

Alpha-2 beta-adrenergic receptor (301–303 I/D) gene polymorphism in hypertension and type 2 diabetes mellitus diseases among Saudi cases in the Qassim region

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Abstract

The hypertension (HTN) and type 2 diabetes mellitus (T2DM) are a common multifactorial disease due to genetics and environmental factors. The alpha 2B adrenergic receptor (α 2B-AR) has relationship with secretion of insulin and mediates the vasoconstriction that elevate blood pressure. This study aimed to determine the association between α 2B-AR gene polymorphism with HTN and T2DM in Saudi cases. 200 cases and 100 healthy controls from Saudi population were recruited from the Internal Medicine clinic, Qassim University. The patients were grouped into: 72 HTN without T2DM; 62 HTN with T2DM and 66 T2DM only. Full medical history, examination and biochemical assays were performed for all participants. Genomic DNA was isolated from

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blood lymphocytes of all subjects for detection of $\alpha 2B$ -AR gene polymorphism by using polymerase chain reaction (PCR). The results found a significant association between D carriers genotype and HTN with T2DM cases ($p < 0.05$) as well as with T2DM-only cases, ($p < 0.05$) compared to control. Regardless of HTN status, only cases with HTN and T2DM as well as those with T2DM were significantly associated with the recessive model DD versus II + ID ($p < 0.05$). So, D carriers genotype was significantly associated with total cases of HTN and T2DM ($p < 0.05$) compared to controls. Our results suggested that there is a relationship between the $\alpha 2B$ -AR I/D gene polymorphism and the risk for T2DM with or without HTN, but no such comparable relationship is evident with HTN-only cases among Saudi population in Qassim region.

Keywords

Insertion/deletion polymorphism, $\alpha 2B$ -AR gene, HTN, T2DM

Introduction

The worldwide economic burden of chronic diseases, particularly cardiovascular diseases, diabetes and obesity is significant.¹ Where diabetes mellitus occurs alongside hypertension, the result is vascular remodelling; and due to insulin resistance, which gives rise to hyperglycaemia and hyperinsulinemia, the volume of body fluids increases placing the peripheral arteries under increased pressure.² Between 90% and 95% of diabetes mellitus (DM) cases are type 2 (T2DM), which is described as being insulin resistant or the level of insulin is relatively low.^{3,4} T2DM is a multi-genic disease; according to the evidence obtained from a several studies, particular populations may carry genetic polymorphisms that depending upon the gene-variant, could variously promote or reduce the risk of developing T2DM.⁵⁻⁷ According to various epidemiological studies, environmental and genetic factors are also implicated in the multifactorial condition, HTN.⁸ Several studies were conducted to determine the role of genetic factors in blood pressure and hypertension; however, there is lack consensus about the identity of the genes postulated as being involved.^{9,10} A theme that emerges in many patients of different ethnicities is a robust relationship between HTN and T2DM. Thus DM and HTN are prevalent diseases in a Saudi population among the study area (Qassim region). Continuously studies have evaluated the frequencies of genotypic variations of candidate genes might be play a role for development of HTN and T2DM in Saudi population. These genes include, beta-2-adrenergic receptors, angiotensin-converting enzyme, cytochrome P450, endothelial nitric oxide synthase and methylenetetrahydrofolate reductase.¹¹⁻¹³

The cardiovascular system is regulated by the central nervous system, which releases catecholamines and activates alpha adrenergic receptors (AR).¹⁴ There are three AR subtypes ($\alpha 2A$, $\alpha 2B$ and $\alpha 2C$) that are implicated in the cardiovascular response to catecholamines (adrenaline and noradrenaline). However, these subtypes receptors are encoded by the $\alpha DR A 2A$, $\alpha DR A 2B$ and $\alpha DR A 2C$ genes.¹⁵ The receptors' pharmacological properties, prevalence in tissue, ability to be phosphorylated and desensitised are variable.¹⁶ The clinical importance of the genetic variation of these subtypes remains unclear.¹⁶ However, a positive correlation between $\alpha 2A$ -AR polymorphism and both HTN,¹⁷ and DM¹⁸ have been identified.

Furthermore, an association between congestive heart failure and the α 2A-CR 322–325 I/D polymorphism was identified.¹⁹ Whereas other study demonstrated that the α 2B-AR polymorphism presents as an insertion/deletion mutation in the α 2B-AR gene, mapped on (2q11.2), which results in 9 or 12 glutamic (Glu.) residues. This manifests as the absence or presence of three Glu. amino acids at sites 301–303 (I/D 301-303, rs29000568) in the third intracellular locus of the α 2B-AR.²⁰ The presence of a relationship between different α 2-AR subtype polymorphisms with vascular diseases, HTN and DM is disputed. Whilst the α 2B-AR (301–303 I/D) polymorphism were confirmed to be related to HTN and coronary arterial disease (CAD)^{21,22} and vascular disease ischaemic stroke,¹⁵ some of these findings have been contested in their study conducted in USA. Whereas, other study did not confirm any correlation between the α 2B-AR (301–303 I/D) gene polymorphism and HTN.²³ On the other hand, studies conducted on Egyptian²⁴ and Malaysian cases²⁵ detected significant relationships between this polymorphism and HTN with or without T2DM. Also, a study of Saudi women reported the risk for gestational diabetes was increased in those with the α 2B-AR polymorphism.²⁶ However, the study of young black adults did not discern a relation between this polymorphism and the predisposition to essential HTN.²⁰ This echoed the results of the study concluded that essential HTN was the product of various environmental and genetic factors.²³ The association between α 2B-AR I/D gene polymorphism and diabetes is due to impairment of α 2B-AR desensitisation which causes prolonged inhibition of insulin secretion. As insulin sensitivity decreases, the requirement for insulin secretion increases, so individuals with an impaired capacity to secrete insulin are predisposed to T2DM.²⁷ An alternative explanation for the association between α 2-AR I/D polymorphism and early-onset diabetes is altered function of the autonomic nervous system that may influence glucose metabolism.²⁸ The possible mechanisms for the relation of DD genotype with hypertension was demonstrated in human in vivo studies includes decrease coronary blood flow, and enhance peripheral resistance on epinephrine infusion.²⁹ The aim of this study was to examine the relationship between the α 2B-AR (301–303 I/D) gene polymorphism with HTN and T2DM diseases in Saudi population among Qassim region.

