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Allelic Diversity of VNTR polymorphism in Monoamine Oxidase A (MAOA) gene in Iraqi Population

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Abstract :

Genetic analysis of population have benefit in behavior population and medical fields .However , genetic study and information on Iraqi population are limited , The gene of monoamine oxidase A (MAOA) was chosen because it has variable number tandem repeats (VNTR) in the promoter region of gene that are known to be correlated with social behavior . The analysis of (VNTR) polymorphism provide valuable information on behavioral disorders and traits in addition to providing information on population variation and differentiation.

Methods: The present study , blood samples were randomly selected from 440 Iraqi volunteers. This study has been conducted in Biology department – collage of science –Babylon university and College of Pharmacy – Ahel AL- Bait University .

DNA was extracted from the peripheral blood of all participants, and the VNTR -MAOA polymorphism were genotyped by using the polymerase chain reaction (PCR) and agarose gel electrophoresis , the different fragment sizes were determined by comparisons to molecular length standards . The results were confirmed by using sequencing PCR technique.

Results: From the sequencing results 3.5R, 4.5R and 5.5R repeats were successfully identified instead of the 3R , 4R, 5R repeats which were identified by previous researchers In addition to the novel repeat **1.5R** it first recorded in Iraqi population . The result of the MAOA – VNTR genotyping revealed that the two common genotype were 4.5/4.5 and 3.5/3.5 with percent about 47.7 % and 25.2% and the two common alleles were 4.5R and 3.5R with percent 54.3 % and 31.2 respectively .

The result of our study compared with data of eleven populations , showed the significant difference between allele frequencies of Iraqi samples with other populations Hispanic (Latino) , White (non- Hispanic) , New Zealand (European origin) , Italian (European origin) , Chinese , Asian (Pacific Islander) , African American and American populations ($P \leq 0$ .05). ,while no significant difference was found with Afrikaner and German, European origin populations (p value= 0.06 , 0.062 and 0.077) respectively .

Conclusion: MAOA allele frequency were differ between racial groups . in addition to establish a new repeat (1.5 R) , the Iraqi population had more 5.5R allele than other populations , .

Key word: Monoamine Oxidase A , VNTR , Polymorphism , *maoa* , Genotype .

INTRODUCTION :

Genetic population studies are useful in providing information on population variation ,differentiation and structure. The *maoa* –VNTR polymorphism are perfect candidates for behavior studies because its role in neurotransmission association gene that connected with range of disorder and traits including aggression , impulsive ,alcohol dependent , violence and bipolar disorder [1-5].

Monoamine oxidase (MAO), as catabolic enzyme regulates monoamine transmitter levels in the central nervous system , the *maoa* gene codes for the production MAOA enzyme which break down certain neurotransmitters ,including serotonin and dopamine [6] .

The activity of this enzyme is partly genetically regulated . The genes encoding the A and B forms of human monoamine oxidase enzymes (MAOA and MAOB) are located together on the short arm of X chromosome , between bands Xp11.23 and Xp11.4. The MAOA gene has a 30 bp functional VNTR polymorphism in promoter region with 2 , 3, 3.5, 4 and 5 or 6 copies [7, 2, 8] . The 3 and 4 repeat alleles are the most common among human populations [7].

The alleles for this polymorphism are typically grouped in to two categories : one group contains alleles that correspond to low enzyme activity and the other contains alleles that correspond to high enzyme activity [9].

The low MAOA activity alleles are not as effective as the high MAOA activity alleles at metabolizing neurotransmitter . as a consequence, the low MAOA activity alleles are typically considered the risk alleles for various psychopathologies and extreme violence [10,11].

The aim of the current study is to determine the genetic variation and allelic frequency of the MAOA – VNTR in Iraqi population and compare the result with similar data from other populations .

MATERIALS AND METHODS :

Blood samples were randomly selected from 440 Iraqi volunteers. This study has been conducted in Biology department – collage of science –Babylon university and College of Pharmacy – Ahel AL- Bait University .

Blood samples (3-5ml) were collected in EDTA tubes from each subject in the study and stored frozen at -20 C˚ until analysis.

Each frozen blood specimen was thawed, genomic DNA was then extracted directly using FAVORGEN tissue genomic DNA extraction kit (Taiwan). DNA purity and concentration were determined using a spectrophotometer (Nanodrop).

Genotyping of *maoa* Polymorphism:

Genotyping was performed using conventional PCR technique of the MAOA – VNTR polymorphism region and DNA sequencing method used for confirmed the presence of tandem repeats. The polymorphism was detected using primers forward:5′ ACAGCCTGACCGTGGAGAAG-’3′and revers:5′ GAACGTGACGCTCCATTCGGA -3′ (Butovskaya *et al*.,2013(B)). The components of PCR working solution were mentioned in table (1).

