



ORIGINAL ARTICLE

Susceptibility to methamphetamine dependence associated with high transcriptional activity alleles of VNTR polymorphism in the promoter region of monoamine oxidase A (*MAOA*)



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KEYWORDS

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Abstract *Background and purpose:* Monoamine oxidase A (*MAOA*, Xp11.3; OMIM: 309850) can modulate the level of neurotransmitters in the central nervous system. A 30 bp variable number of tandem repeat (VNTR) genetic polymorphism on the promoter region of the *MAOA* can modulate the transcriptional activity of the gene. Association between this polymorphism and dependency to methamphetamine was investigated.

Subjects and methods: A total of 65 methamphetamine abusers (52 males and 13 females) and 635 healthy controls (525 males and 110 females) were included in the present case–control study. Genotypic analysis for the *MAOA* VNTR polymorphism was determined by conventional PCR. Based on transcriptional activity of the VNTR alleles, the alleles were categorized into two classes: L allele (2R and 3R alleles) and H allele (3.5R, 4R and 5R alleles), which have low and high transcriptional activities, respectively.

Results: Our data show that the H allele significantly increases the risk of methamphetamine dependence in males (OR = 2.03, 95% CI: 1.04–3.67, $P = 0.037$). The H allele seems positively associated with the risk of dependency to methamphetamine among females, but the observed OR did not reach the significance level, probability due to small sample size of the patients.

Conclusion: The present study supports the role of the VNTR polymorphism on the promoter region of the *MAOA* on methamphetamine dependence.

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1. Introduction

Monoamine oxidase A (*MAOA*, Xp11.3; OMIM: 309850) oxidizes neurotransmitters. It can modulate the level of neurotransmitters in the central nervous system and plays an

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important role in many behaviors such as violence, criminal, and drug dependence [1–6]. Previously several genetic polymorphisms were reported in the gene encoding *MAOA* (). A 30 bp sequence within the core promoter region of the *MAOA* was found to occur as a polymorphic element, either singular or as tandemly repeated 2, 3, 3.5, 4 or 5 times [7–9]. This polymorphic site is located 1.2 kb upstream of the *MAOA* coding sequence. Studies have shown that the 3.5R, 4R and 5R alleles of this variable number of tandem repeats (VNTRs) were more active than the other alleles (2R and 3R) [8].

There were several studies investigating the association between this genetic variation and susceptibility to drug dependence, including alcohol, heroin and methamphetamine [2–4,6,10–13]. However, the results were not consistent. Based on our knowledge there was only one study investigating the association between the *MAOA* VNTR polymorphism and susceptibility to methamphetamine dependence in the Japanese population [3]. There was no study from Caucasian populations. Therefore, the present case–control study was carried out.

2. Subjects and methods

2.1. Participants

A detailed description of the study subjects has been reported in our previous reports [14,15]. A total of 65 methamphetamine abusers (52 males and 13 females) and 635 healthy controls (525 males and 110 females) were included in the present case–control study. The mean age (SD) of the patients and the controls were 35.0 (8.5) and 33.6 (9.0) years, respectively. The patients were assessed using the Structured Clinical Interview based on *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) criteria for methamphetamine dependence. Moreover, urine drug screens were obtained. All patients were interviewed by a senior psychiatrist.

Considering that in the genetic association studies we are going to compare the frequency of the genotypes (or the alleles) between cases and controls, it is necessary to select both groups from a gene pool (because allelic frequency may show a difference between gene pools). This point strongly recommended by STrengthening the REporting of Genetic Association studies (STREGA) [16]. However, it should be noted that the Iranian gene pool is one of the most heterogeneous gene pools [17–19]. Therefore, we select our patients and controls from Persians (Caucasians/Muslims) living in Fars province. Data on ethnicity were collected using simple questions like the parental and grandparental ethnicity (and also the religion) of each participant. Participants whose mothers and fathers (and also their grandparents) did not belong to Persians or to non-Muslim religious communities were excluded.

This study was approved by the Shiraz University ethics committee and informed consent was obtained from each subject before the study. The work has been carried out in accordance with The Code of Ethics of the World medical association (Declaration of Helsinki) for experiments in humans.

Using the QUANTO (<http://biostats.usc.edu/software>) software, to detect a real difference in genotypic frequency

with a power of 0.80, $\alpha = 0.05$, OR = 1.75, 40% frequency of the minor allele, and if the ratio of case/control be equal to 1/10; a minimum sample of 136 would be necessary.

