Preliminary study on association of β2 - Adrenergic Receptor Polymorphism with hypertension in hypertensive subjects attending Balok Health Centre, Kuantan

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SUMMARY

Polymorphisms within the β2-adrenergic receptor (ADRB2) gene have been repeatedly linked to hypertension. Among the ADRB2 polymorphisms detected, Arg16Gly and Gln27Glu codons are considered the two most important variations. The amino acid substitution at these codons may lead to abnormal regulation of ADRB2 activity. The aim of the present study was to assess the association between ADRB2 polymorphisms and hypertension. This case-control study consisted of 100 unrelated subjects (50 hypertensive and 50 matched normal controls). Arg16Gly and the Gln27Glu polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism assay. There were no significant evidence of association in allelic and genotypes distribution of Arg16Gly and Glu27Gln with blood pressure and hypertension. These findings suggest that the variation within codon 16 and 27 of ADRB2 gene were unlikely to confer genetic susceptibility for hypertension in our population samples.

KEY WORDS:
ADRB2 gene, single nucleotide polymorphism, hypertension, blood pressure

INTRODUCTION

Hypertension is a complex trait, influenced by multiple environmental and genetic factors where the estimated heritability for systolic and diastolic blood pressure are 34 and 37% respectively[1]. It affects almost 4.8 million of Malaysian individuals and is responsible for significant comorbidities, such as stroke, cardiac dysfunction, heart failure and renal failure [2].

The human ADRB2 is a G-protein-coupled receptor found in a variety of tissue types and is a target of the catecholamines. It has been suggested that the polymorphic variants of this receptor manifest different regulation upon agonist stimulation in cultured cells[3]. The genetic variations are responsible for differing response of treatment among individuals toward adrenoreceptor drugs in asthmatic cases [4,5]. The ADRB2 gene (MIM number: ID+109690, gene locus: 5q32-34) has 9 different allelic variants; in which, four of these variants involve the change of amino acids at residues 16, 27, 34 and 164. The most polymorphic substitution is the substitution of Glycine for Arginine at codon 16 (Arg16Gly) and the substitution of Glutamic acid for Glutamine at codon 27 (Gln27Glu) [6]. The evidence for ADRB2 gene association with hypertension is not conclusive. There are conflicting reports on the association of these polymorphism with hypertension. Several reports have suggested the association of the above variants with the prevalence of hypertension and systolic blood pressure (BP)[7,8,9] whilst others refuted the association[10,11]. The role of this gene in hypertension was revisited by Masuo(2010) in his latest review who suggested likely pathophysiological linked between adrenoceptor polymorphism, hypertension and metabolic syndrome [12].

Although the evidence for ADRB2 gene association with hypertension is not conclusive, studies have suggested that the functional alteration of the sympathetic system contributes to hypertension in spontaneously hypertensive rats [13,14]. Taking into account that ADRB2 is an important target of many drugs and endogenous substances, inter-ethnic differences in this gene may explain differences in drug response and disease susceptibility[15]. Therefore, the present case-control study was undertaken to see the association between the three functional variants of ADRB2 (Arg16Gly, Gln27Glu and Thr164Ile) and hypertension in our hypertensive subjects at the Balok Health Centre, Kuantan. Arg16Gly and Gln27Glu were selected as substitutions of amino acid at these positions are known to cause abnormal regulation of ADRB2 activity [16]. We also selected the Thr164Ile polymorphism for similar association as evidence from in-vivo study have suggested the role of this polymorphism with vascular sensitiveness towards beta2-receptor agonist [17].

MATERIALS AND METHODS

Subjects

The study included 50 unrelated hypertensive patients (16 males and 34 females with mean age: 53.2 ± 8.7 years) and 50 healthy unrelated controls (28 males and 22 females with mean age: 48.0 ± 5.9 years). In both groups, more than 85% of the participants were Malays. Four were Chinese (6%) and one was Indian (2%) for controls and cases respectively. All the hypertensive subjects were recruited from Balok Goverment Health Clinic in Kuantan, Pahang. The diagnosis

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of hypertension were made by experienced family medicine specialist according to the WHO set criteria for hypertension18 and Malaysian Ministry of Health guidelines on management of hypertension[2]. Informed written consent was taken before the start of the study, which was approved by the Ethical committee of the Kulliyah of Medicine, International Islamic University Malaysia. The control subjects were recruited within the university communities who were age and race matched. The sample size was calculated using the OpenEpi for unmatched case control study[19]. Using data from Ranade et.al (2001)[7], twenty percent out of calculated total sampel size of 460 subjects were recruited for this preliminary study.

