	0	2
2, 4	1	
3, 3	39	87
3, 3.5	1	
3, 4	112	
3, 5	2	
3.5, 3.5	0	7
3.5, 4	2	
4, 4	123	149
4, 5	3	
5, 5	0	1
Total	283	246

P2 VNTR		
Genotype	Female	Male
8, 8	0	2
8, 10	3	
9, 9	179	175
9, 10	76	
9, 11	19	
10, 10	15	49
10, 11	4	
10, 12	1	
11, 11	2	15
Total	299	241

Table III*MAOA* P1 and P2 Haplotype Distribution in the Iowa Adoption Studies

P1 Genotype	P2 Genotype	Observed
2R	10R	1
3R	8R	2
3R	9R	17
3R	10R	46
3R	11R	13
3.5R	9R	6
4R	9R	136
5R	9R	1

* Because haplotypes may differ as a function of ethnicity, the current results are only for the White subjects. Results for the other subjects are available on request.

Table IV

Promoter Scan Analysis of MAOA P2 Alleles

Allele	DNA Segment Length	Forward Strand Score	Reverse Strand Score
8R	646 bp	59.61	97.91
9R	656 bp	109.53	97.91
10R	667 bp	109.53	97.91
11R	677 bp	99.88	97.91