2.2. Genotyping analysis

Peripheral blood samples were collected from the participants. Genomic DNA was isolated from EDTA treated blood samples. Genotypic analysis for the *MAOA* VNTR polymorphism was determined by the PCR assay, using specific primers as described previously [6]. In brief, conventional PCR amplification was performed using the following with 5'-ACA GCC TGA CCG TGG AGA AG-3' (forward) and 5'-GAA CGG ACG CTC CAT TCG GA-3' (reverse) primers [6]. PCR conditions consisted of an initial denaturation step of 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 68 °C for 45 s and 72 °C for 45 s, a final extension of 72 °C for 5 min. PCR products were subjected to 2.5% agarose gel electrophoresis and then visualized with a gel imaging system to determine genotypes.

Alleles containing 2, 3, 4 or 5 repeats gave products with the following lengths of 294, 324, 354, and 384 bp, respectively. Based on transcriptional activity of the VNTR alleles, the alleles were categorized into two classes. The 2R and 3R alleles (L allele) have lower transcriptional activity corresponding with lower expression level, while the 3.5R, 4R and 5R (H allele) have higher transcriptional activity corresponding with higher expression [8].

2.3. Statistical analysis

Chi-square test was performed for the polymorphism to determine if the control group demonstrated the Hardy–Weinberg equilibrium. The association between the genotypes and risk of dependence to methamphetamine was assessed by calculating odds ratios (ORs) and 95% confidence intervals (CIs). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) (version 11.5). A probability of $P < 0.05$ was considered statistically significant. All P values were two-tailed.

3. Results

The gene encoding *MAOA* is located on the human chromosome X, therefore we should report the genotypes of the *MAOA* VNTR polymorphism in each gender. Table 1 shows the genotypic frequency of the polymorphism in cases and healthy controls. Table 2 shows the distribution of the genotypes according to the L and H alleles.

There was no significant difference between gender groups for the frequency of the H and L alleles ($\chi^2 = 3.37$, $df = 1$, $P = 0.066$). The observed genotypic frequency in the females of the control group was in agreement with Hardy–Weinberg equilibrium ($\chi^2 = 1.58$, $df = 1$, $P = 0.208$).

The present data indicate that the H allele significantly increases the risk of methamphetamine dependence in males (OR = 2.03, 95% CI = 1.04–3.97, $P = 0.037$) (Table 2). Our statistical analysis revealed that the combination genotypes of LH + HH (versus the LL genotype; dominant penetrance condition) were not associated with the risk of methamphetamine dependence among females (OR = 3.71,

Table 1 Genotypic distribution of the *MAOA* VNTR polymorphism in healthy control and methamphetamine dependent subjects, cases stratified by gender.

Genotypes/Gender	Controls	Cases
<i>Females</i>		
2R/2R	1	–
2R/4R	1	–
3R/3R	25	1
3R/4R	44	7
3R/5R	3	–
4R/4R	34	5
4R/5R	2	–
Total	110	13
<i>Males</i>		
2R	6	–
3R	193	12
4R	306	38
5R	20	2
Total	525	52

Table 2 Association between genetic polymorphism of 30 bp VNTR polymorphism of *MAOA* and risk of methamphetamine dependence.

Genotypes/Alleles	Controls	Cases	OR	95% CI	P-value
<i>Males</i>					
L	199	12	1.0	–	–
H	326	40	2.03	1.04–3.97	0.037
<i>Females</i>					
LL	26	1	1.0	–	–
LH	48	7	3.79	0.44–32.5	0.224
HH	36	5	3.61	0.39–32.7	0.254
LH + HH	84	12	3.71	0.46–29.9	0.218
L	100	9	1.0	–	–
H	120	17	1.57	0.67–3.68	0.296

95% CI: 0.46–29.9, $P = 0.218$). The H allele seems positively associated with the risk of dependency to methamphetamine among females (OR = 1.57, 95% CI: 0.67–3.68, $P = 0.296$), but the observed OR did not reach the significance level, probability due to small samples size of the patients (Table 2).

4. Discussion

It should be noted that only one study has reported the association between *MAOA* VNTR polymorphism and dependency to methamphetamine in a Japanese population, which reported no significant association [3]. However, our present study indicating that the H allele of the study polymorphism was associated with the risk of methamphetamine abused at least in males (Table 2).

We know that association between a genetic polymorphism and a multifactorial trait was not similar between different ethnic groups [20–23]. It is possible that the discrepancy between findings of the present study with the study of Nakamura et al., [3], at least in part, may be interpreted by the ethnicity of the participants (Asians and Caucasians).

However, it is very premature to conclude in which ethnic groups this polymorphism is associated with risk of methamphetamine dependence.

It should be noted that very recently we reported no significant association between the study polymorphism and the risk of heroine dependence in our population [13]. It might be concluded that the mechanisms involved in dependency to heroin and methamphetamine is not similar, at least in our population.

Finally, the small sample size (especially in the methamphetamine dependent cases) is the major limitation of the present study. Therefore, the contribution of the VNTR genetic polymorphism on the promoter region of the *MAOA* in dependency to methamphetamine should be further researched on other populations.

Disclosure statement

The authors declare no conflict of interest.

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