Genotyping

Peripheral blood were collected from all subjects and the DNA were extracted by using Magtration system 12 G/C (Precision, Japan). Quantity and quality of DNA were measured using biophotometer plus (Eppendorff, USA). The three polymorphic sites of the ADRB2 gene (codon 16, 27 and 164) were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. PCR was performed in 20µL reaction volume that includes; 10µL of HotStarTaq Plus Master Mix (Qiagen), 40pmol from each primer and 150-200ng of DNA. The information regarding the primers and restriction enzymes used in the assay is given in Table I. The products of codon 16, 27 and 164 were then digested at 37ºC over night with Kpn1, Fnu4H1 and MnII respectively, followed by electrophoretic separation on 3% agarose gel.

Validation of genotyping assay by direct sequencing

To determine the exact genotype at codons 16 and 27, PCR products from four randomly chosen subjects with different genotype findings were subjected to automated direct DNA sequencing using ABI PRISM 3130 genetic analyze (Applied Biosystems, USA). The PCR products were first purified using QIAquick PCR purification kit (Qiagen, Germany) followed by cycle sequenced of the product using the forward primer and Big Dye Terminator v3.1 cycle sequencing kit. The cycle sequenced products were purified using ethanol precipitation method. The rs1042713 and rs1042714 reference sequences (www.ncbi.nlm.nih.gov/SNP/) were used for codon 16 and codon 27 respectively. (See Figure1)

Meta-analysis

The Singapore Genome Variation Project found that the genetic variation of Malays and Chinese were relatively similar[20]. Meta-analysis was perfomed by combining the current study with two previous studies done on Chinese 7 and Japanese[21] ethnic groups. The combined of 1467 cases and 936 controls had more than 95% power to detect a genetic risk factor of OR=1.2 (α=0.01) if the minor allele is very frequent (approaches 0.50)[22].

Statistical analysis

The statistical analysis was performed by using SPSS software 17.00. Hardy–Weinberg equilibrium for the genotype distribution of every SNP was tested in controls by χ^2 test with 1 df. The allele frequencies and genotype distribution of the 2 SNPs of ADRB2 were assessed between the hypertensive and normotensive subjects by χ^2 test. The association of the ADRB2 genotype with blood pressure was tested by ANOVA. The haplotype frequencies and diplotype distributions of ADRB2 were estimated by χ^2 test. Data were expressed as Mean ± Standard deviation (SD), and a p value of < 0.05 was considered statistically significant. Meta-analyses were performed using Comprehensive Meta-analysis Version 2 (2005) software[23].

RESULTS

The demographic and clinical data of the subjects in this study are as shown in Table II and III respectively. There was no statistical difference for both age and race between hypertensive and non hypertensive groups. The genotype distributions of Arg16Gly, Glu27Gln and Thr164Ile of ADRB2 gene were in Hardy–Weinberg equilibrium for both cases and controls group. For codon 164, only homozygote for Thr164 was found in the cases and controls, thus this polymorphism were not considered for futher analysis. Allele and genotype frequencies of the two SNPs (Arg16Gly and Glu27Gln) between patients and controls are shown in Table IV. There was no significant association in allele or genotype distribution of the these polymorphisms of ADRB2 gene with hypertension.

When further analysis of possible combination of the two SNPs was made, three out of 4 possible haplotypes structures (Arg16/Gln27, Gly16/Gln27, and Gly16/Glu27) and 8 haplotype combinations (diplotypes) were observed. All possible haplotype combinations showed no significant differences between cases and controls (Table V). Multivariate analysis revealed that there was no association between Arg16Gly and Gln27Glu genotypes and the level of blood pressure (Table VI). The meta-analyses result for codon 16 and 27 were shown in Figure 2 and Figure 3 respectively. There were no siginificant association between both of the codons and hypertension in this combined data.

