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The Trp64Arg Polymorphism in the ADRB3 Gene is Associated with Hypertension in Syrians

Hiba Kady[1], Mohammad Imad Balid[2] and Mohammad Yaser Abajy[3]

[1]*Postgraduate Student (MSc), Dept. of Biochemistry and Microbiology, Faculty of Pharmacy, Aleppo University, Syria*
[2]*Dept. of Laboratory Medicine, Faculty of Medicine, Aleppo University, Syria*
[3]*Dept. of Biochemistry and Microbiology, Faculty of Pharmacy, Aleppo University, Syria*

Abstract: *Genes involved in the regulation of catecholamine function may be important in the development of hypertension. The β-adrenergic receptors importantly influence vascular reactivity and may regulate blood pressure. Genetic polymorphisms of the β-adrenergic receptors' genes have been shown to alter the function of several adrenergic receptor subtypes and thus to modify the response to catecholamine. To determine if the common single nucleotide polymorphism (SNP) Trp64Arg in β_3-adrenergic receptor gene (ADRB3) is associated with hypertension in adults, we recruited 78 individuals involving 37 healthy normotensive controls and 41 hypertensive patients who are between 36 and 70 years old in a case - control study which will be discussed in this paper. Results suggest that the Trp64Arg polymorphism contributes to hypertension in our population.*

Keywords: *ADRB3, adrenergic receptor, hypertension, PCR-RFLP, polymorphism, SNP, Trp64Arg,*

1. INTRODUCTION

Hypertension is an important risk factor contributing to considerable mobility and mortality of stroke, heart failure, coronary heart disease and renal failure [1]. It is a major public health problem as the World Health Organization (WHO) indicated that hypertension affects 26.4% of the world's adult population [2]. Approximately 20%–40% of the variation in blood pressure among the general population is determined by genetic factors [3]. Hypertension is a multifactorial disorder that results from the interplay of multiple genes and environmental factors. It is clear from familial and epidemiological studies that hypertension occurs as a result of a complex interplay between genetic and environmental lifestyle exposures [4]. It results essentially from an imbalance between the vasoconstrictive and vasodilatory mechanisms, partly due to pre- and post-synaptic sympathetic dysfunctions [5]. A generalized decrease of the β- adrenergic response has been recognized in systemic hypertension in human [6]. β_3- adrenergic receptor present on endothelial cells have been identified to induce vasorelaxation of human coronary arterioles in invitro studies [7].

The β_3-adrenergic receptor (β_3-AR) is a member of the family of the adrenergic receptors (adrenoceptors) comprising of the α-adrenoceptors and β-adrenoceptors [8]. β_3-AR is a guanine binding protein coupled receptor (GPCR) which its general structure characterized by seven transmembrane domains with three intracellular and three extracellular loops [9]. A missense mutation in the first transmembrane domain or the most proximal residue of the first intracellular loop of the human β_3-AR was reported. The mutation produces a Thymine (T) to Cytosine (C) transition at nucleotide position 190 causing the replacement of Tryptophan (Trp/W) by Arginine (Arg/R) at amino acid position 64 (Trp64Arg) [10]. The exact functional effect of the expression and activity of the *ADRB3* gene is still unclear. There are only few data about the potential role of vascular β3-AR in hypertension. However, some studies showed that the Trp64Arg polymorphism has been associated with hypertension [11] [12] [13], but findings are still controversial since some studies found no association [14] [15] [16].

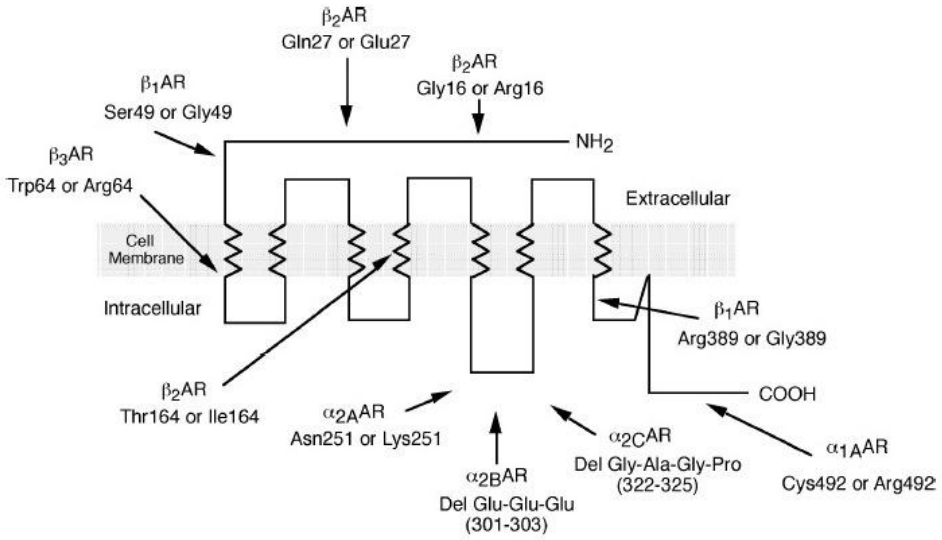

Fig.1: General structure of human adrenoceptors and the approximate locations of polymorphisms of human adrenergic receptors. [9]