Table (1): The Master Mix components of PCR :

Component	Amount (µl)	Concentration
Master Mix	12.5	1X
DNA	3	50-150 ng/µl
MAOA primers	2	10pomol
DNas free water	Up to 25µl	-
Total volume	25µl	-

Table (2): The PCR protocol for *MAOA-VNTR* polymorphism detection.

No.	Steps	Temperature °C	Time	cycle	Product size (bp)	Reference
1.	Initial denaturation	94	10 min.	1	291,321,336,351,381	11
2.	Denaturation	94	1 min.	35		
3.	Annealing	66	1 min.			
4.	Extension	72	1 min.			
5.	Final extension	72	5 min.	1		
6.	Hold	4	5 min.	1		

PCR Protocol:
PCR was performed in a thermo cycler under the following conditions adopted in table (2).
The amplified products of *maoa* VNTR polymorphism was analyzed by different fragment sizes which were determined by comparisons to molecular length standards and confirmed by software analysis (Gel analyzer 2010) the electrophoresis was achieved in 2.5 % agarose gel stained with 1µl (10mg /ml) Ethidium Bromide, at 75 V for 1.5/ and or 2 hours using 1X/or 0.5X TBE buffer, then visualized under UV light using ultraviolet Gel documentation. DNA ladder (100 bps Promega) were used as a comparative . The lengths of PCR products of 2,3,4 and 5 repeats were 291,321,351 and 381 respectively.

Purification of PCR Products:
Purification of PCR Products is accomplished according to Megaquick-spin^{TM} total fragment DNA purification kit-iNtRON company protocol .PCR products are selectively adsorbed in silica gel –based MEGAquick – spin ^{TM} column and then was washed away other components. The elution of DNA was performed, and then can be discard the column. PCR purification product was high quality DNA which can be used for DNA sequencing .

PCR –Sequencing
PCR- Sequences of MAOA –VNTRs were performed by MACROGEN company / korea, for confirming the presence of tandem repeat in mentioned gene . The samples were primed and amplified for the mentioned genes above by using above mentioned primers . The samples were sent for analytic sequences. Sequencing results were viewed by Company Macrogen / korea, using sequence scanner machine AB13730XL Applied Biosystems. Number of samples for detection of polymorphisms depended on processing successful of DNA-Sequences related with source of company.

Analysis of Data:
Data analysis were conducted in 2 ways :
Analysis of sequence results for both strand (forward and reverse)by (Bio Edit version 7.2.5 program .
Statistical analysis :
Statistical analysis was carried out using SPSS version 23.categorical variables were presented as frequencies and percentage . Chi-square test and fisher exact test were used to compare between percentages (frequencies) of some populations with present data . P value for all tests was considered significant if ≤ 0.05

RESULTS AND DISCUSSION :

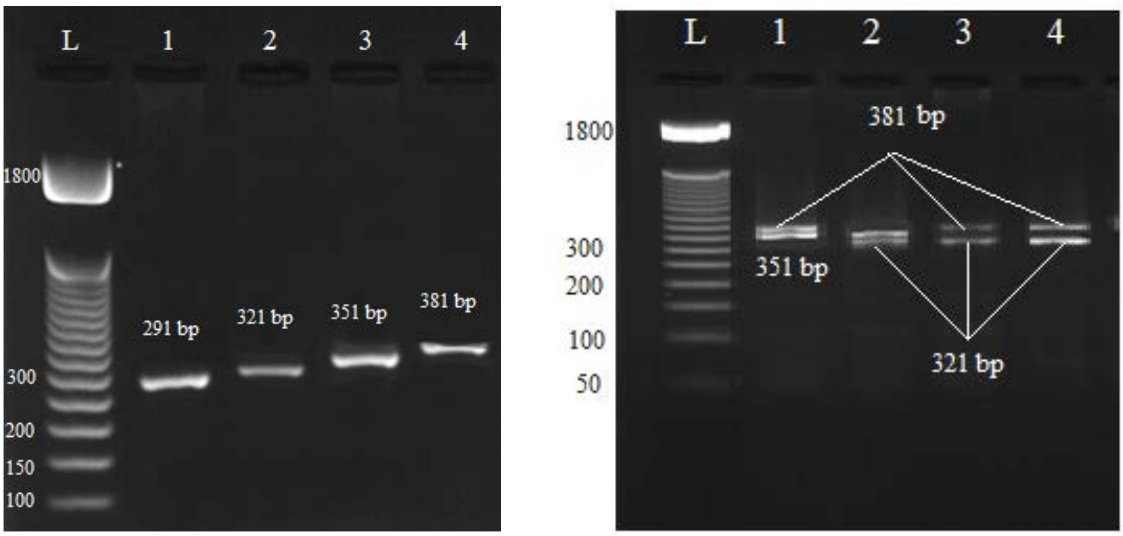
Genotypes and allele frequencies for *MAOA-VNTR* polymorphism :