DISCUSSION

To test the relationships between essential hypertension and ADRB2 polymorphisms, we performed association studies of 2 functional SNPs within the coding region of this gene. The Gly16 allele was slightly more frequent among hypertensive individuals than the Arg16 allele (51% and 49% respectively). As for the Glu27 allele, it was less frequent among hypertensives compared with the Gln27 allele but was not significantly associated with the occurrence of hypertension (7% and 93% respectively). These are quite similar to the data reported by Kato et.al (2001)21 in Japanese hypertensive individuals. There was also no statistical difference between systolic and diastolic blood pressure and each SNPs (Arg16Gly and Gln27Glu) of ADRB2 gene. The haplotype analysis showed no significant difference with regard to the prevalence of the Gly16Glu27, Gly16Gln27 and Arg16Gln27 haplotypes in hypertensive patients compared with normal subjects ($p = 0.455$). This study observed three out of the four possible haplotypes (Arg16/Gln27, Gly16/Gln27, and Gly16/Glu27) and 8 diplotypes. In contrast to Aynacioglu (1999)[24] , we found no occurrence of Arg16 together with Glu27.

Table I: Primers used in genotyping assay of the three variants of ADRB

Marker	Primer Sequence	Enzyme	References
Codon 16	F 5′ AGCCAGTGCGCTTACCTGCCAGAC-3′		
	R 3′-CATGGGTACGCGGCCTGGTGCTGCAGTGC-5′	KpnI	Garovic et al., 2002
Codon 27	F 5′ -GGCCCATGACCAGATCAGCA- 3′		
	R 5′-GAATGAGGCTTCCAGGCGTC-3′	Fnu4HI	Large et al., 1997
Codon 164	F 5′ -GGACTTTTGGCAACTTCTGG-3′		
	R 5′-ACGAAGACCATGATCACCAG-3′	MnlI	Large et el., 1997

Table II: Demographic data

Variables	Hypertensives (n= 50) N (%)	Controls (n= 50) N (%)	$\chi 2$	df	p value
Race					
Malay	43 (86%)	6 (12%)			
Chinese	6 (12%)	4 (8%)	0.754	2	0.686
Indian	1 (2%)	2 (4%)			
Sex					
Male	16 (32%)	28 (56%)	5.844	1	0.016*
Female	34 (68%)	22 (44%)			

Chi-square test, p <0.05 is taken as statistically significant at 95% confidence interval, *significant difference.

Table III: Age and blood pressure in hypertensive and control groups

Variables	Hypertensive (n= 50) mean (SD)	Control (n= 50) mean (SD)	t-stat	p value
Age (years)	53.18 (8.71)	48.04 (5.85)	3.462	0.07
Blood Pressure(mmHg)				
Systolic	138.16 (18.26)	124.38 (8.72)	4.815	0.001*
Diastolic	82.68 (10.84)	80.56 (5.36)	1.239	0.218

Independent t-test, p <0.05 is taken as statistically significant at 95% confidence interval, *significant difference.

Table IV: Allele frequency of the ADRB2 SNPs (Gly16Arg and Gln27Glu) in hypertensives and controls

SNPs	Genotype	MAF	Allele OR (%95 CI)	p value
Gly16Arg	GG/GA/AA			
Case	14/23/13	0.49	1.085 (0.491-2.395)	0.479
Control	17/22/11	0.44		
Gln27Glu	CC/CG/GG			
Case	1/5/44	0.07	0.638 (0.168-2.413)	0.189
control	1/10/39	0.12		

MAF; Minor allelic frequencies, OR: odds ratio, CI: confidence interval. Chi-square test, p <0.05 is taken as statistically significant at 95% confidence interval.

Table V: Haplotype distribution of Arg16Gly and Gln27Glu of ADRB2 gene

Haplotypes	Hypertensives	Controls	p value
Arg16/Gln27	12	10	
Arg16/Glu27	0	0	
Arg16/Gln27Glu	1	1	
Gly16/Gln27	11	12	
Gly16/Glu27	1	0	0.455
Gly16/Gln27Glu	2	5	
Arg16Gly/Gln27	21	17	
Arg16Gly/Glu27	0	1	
Arg16Gly/Gln27Glu	12	4	

Chi-square test, p <0.05 is taken as statistically significant at 95% confidence interval.