2. MATERIALS AND METHODS

2.1 Subjects:

The population studied consisted of 78 native Syrians aged from 36 to 70 years (average 50.1 ± 9.7) living in Aleppo, Syria. Of the population, 37 were healthy normotensive individuals (25 men and 12 women), while the other 41 were previously diagnosed with hypertension in Aleppo University Cardiology Hospital (23 men and 18 women). Peripheral blood samples were collected in EDTA containing anticoagulation tubes and were anonymously coded and stored.

2.2. DNA isolation:

Genomic DNA was extracted from 200 µl venous blood using a spin column format kit (QIAmp™DNA Blood Mini Kit; QIAGEN, USA). The procedure was carried out according to the manufacturer instructions.

2.3. Polymerase Chain Reaction (PCR) amplification of the SNP-containing fragment:

A 210 bp fragment of the *ADRB3* gene containing the target SNP location was amplified with the forward primer 5'- CGCCCAATACCGCCAACAC -3' and the reverse primer 5'- CCACCAGGAGTCCCATCACC -3' using the PCR method as described by Widén *et al*., 1995 [10] with one modification in using 4% dimethyl sulfoxide (DMSO) instead of 4% formamide as they both are PCR enhancers that increase specificity and efficiency, lower T_m and reduce secondary structures [17].

2.4. Determination of Trp64Arg Polymorphism:

Genotyping was carried out using PCR-RFLP technique according to Widén E. *et al.*, 1995 [10]. Digestion of the amplification product with *Mva*I restriction enzyme produced fragments of the following sizes: 99, 62, 30, 12, and 7 bp in Trp64 homozygotes; 161, 99, 62, 30, 12, and 7 bp in Trp64/Arg64 heterozygotes; and 161, 30, 12, and 7 bp in Arg64 homozygotes. The smallest of these fragments (30, 12, and 7 bp) were too small to be resolved on the gel.

2.5. Statistical Analysis:

Analysis was performed using the SPSS (IBM® SPSS® Statistics 22) for windows statistical package. Descriptive statistics including the mean ± SD were calculated. Differences were significant at *P* value less than 0.05. The Relation between the polymorphism and hypertension was assessed by Logistic Regression analysis. It was used to evaluate the impact of the polymorphism on the development of hypertension. Crosstabs were

used to display the frequency distribution of the variables. R Square was also used to measure the predictive power of the studied polymorphism to develop hypertension.

3. RESLUTS

3.1. Genotyping and Allele Frequencies:

Genotype and allele frequencies are shown in Table 1. 39 subjects were homozygous for Trp, three were homozygous for Arg64 and 36 were heterozygous. The overall allele frequency of the Arg allele in the studied population is 26.9%.

Table 1. Genotype and Allele Frequencies of Trp64Arg in Studied Subjects

		Total (%)	Normotensive (%)	Hypertensive (%)
Genotype		N = 78	n = 37	n = 41
	Trp/Trp	39 (0.500)	25 (0.32)	14 (0.179)
	Trp/Arg	36 (0.461)	11 (0.141)	25 (0.32)
	Arg/Arg	3 (0.038)	1 (0.012)	2 (0.022)
Allele		N = 156	n = 74	n = 82
	Trp	114 (0.731)	61 (0.391)	53 (0.34)
	Arg	42 (0.269)	13 (0.083)	29 (0.186)

