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Assessment of the role of a functional VNTR polymorphism in MAOA gene promoter: a preliminary Study

Chung-Yen Pai, [1,*] Ph.D. ; Su-Lien Chou,[1,2] M.S. ; Frank Fu-Yuan Huang,[3] Ph.D.

[1]Department of Forensic Sciences, Central Police University, Kueishan, Taoyuan 33304, Taiwan, ROC
[2]Department of Biomedical Engineering and Environmental Sciences, National Tsing Hua University, Hsinchu 30043, Taiwan, ROC
[3]Department of Crime Prevention and Correction, Central Police University, Kueishan, Taoyuan 33304, Taiwan, ROC

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Abstract

The monoamine oxidase A (MAOA) is involved in the degradation of various biogenic amines which have been hypothesized to have a relationship with personality traits. In literatures, however, inconsistent results appeared in a number of case-control studies using the functional MAOA-uVNTR marker. We aimed to further look into the uVNTR polymorphism among the Asian population and to preliminarily investigate its transcriptional function. Gene fusion and transfection experiments have been manipulated for the uVNTR in transfected human cervical carcinoma and neuroblastoma cell lines. For luciferase activity assay, the pGL3-Enhancer construct carrying the allele of 4 tandem repeats was chosen for proto-construct. By using a vector-anchoring strategy, the 2-, 3-, and 5-copy alleles contained in the recombinant pGEM-T vectors were reconstructed into pGL3-Enhancer vector carrying identical core promoter sequence. Four alleles, 2-, 3-, 4-, and 5-tandem repeat, were identified from regular male Chinese Han in Taiwan (n = 474). The most common allele (3-repeat) and the second frequent one (4-repeat) constituted >99% (472 out of 474) of the observed alleles. Interestingly, in conflict with our data, the two most common alleles have an opposite ratio composition in Caucasian. Functional characterization in a luciferase assay demonstrated that the 2-repeat allele was more active than others. Together with the observation, some disagreements among the results of transcriptional activities of MAOA gene constructs were seen in literature. These discrepancies imply that the MAOA-uVNTR functional polymorphism might not play a crucial role in behavioral or physiological variability in humans.

Keyword: MAOA promoter, u-VNTR, luciferase activity, antisocial behavior, forensic science

Introduction

Human and animal studies have provided several evidences of a relationship between antisocial behaviors and alterations of the monoamine oxidase A (MAOA) gene. In a Dutch family, a nonsense mutation in the MAOA gene was associated with impulsive, aggressive, and violent behavior in the affected males [1]. Further, aggressive behavior was observed in male transgenic mice with deletion of the MAOA gene [2]. These studies implicated that the disordered phenotypes were linked to a level of MAOA activity, which has been shown to be genetically determined [3].

In literatures, DNA polymorphisms in the structural and regulatory region of MAOA gene have been shown to influence transcriptional activity [4]. One of them was recently focused: a 30-bp variable tandem repeat (VNTR), located at 1.2 kb upstream of the MAOA

*Corresponding author, e-mail: paicy@mail.cpu.edu.tw

coding sequence [5]. This polymorphism is characterized as a repeat sequence with 3, 3.5, 4, or 5 copies, designated as MAOA-uVNTR (i.e., upstream element). Alleles with 3.5 or 4 repeats were reported to express 2- to 10-fold more efficiently than alleles containing 3 or 5 repeats, implicating a functional genetic marker for antisocial traits. However, in Sabol's report [5], substantial variations in allele frequency of different ethnic/racial groups were observed. The expression and regulation of MAOA gene of most individuals in variety of general ethnic/racial populations are presumably to be normal, reflecting to the natural setting. That is to say that it would be very unimaginably and unreasonably to find most citizens of a specific ethnic/racial population possessing abnormal behavioral and physiological phenotypes. Based on this logical premise, if the MAOA-uVNTRs do serve as a prerequisite in regulation of gene expression at transcriptional level, alleles with 3.5 or 4 repeats identified as the optimal length for regulatory region should be the wild type among a variety of common populations.