Table VI: Blood pressure characteristics by genotyping

	SNP genotypes (n=100)	Blood pressure mean (SD)	p value
Systolic BP	Arg16Gly		
	AA	130.54 (10.60)	
	GA	131.82 (19.50)	0.946
	GG	131.03 (13.48)	
	Gln27Glu		
	GG	131.58 (16.31)	
	CG	128.40 (11.67)	0.572
	CC	140.00 (28.28)	
Diastolic BP	Arg16Gly		
	AA	82.25 (7.67)	
	GA	80.78 (9.81)	0.678
	GG	82.35 (7.36)	
	Gln27Glu		
	GG	81.88 (8.41)	
	CG	78.67 (7.29)	0.067
	CC	93.00 (18.38)	

ANOVA test, p <0.05 is taken as statistically significant at 95% confidence interval.

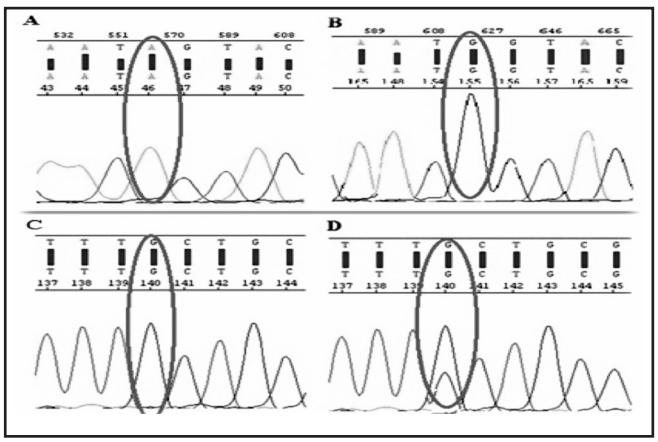

Fig. 1: Direct nucleotide sequencing of selected sample. A: Homozygous Arg16. B: Homozygous TAG to TGG transition resulting change of Arginine to Glycine at codon 16 (Gly16). C: Homozygous Glu27. D: Heterozygous TTG to TTC/G transition at codon 27 (Gln27Glu).

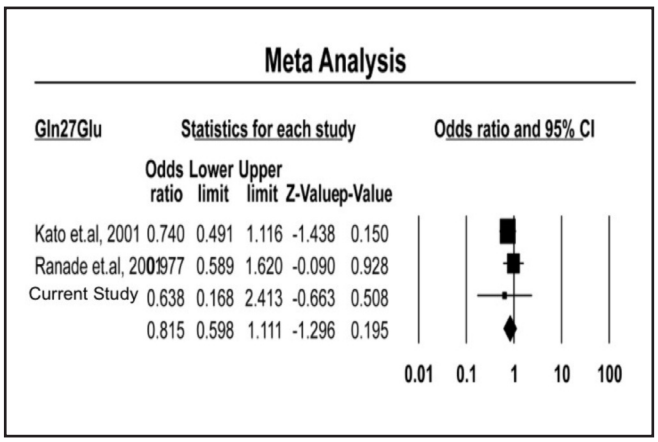

Fig. 3: Meta-analysis of codon 27 variant of ADRB3 gene. The combined OR=0.815 (0.598-1.111, 95% CI), p=0.195.

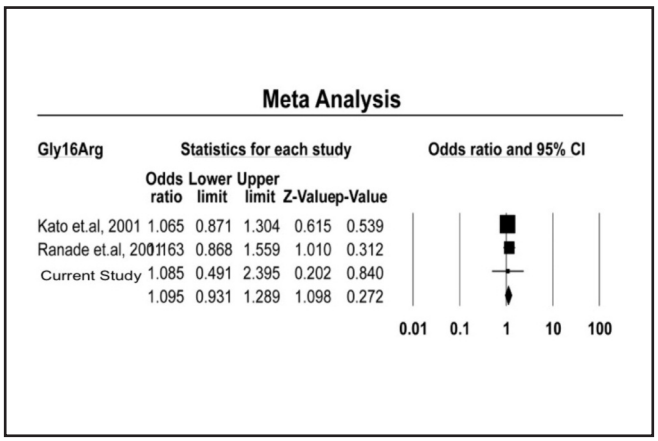

Fig. 2: Meta-analysis of codon 16 variant of ADRB3 gene. The combined OR=1.095 (0.931-1.289, 95% CI), p=0.27.