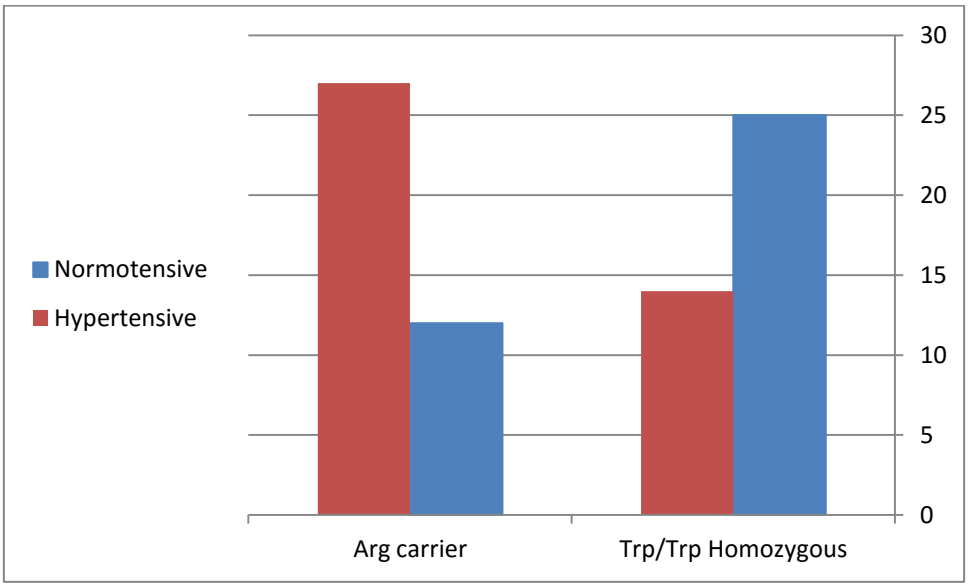

Fig. 1. Trp64Arg Polymorphism Count in Studied Population

3.2. Effect of theTrp64Arg Polymorphism on Development of Hypertension:

Due to the small number of mutant homozygotes, comparisons were made between carries of the Arg Allele and the wild-type Trp/Trp homozygote.

The contribution of Trp64Arg polymorphism with the development of hypertension was assessed by using the logistic regression test. We found a significant association of the presence of the Arg allele (Trp/Arg + Arg/Arg) with the development of hypertension when compared with Trp/Trp homozygote. The Logistic Regression

analysis showed a significant relationship between the Trp/Arg polymorphism and hypertension (Wald = 8.344, *P* = 0.004) suggesting that carriers of the Arg allele are four folds likely to develop hypertension (OR = 4.018). Carrying this allele is accounted for 14% of the variance (R^2 = 0.143).

4. DISCUSSION

β_3-AR is located mainly in the adipose tissue and is involved in the regulation of lipolysis and thermogenesis. In addition to its rule in metabolic function, β_3-AR regulates cardiac inotropy, angiogenesis and endothelium-dependent vasorelaxation [7]. Some experimental data showed that β_3-AR can modulate peripheral vascular tone and increase blood pressure [10].

Our study shows that the substitution of Arg for Trp in codon 64 is present at an allelic frequency of 26.9%, and the homozygous genotype occurs at a frequency of 3.8% in a sample that is representative of the Syrian population which is Caucasian of predominantly Arab ancestry.

We also reported that the Trp64Arg mutation is associated with hypertension similar to findings of Tonolo *et al*., 1999 [11] who studied this Arg64 variant in Sardinian population. Ringel *et al*., 2000 [12] reported that this association was found only in men of Caucasian (German) population, but we could not conduct a gender-based analysis due to the small number of subject in our study. In the contrary, our findings disagree with Pamies-Andreu *et al*., 2000 [15] and Thomas *et al*., 2000 [16] who found no significant association of the Trp64Arg variants and hypertension in Caucasian (Spain) population and Southern Chinese population respectively.
It makes sense that our data are consistent with the findings of Tonolo *et al*., 1999 and Ringel *et al*., 2000 since they both studied the Caucasian population. Disagreeing with Thomas *et al*., 2000 who studied the Chinese population might be due to the effect of race. However, disagreeing with Pamies-Andreu *et al*., 2000 who also studied the Caucasian population indicates that the Trp64Arg polymorphism's contribution to the development of hypertension might vary according to race, environmental factors and lifestyle. This dissimilarity in findings might be also because of the small sample studied by Pamies-Andreu *et al*., 2000 (N = 87) when compared to Tonolo *et al*., 1999 (N = 494) and Ringel *et al*., 2000 (N = 417) which makes their findings more reliable. Another possibility is that this polymorphism might not be directly involved in rising blood pressure, but may act as a marker for still unknown genes nearby.

It is mention worthy that hypertension is a multi-genic and multifactorial disease and it is doubtful that a single amino acid change in the β_3-adrenoceptor would be sufficient to explain the onset of the disease, although it might contribute to the development or aggravation of the condition.

5. CONCLUSION

Our study has shown an association between carrying the Allele Arg64 in the *ADRB3* gene and the development of hypertension in the Syrian population. It is important to consider applying interventions to prevent or delay hypertension and its complications when carrying this polymorphism is confirmed.
Since pathogenesis of hypertension is complicated and might be influenced by multi genes as well as environmental factors, further study with a bigger sample size and more genes to be analyzed is suggested.

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