As well known, the transcriptional control region of eukaryotic genes can be separated into two categories, a core promoter and upstream (or downstream) elements. The presence of sequence variation in MAOA core promoter has been described [6, 7]. However, the promoter fusion plasmid construction designed by Sabol et al. [5] includes two variables: sequence polymorphism in core promoter and the length polymorphism in upstream element (MAOA-uVNTR), which would then complicate the result of luciferase activity assay for different uVNTR alleles.

To evaluate whether the uVNTR *per se* play a functional and anonymous role in MAOA-gene expression, it is important to assay whether different alleles would modify the MAOA enzyme expression and to investigate the MAOA-uVNTR polymorphism among ethnic populations.

Materials and Methods

Identification of MAOA-uVNTR alleles

A total of 474 male volunteers of subjects, without any previous convictions, coming from various parts of Taiwan participated in this study. They are all Han Chinese. Informed consents were obtained from all the subjects. The 30-bp repeat polymorphisms of MAOA-uVNTR were screened and identified using the male DNA samples. The procedures are briefly described. The DNA samples were extracted using salt chloroform method [8] and amplified by PCR with the primer set closely flanking to the uVNTR site: MAO-Mlu (5'-AGCACGCGTGCCTCAGCCTCCTTCCCCGGC-3') and MAO-Bgl II(5'-CCGAGATTCGGCGGGCCCTCCGCCTTG CGC-3'). The PCR was preheated to 94℃ for 5 min, followed by 35 cycles of 94℃ for 1 min, 60℃ for 1 min, 72℃ for 1 min and 20 sec. Horizontal electrophoresis was performed on 2.5% of SeaKem LE agarose gels (FMC Crop., Rockland, ME) in TBE buffer. The lengths of PCR products of 2, 3, 4, and 5 repeats were 140bp, 170bp, 200bp, and 230bp, respectively.

PCR amplification of the whole promoter region

PCR fragments containing the whole promoter region were amplified from genomic DNA using primers: -1396Mlu (5'-CGGAATTCCGCTGGTCTCTAAGAGTGGGTAC-3') and -1Bgl II (5'-GAAGATCTTCGCTTTGGCTGACAC GCTCC-3')Two-step PCR was performed: 35 cycles of 94℃ for 1 min and 68℃ for 2.5 min.The length of PCR product is about 1.4 kb.

Plasmid construction (I) and core promoter sequencing

The 1.4 kb PCR fragments containing both upstream uVNTR repeat and MAOA core promoter region were codigested with Mlu I and BglII, A-tailing treatment, and then ligated into a linear form of pGEM-T vector. After transformed to competent E.coli cells, the construct formation was confirmed by same double restriction digestion. The DNA sequences of core promoter from various uVNTR alleles were also screened and verified by ABI terminator cycle sequencing using ABI 377 model.

Plasmid construction (II) with identical core promoter

For investigating the regulatory role of upstream uVNTR, the double digested 1.4kb PCR products were ligated into pGL3-Enhancer luciferase reporter vector (Promega). The DNA sequence variations in MAOA core promoter could be the potential binding sites for transcriptional factors and thus result in a fluctuation in the luciferase activity assay. Therefore, the pGL3-Enhancer construct carrying the allele of 4 tandem repeats was chosen for proto-construct. The recombinant pGEM-T vectors containing 2, 3, and 5 repeats were codigested with MluI/AgeI, respectively. Resulting

DNA fragments were ligated into the pGL3-Enhancer constructs containing other alleles (ie., 2, 3, and 5 repeats) treated with same double digestion. From this replacement manipulation, different uVNTR allele constructs with identical core promoter sequence were thus obtained, and hopefully the accurate relationship between repeat alleles and MAOA gene expression could be measured.