The ADRB2 gene locus has been linked both to systolic and diastolic BP in large populations studies in United States[25,26], similar association was also found in Asian Chinese and in African Caribbean populations[7,27]. The Gly16Arg and Gln27Glu variants were also significantly associated with the prevalence of hypertension and systolic BP in two relatively recent studies[8,28]. The negative association between the 2 SNPs (Gly16Arg and Gln27Glu) and hypertension in our study was similar to the association studies conducted on black African and black American ethnic groups[10,11].

The relatively low number of subjects could have contributed to the non significant relationship in our study. Nevertheless, our genotypes data revealed that the Hardy–Weinberg equilibrium did not deviated for both codons. Gly16 in particular was very frequent in our population sample. Therefore, our sample size was good enough to detect association of major impact if there was any. Conversely, minimal impact due to gene-gene interaction would only be detected by large population studies.

The confusion on the exact role of these polymorphisms with the occurrence of hypertension and elevation of systolic blood pressure could probably be solved by the recent most

extensive population study done by Hindorff et.al (2005)[29] on over 5000 participants where they found no significant effect of the Arg16Gly and Gln27Glu polymorphisms on BP control and hypertension. However, as the study was done on mainly Black and Caucasian American hypertensive subjects, the association could not be generalized to the other ethnic groups.

Inconsistent results of ADRB2 polymorphisms with hypertension have also been reported in studies of two well known candidate genes of hypertension, the Angiotensin 1-Converting Enzyme and Angiotensinogen genes[30]. These inconsistencies could be related to statistical power of study as frequencies of many genetic variants vary in different population. The statistical power is also affected by heterogenous ethnic groups of a given population. This could mean that, a given susceptible gene may exert detectable blood pressure regulating effects in one ethnic group but not in others group. Therefore, results of a single study might not be able to lead to a conclusive claim. Further replication and meta analysis studies have been suggested to overcome this limitation as have been proven in genetic association studies of other complex diseases[31,32,33]. The meta-analysis was performed based on the assumption that, genetic variation of Malays and Chinese were relatively similar as suggested by Singapore Genome Variation Project [20]. Combining allele frequency result from three studies, we found that there was no significant evidence of association between these two SNPs and hypertension, eventhough all the three studies have shown similar direction of susceptibility.

The molecular variations of ADRB2 gene might cause attenuated vasodilatation, leading to increased total peripheral resistance and hence ultimately resulting in hypertension. In studies among asthmatic patients, Arg16Gly substitution exaggerates agonist-mediated receptor down-regulation, whereas Gln27Glu reduces it[34]. Moreover, resistance to the β2-mediated venodilation was found in subjects homozygous for Gly16 and Glu27 or Gln27 but not in those homozygous for Arg16[17]. We postulate that having these polymorphisms does not necessarily lead to susceptibility of getting hypertension. Nevertheless, knowing the exact allele of the patients may help in the management of their hypertension as SNPs variation in ADRB2 gene is known to modulate the receptor susceptibility toward its target drugs[35]. In our study, almost 51% and 7% of hypertensive patients have Gly16 and Glu27 variant respectively.

In conclusion, we examined 2 common single nucleotide polymorphisms of ADRB2 (Arg16Gly and Gln27Glu) in an association study to reveal the relationship of these polymorphisms and hypertension. In the homozygous form, a Glu27 variant was uncommon in our hypertensive individuals as compared with Gln27 variant. Similarly, Gly16 variant was more common in the hypertensives groups than in control groups. Genetic variability in the human ADRB2 gene could not be of major importance for hypertensive in our population sample eventhough there are possibilities that the analysis of allele and genotyping frequencies may show significant associations in larger study groups. Future studies on ADRB2 may also be conducted to establish whether the polymorphism profile of a given individual can help the clinician to treat hypertension with greater specificity.

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