1- Analysis of *maoa* VNTR polymorphism :

The human *maoa* gene contains 30 bp VNTR polymorphism in promoter region , this polymorphism varies from 2 to 5copies the polymorphism was analyzed by the PCR and the different fragment sizes were determined by comparisons to molecular length standards and confirmed by software analysis (Gel analyzer 2010) (figure 1) .Ten samples were sequenced to established the polymorphisms and from the sequencing results the 1.5R, 3.5R, 4.5R and 5.5R alleles were successfully identified instead of the 3R ,4R, 5R alleles which were identified by previous authors [7, 2] ,while the **1.5 R** alleles was novel allele and it found at the first time in Iraqi population . The results were compared with data obtained from Gene Bank published (accession no. M89636.1) which is available at the NCBI and aligned by using Bio Edit software program (figure 2).
The difference between results from this study and the previous mentioned studies were due to a difference regarding half of the 30 bp repeat sequence. The original researches did not include half of the repeat when allele calling was performed. Authors such as Das *et al*. (2006)[13] , Malan (2014) [14] and Altayie and Jebor (2017)[15] have also noted this discrepancy .

Genotypeś Distribution of *maoa* VNTR Polymorphism with Allele Frequency

The distribution of the observed *maoa* VNTR genotype and allele frequencies in Iraqi population are shown in table (3).The highest genotype was 4.5/4.5 (47.7%),followed by 30.2% for 3.5/3.5 genotype , 6.8 % for 5.5/5.5 genotype , 5.5% for both 4.5/3.5 and 5.5/3.5 genotypes and (1.1% and 3.2%) for (1.5/1.5 and 5.5/4.5) genotypes respectively.
Two common alleles (4.5R and 3.5R) were observed with frequency 52% and 35.8% respectively , 11.1 % for rare 5.5 R allele and one rare novel alleles (1.5R) with frequency (1.1%) were observed in our samples.
The results pointed out that there was a significant difference ($p \leq 0$ .05) among all genotypes , meantime there was significant difference among 4.5R and 3.5R alleles ($p = 0.003$) in study samples.



(A) **(B)**

Figure (1): The Electrophoresis pattern of PCR product for *maoa* gene this amplification products were representing the VNTR polymorphism for gene in 2.5% agarose ,75 V, for 2 h (10 µl of PCR product loaded in each well).

(A) Different *VNTRs* of *homozygote maoa* gene:

- Lane L: DNA ladder (50bp)
- Lane 1 : band 291 bp ,represent the homozygotes genotype 1.5/1.5 repeat
- Lane 2: band 321 bp ,represent the homozygotes genotype 3.5/3.5 repeat .
- Lane 3 : band 351 bp ,represent the homozygotes genotype 4.5/4.5 repeat .
- Lane 4 : band 381 bp ,represent the homozygotes genotype 5.5/5.5 repeat .

B))) Different *VNTRs* of *heterozygote maoa* gene:

- Lane L: DNA ladder (50bp) .
- Lane 1: both bands 381& 351 bp ,represent the heterozygotes genotype 5.5/4.5 repeats .
- Lane 2: both bands 351& 321 bp ,represent the heterozygotes genotype 4.5/3.5 repeats .
- Lane 3 & 4: both bands 381& 321 bp ,represent the heterozygotes genotype 5.5/3.5 repeats .

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-       5.5 R         GGCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT
-       4.5 R         GGCGGCACAAGCACCTGCACCAGTATCCCCACCAGCACCGGCACCGGCACCAGTACCCGCACCAGT
-       3.5 R         GGCGGCACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT
-       1.5 R         GGCGG~ACCGGCACCGGCACCAGTACCCGCACCAGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
-       M89636.1      GGCGGCACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT

-       5.5 R               ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT
-       4.5 R               ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT
-       3.5 R               ACCGGCACCGGCACCAGTACCCGCACCAGT~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
-       1.5 R               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
-       M89636.1            ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCAGTACCCGCACCAGT

-       5.5 R               ACCGGCACCGGCACCAGTACCCGCACCAGTACCGGCACCGGCACCGAGCGCAAGG
-       4.5 R               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ACCGGCACCGGCACCGAGGGCAAGG
-       3.5 R               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ACCGGCACCGGCACCGAGCGCAAGG
-       1.5 R               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ACCGGCACCGGCACCGAGCGCAAGG
-       M89636.1            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ACCGGCACCGGCACCGAGCGCAAGG
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Figure (2): Nucleotide sequence alignment of *maoa*- VNTR sequences identified (5.5 .4.5 and 3.5 and **novel repeat 1.5R** alleles) and a reference sequence from Gen Bank (Accession number M89636.1), The 30 bp repeat sequences are highlighted consecutively yellow and red, half repeat indicated by green.