Cell lines and luciferase activity assay

Two types of cell lines, Hela (Human cervical carcinoma cells) and human neuroblastoma cell line (IMR-32), were used for transfection. The cell lines were co-transfected with a mixture of 2 μg DNA from the MAOA pGL3-Enhancer construct and 0.6μg from pRSV40-lacZ as a control. Aliquots of 80 μl of cell extracts were incubated with luciferin reagent (Promega) to measure luciferase activity. For independent experiments in triplicate using different plasmid preparations were performed.

Results and Discussion

MAOA-uVNTR polymorphism and PCR product of the whole promoter region

Four different upstream MAOA-uVNTR alleles containing 2, 3, 4, and 5 tandem repeats were identified from the present sample subjects (Fig. 1). Two infrequent alleles, 2- and 5-repeat, were found only from single individual, respectively. The two single individuals with 2-, and 5-allele were excluded from statistical analyses because of its low frequency in the Chinese Han population. The PCR products of the MAOA whole promoter region containing the four types of uVNTR alleles are shown in Figure 2. There was no 3.5-copy as reported by Sabol et al. [5] in this study. Moderate DNA sequence variations in core promoter from the DNA samples of four-uVNTR alleles were also observed (data not shown) using ABI terminator cycle sequencing, ABI 377 model.

Comparison of the MAOA-uVNTR allele frequencies

The frequencies of 3-, 4-repeat alleles of MAOA-uVNTR were 64.7% and 34.8%, respectively in our sample size with the very rare alleles of 2- and 5-copy. The allele frequency results among ours and others [5, 9, 10] are shown in Table 1. It is worthy of notice that both the Chinese Han subjects (see Table 1) in Taiwan had higher frequency of the 3-repeat allele, and there was no statistically significant difference between the two groups (p value=0.099, x^2=2.706, df=1), suggesting the similarity of allele frequency distribution. Also, in line with several prior studies, the 3-repeat and 4-repeat alleles were very frequently found in Asian groups (Table 1). Statistical result revealed no difference among the populations of Eastern Asia (p value=0.414, x^2=2.858, df=3). However, when incorporated with the White/Non-Hispanic (Caucasian) subjects, significant difference was seen (p value<<0.0001, x^2=183.855, df=4).

Kunugi et al. [10] examined the uVNTR polymorphism for the mood disorders in Japanese subjects. They demonstrated that the frequency distributions of 3- and 4-repeat alleles in control, bipolar, and unipolar subjects were (62% vs. 38%), (59% vs. 41%), and (57% vs. 43%), respectively, showing the higher 3-copy allele in various subject groups. Lu et al. [9] studied the association of the uVNTR with alcoholism among the Han males in Taiwan. Three alleles of 2, 3 and 4 repeats were identified from 77 control Han people with the frequencies of 1.3%, 54.5%, and 44.2%, respectively, and from 214 alcoholic subjects with the frequencies of 0.5%, 57%, and 42.1%, respectively. Lu et al. [9] also reported a higher frequency of the 3-repeat allele and their Taiwanese Han data was quite similar to ours. Furthermore, a report by Sabol et al. [5] revealed a frequency of 61% for 3-repeat and 37.8% for 4-repeat in Asian subjects and Pacific islanders. All these reports, including ours, consistently demonstrated that the 3 and 4 repeats in Asian populations were the two most frequent alleles, with the 3-repeat allele being more frequent than the 4-repeat.

In contrast, the 4-repeat allele was reported to be more common than the 3-repeat in Caucasian Australians [11], White/nonHispanics, German, and Italian subjects [12]. For example, Hamilton et al.[13] observed allele frequencies of 3 repeats (36.2%), 3.5 repeats (2.9 %), 4 repeats (60.5 %) and 5 repeats (0.4 %) among 620 American individuals [13]. Clearly, there were substantial variations in frequency distributions of 3- and 4-alleles among different ethnic/racial groups.

