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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 bp 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.

1. 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 repeats (VNTRs) 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 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 (G × E) effects for ASPD and ASPD spectrum behavior (Cadoret et al., 1995, 2003; Riggins-Caspers et al., 2003). Spurred by the seminal findings of Caspi and colleagues who demonstrated significant G × E 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

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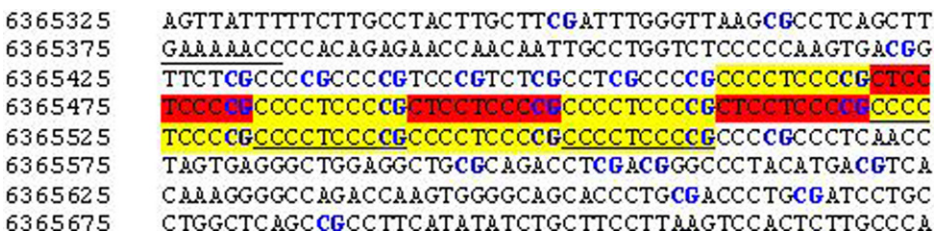

Fig. 1. The sequence and structure of the *MAOA* P2 VNTR region. Sequence numbering is from the GRCh37 reference assembly. Two decamer repeat units are found in the region: CTCCTCCCCG (red) and CCCCTCCCCG (yellow). In areas with consecutive repetitive repeats, the boundary between the repeated domains 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 is 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. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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 bp 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 G × E interplay at this locus may help improve our prediction of antisocial personality disorder (ASPD) in women.

2. 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 80–90% 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 Fig. 1). Sequence of each construct is contained in supplemental Fig. 1. All constructs are available upon request to RAP.

DNA methylation measurements were conducted as previously described (Philibert et al., 2007, 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 h 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., 2008a, 2010). 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-GTTTGATTTTTAGATAG 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, 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.

2.1. 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 and Muthen, 1998) 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