Comparison of the *maoa*- VNTR allele frequencies with other Populations

The allele frequency results among ours and others researches are shown in Table (4) , it is worthy of notice that the higher allele frequency was 4-repeat allele in line with several prior studies, suggesting the similarity of allele frequency distribution and there was no statistically significant difference between our study with Afrikaner and German, European origin populations (p value= 0.06 , 0.062 and 0.077) respectively , the 2-repea, 3.5 repeat and 5-repeat were infrequent alleles found in several populations (Table 1). Statistical result revealed the significant difference when incorporated with Hispanic (Latino) , White (non- Hispanic) , New Zealand (European origin) , Italian (European origin) , Chinese , Asian (Pacific Islander) , African American and American populations ($P \leq 0$ .05).

Table (3): Genotypeś distribution of *maoa* VNTR polymorphism with allele frequency in Iraqi populations .

***maoa* VNTR Polymorphism**	**NO.(%)**	**Sig.**
Genotype		
Homozygote 4.5/4.5	210(47.7)	
Homozygote 3.5/3.5	133(30.2)	**0.012***
Homozygote 5.5/5.5	30(6.8)	**<0.001***
Homozygote 1.5/1.5	5(1.1)	**0.03***
Heterozygote 4.5/3.5	24(5.5)	**<0.0001***
Heterozygote 5.5/3.5	24(5.5)	**<0.0001***
Heterozygote 5.5/4.5	14(3.2)	**0.001***
Total number	**440**	
Allele		
4.5R[a]	458(52)	
3.5R	314(35.8)	**0.003***
5.5R	98(11.1)	0.28
1.5R	10 (1.1)	0.53

*P value ≤ 0 .05
[a] reference

Population	**Number of tested**	***maoa* VNTR allele frequency (%)**							**Reference**
		1.5R	**2R**	**3R**	**3.5R**	**4R**	**5R**	**Sig.**	
Iraqi	440	1.1	0	35.8	0	52	11.1	-------	Present study
Hispanic/Latino	92	0	0	29.3	0	70.7	0	**0.001***	7
Afrikaner	196	0	0	28.1	0	68.4	3.5	0.06	16
White/non- Hispanic	1629	0	0	32.7	0.5	64.9	1.8	**0.044***	7
New Zealand, European origin	1040	0	0.3	33.8	0.9	63.8	1.2	**0.04***	3
German, European origin	131	0	0	35.9	0.8	61.1	2.2	0.062	17
German, European origin	390	0	0.8	35.9	0.8	61	1.5	0.077	18
Italian, European origin	180	0	1.7	40	0	56.6	1.7	**0.046***	17
Chinese	214	0	0.5	57	0	42	0.5	**0.003***	19
Asian/Pacific Islander	82	0	0	61	1.2	37.8	0	**0.000***	7
African American	88	0	0	59	2.3	36.4	2.3	**0.001***	7
American	620	0	0	36.2	2.9	60.5	0.4	**0.003***	20

Table 4 : Count of alleles frequencies for *maoa* - VNTR in different/ethnic populations .

*P ≤ 0 .05

In contrast, the 4-repeat allele was reported to be more common than the 3-repeat in Caucasian Australians [21], White/non-Hispanics, German, and Italian subjects [17]. Among Caucasians the 4 repeat allele frequency (0.684) of the VNTR is twice as common as the 3- repeat allele frequency (0.331) [7]. Hamilton *et al* .(2000)[20] 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. In contrast , Sabol *et al*. (1998)[7] revealed a frequency of 61% for 3-repeat and 37.8% for 4-repeat in Asian subjects and Pacific islanders. All these results, including ours, consistently demonstrated that the 3 and 4 repeats in Asian populations were the two most frequent alleles. Clearly, there were substantial variations in frequency distributions of 3- and 4-alleles among different ethnic or racial groups, These discrepancies imply that the *maoa* VNTR functional polymorphism might not play a crucial role in behavioral or physiological variability in humans [22, 23].

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