Luciferase activity assay for MAOA-uVNTR repeats

Sabol et al. [5] was the first group performing gene fusion and transient transfection experiments to investigate the transcriptional effect of MAOA-uVNTR repeats in human neuroblastoma and placental choriocarcinoma cell lines. They concluded that the

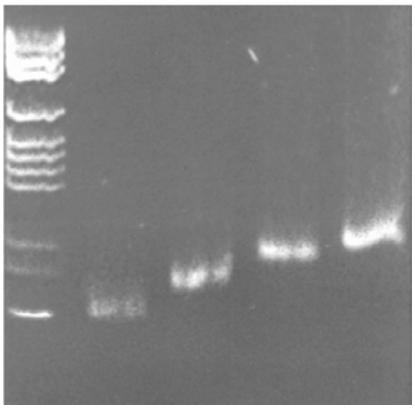

Figure 1: Observed four different MAOA-uVNTR alleles containing 2, 3, 4, and 5 tandem repeats were identified from the 474 Chinese Han subjects. Lane 1: pGEM DNA weight marker (Promega). Lanes 2, 3, 4, and 5 are 2-, 3-, 4-, and 5-repeat alleles with the length of 140, 170, 200, and 230 bp, respectively. The 2- and 5-copy types are infrequent repeat alleles, rarely reported in literatures.

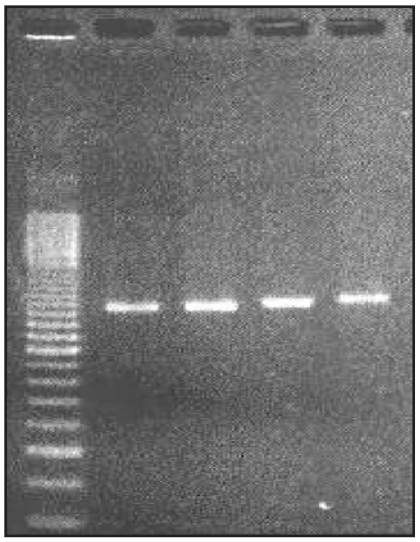

Figure 2: The PCR products of the whole promoter region containing 2-, 3-, 4-, and 5-tandem repeat were amplified. Lane 1 is the 100 bp-ladder marker (Promega), and lanes 2, 3, 4, and 5 are 2-, 3-, 4-, and 5-repeat alleles, respectively. The length of the PCR products calculated by analyzer program is 1333.3 bp, 1370.7 bp, 1408.9 bp, and 1448.4 bp for the four alleles, respectively.

(a) (b)

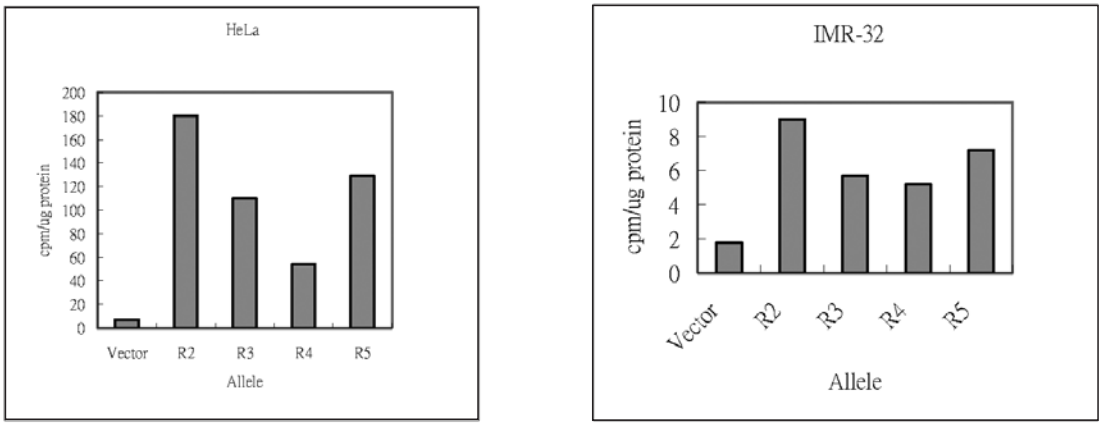

Figure 3. Transcriptional activity of MAOA gene promoter repeat constructs transfected into human cervical carcinoma Hela cells (a) and human neuroblastoma IMR-32 cells (b). Data are mean (± SD) relative luciferase activities of four independent experiments in triplicate. Constructs with 2 and 5 repeats were more active than the constructs containing 3 and 4 repeats.

Table 1 Count of alleles and polymorphism for MAOA-uVNTR in different/ethnic populations

MAOA-uVNTR (frequency × 100%)						
Populations	Sample size	2R	3R	3.5R	4R	5R
The present Chinese Han in Taiwan	474	1(0.2)	307(64.7)		165(34.8)	1(0.2)
Chinese Han in Taiwan [9]	77	1(1.3)	42(54.5)		34(44.2)	
Japanese [10]	125		78(62)		47(38)	
Asian/Pacific Islander [5]	82		50(61)	1(1.2)	31(37.8)	
White/Non-Hispanic [5]	1612		539(33.1)	8(0.5)	1056(64.8)	26(1.6)

alleles of 3.5- and 4-copy could induce a 2 to10-fold increase in transcription activity than the alleles containing 3- and 5-repeat, based on promoter fusion assays using the luciferase reporter gene. We had also fused an upstream 1.4kb DNA fragment encompassing uVNTR with the pGL3-Enhancer luciferase vector and transiently transfected the vector into two different human cell lines, the human cervical carcinoma (Hela) and the human neuroblastoma (IMR-32). To acquire accurate luciferase activity measurement, we abolish the sequence variation in MAOA core promoter by manipulating a vector-anchoring replacement. The serial pGL3-Enhancer constructs so obtained would have the same core promoter and carry different repeat alleles. Our results showed that the alleles with 2- and 5-copy had a higher luciferase activity in both the human cell lines (Figure 3).

Several discrepant reports concerning the transcriptional activity of MAOA have ever been raised. The results of Deckert et al. [12] are contradictory to those of Sabol et al. [5] as they showed that the allele containing five repeats had an increased luciferase activity in the same neuroblastoma cell line. This disagreement might result from the slightly different constructs used in both transfection experiments. Nevertheless, in accordance with Sabol et al.[5], Denny et al. [14] found a significant reduction in MAOA activity in human fibroblast cultures containing 3-repeat. A recent report [15] studied the relationship between uVNTR genotypes and concentrations of monoamine metabolites in lumbar cerebrospinal fluid in healthy human brains. It revealed that women carrying the 3.5- or 4-repeat alleles had significantly higher HVA (homovanillic acid) and 5-HIAA (5-hydroxy-indole-3-acetic acid) levels than women without these alleles. In men, however, a trend in the opposite direction was found.

Two studies [16, 17] enrolling Caucasian descent subjects reported that the less active 3-repeat allele might contribute to antisocial alcoholics, and modestly to the dimension of over- and under-reactive behaviors. However, in contrast, in an Australian general population samples, no association was detected between the MAOA-uVNTR polymorphism and both depression symptoms and personality traits that predispose to antisocial behaviors [11].

Conclusions

These inconsistent findings coupled with the discrepancy of ethnic/racial group-specific of MAOA-uVNTR polymorphism could reach to two interesting implications. First, the MAOA-uVNTR polymorphisms probably do not play a strong functional role in regulating MAOA enzymatic expression, but still has some influence on the transcriptional efficiency and activity of MAOA, and in vivo CNS serotonergic function. Second, the MAOA-uVNTR element *per se* could not play a critical role in modulating abnormal behavior, personality [18], and psychiatry disorder [17, 19]. Explanation for the complex behavioral traits based on a single factor (e.g., uVNTR frequency) may result in a spurious conclusion.

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References

1. Brunner HG, Nelen MR, van Zandvoort P, Abeling NG, van Gennip AH, Wolters EC, Kuiper MA, Ropers HH, van Oost BA. X-linked borderline mental retardation with prominent behavioral disturbance: phenotype, genetic localization, and evidence for disturbed monoamine metabolism. Am J Hum Genet 1993; 52:1032-9.

2. Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 1995; 268:1763-6.

3. Devor, CR, Cloninger, PL, Hoffman, B. Tabakoff. Association of monoamine oxidase (MAO) activity with alcoholism and alcoholic subtypes. Am J Med Genet 1993; 48: 209–13.

4. Manuck SB, Flory JD, Ferrell RE, Mann JJ, Muldoon MF. A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. Psychiatry Res. 2000; 95: 9-23.

5. Sabol S.Z., Hu S., Hamer, D. A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet. 1998;103: 273-9.

6. Denney RM, Sharma A, Dave SK, Waguespack A. A new look at the promoter of the human monoamine oxidase A gene: mapping transcription initiation sites and capacity to drive luciferase expression. J. Neurochem. 1994; 64: 843-56.

7. Zhu QS, Shih JC. An extensive repeat structure down-regulates human monoamine oxidase A promoter activity independent of an initiator-like sequence. J Neurochem 1997; 69: 1368-373.

8. Mullenbach R, Lagoda PJL, Welter C. An efficient salt chloroform extraction of DNA from blood and tissues. Trends Genet 1989; 5: 391.

9. Lu RB, Lee JF, Ko HC, Lin WW, Chen K, Shih JC. No association of the MAOA gene with alcoholism among Han males in Taiwan. Progress in Neuro-Psychopharmacology and Biological Psychiatry 2002; 26: 457-61.

10. Kunugi H, Ishida S, Kato T, Tatsumi M, Sakai T, Hattori M, Hirose T, Nanko S. A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders. Mol Psychiatry 1999; 4:393-5.

11. Jorm AF, Henderson AS, Jacomb PA, Christensen H, Korten AE, Rodgers B, Tan X, Easteal S. Association of a functional polymorphism of the monoamine oxidase A gene promoter with personality and psychiatric symptoms. Psychiatr Genet 2000;10: 87-90.

12. Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova O, Di Bella D, Nothen MM, Maffei P, Franke P, Fritze J, Maier W, Propping P, Beckmann H, Bellodi L, Lesch KP. Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 1999; 8: 621-4.

13. Hamilton SP, Slager SL, Heiman GA, Haghighi F, Klein DF, Hodge SE, Weissman MM, Fyer AJ, Knowles JA. No genetic linkage or association between a functional promoter polymorphism in the monoamine oxidase-A gene and panic disorder. Mol Psychiatry 2000; 5: 465-6.

14. Denney RM, Koch H, Craig IW. Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promoter-associated variable number tandem repeat. Hum Genet 1999; 105: 542-51.

15. Jonsson EG, Norton N, Gustavsson JP, Oreland L, Owen MJ, Sedvall GC. A promoter polymorphism in the monoamine oxidase A gene and its relationships to monoamine metabolite concentrations in CSF of healthy volunteers. J Psychiatr Res 2000; 34: 239-44.

16. Samochowiec J, Lesch KP, Rottmann M, Smolka M, Syagailo YV, Okladnova O, Rommelspacher H, Winterer G, Schmidt LG, Sander T. Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial alcoholism. Psychiatry Res 1999; 86: 67-72.

17. Schmidt LG, Sander T, Kuhn S, Smolka M, Rommelspacher H, Samochowiec J, Lesch KP. Different allele distribution of a regulatory MAOA gene promoter polymorphism in antisocial and anxious-depressive alcoholics. J Neural Transm 2000; 107: 681-9.

18. Koller G, Bondy B, Preuss UW, Bottlender M, Soyka M. No association between a polymorphism in the promoter region of the MAOA gene with antisocial personality traits in alcoholics. Alcohol Alcohol 2003; 38: 31-4.

19. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. Science. 2002; 297: 851-4.