Material and methods

Subjects

In this hospital based case-control study, 200 cases of both gender and 100 healthy controls that were available in the study period from May 2019 until January 2020 were recruited from the Internal Medicine clinic, Qassim University. All selecting participants were Saudi and their small number was a limitation of our study. Cases were allocated to three groups based on clinical evaluations: HTN without T2DM ($n = 72$), HTN and T2DM ($n = 62$) and T2DM only ($n = 66$). The cases were divided into two subgroups total HTN ($n = 134$) and total T2DM ($n = 128$). The control group were apparently healthy normotensive individuals who visited the hospital for routine check-up, have normal blood glucose and no relatives with

either HTN or T2DM. The inclusion criteria were applied; for HTN cases, their systolic blood pressure needed to be ≥ 140 mmHg and diastolic ≥ 90 mmHg, or receiving antihypertensive treatment. For T2DM cases, their FBG had to be ≥ 126 mg/dl or being treated for T2DM. Patients who had renal, hepatic, respiratory diseases and a family history of hypercholesterolemia or congenital heart diseases were excluded. In addition any cases as well as healthy individuals who refused to participate/sign the informed consent of the study were excluded. Each participant underwent a general medical examination; full medical histories and **written** informed consent were obtained. The subcommittee of health research ethics, deanship of scientific research, Qassim University, KSA approved the research to be carried out (Code NO 01-05-2019).

Biochemical analysis

Five ml of peripheral blood samples were collected from each subjects in EDTA tubes. This process was performed in the clinical pathology laboratory. To separate the plasma, 3 ml from collected samples were centrifuged at 4000 rpm/20 min, were stored at -20°C until for biochemical assays. To analyse the total cholesterol, triglycerides and HDL, colorimetric methods were used in accordance with the protocol described by El-Moghazy et al.³⁰ Analysis kits were provided by the Crescent Company, KSA. To estimate LDL, the Friedewald formula was used. Glycated haemoglobin was measured using the Quo-labA1C analyser system.

Genotyping analysis

Genomic DNA was isolated from 2 ml blood lymphocytes of all subjects using an Accuprep genomic DNA extraction kit (K-3032) from the Bioneer Company (Republic of Korea). The concentration of DNA was estimated by UV spectrophotometer. The 20- μl reaction mixture contained 1 μl of template DNA, 2 μl (10 pmol/ μl) of every primer, 10 μl ($2\times$ PCR master mix) and 5 μl of sterile water. The master mix contained 1 unit Taq DNA polymerase, 250 μM of each dNTP (dATP, dGTP, dTTP and dCTP) and 1.5 mM MgCl. PCR running was performed for DNA amplification by using the following protocol: initial pre-denaturation cycle of 5 min at 95°C for one time, 35 denaturation cycles at 95°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 45 s, and followed by one extension cycle of 5 min at 72°C . Finally stop the reaction at 4°C . PCR was run using the forward primer 5'AGGGTGTGGGGGCATCT3' and the reverse primer 5'CAAGCTGAGGCCGAGACACT3'. PCR master mix and the primers were obtained from the Bioneer Company (Republic of Korea V2/2017-03-16).

The $\alpha 2\text{B-AR}$ gene subtypes II, I/D and DD were detected by running 2% agarose gel electrophoresis. The PCR amplified products were revealed by using ultraviolet beams after staining with ethidium bromide. 100-bp DNA ladder (Promega, US) was used for demonstration of the D allele fragments (with size 103 bp) and the I allele fragments (with size 112 bp) (Figure 1).

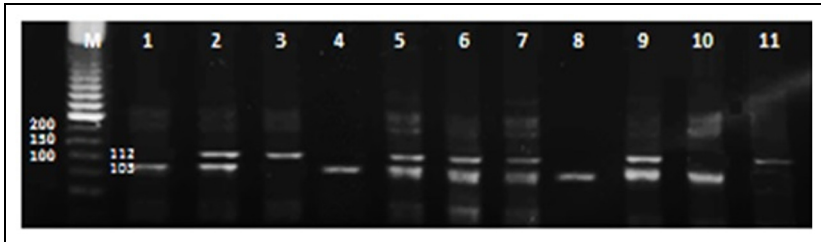


Figure 1. Two percentage agarose gel electrophoresis of PCR products reveal that: lanes 2, 5, 6 and 7, show heterozygous ID genotypes (112/103 bp.); lane 3 show homozygous II genotypes (112 bp) whereas lane 1, 4 show homozygous DD genotype (103 bp). M represents DNA ladder.

Statistical analysis

Data were analysed by using SPSS version 27. For comparing all demographic and clinical data of all participants, we used one way ANOVA test (post hoc Tukey's test) for comparing all pair groups with each other. Hardy-Weinberg equilibrium (HWE) tests were conducted to establish the distribution of genotype in all subjects. Chi-square test (χ^2), odds ratios (ORs) and confidence interval (95%CI) by using logistic regression test for genotyping and allelic frequencies among all subjects.³¹ The threshold for statistical significance was set as $p < 0.05$.

Results

Demographic and clinical data of all participants

The mean age of the 200 patients of both gender in this study was (52.92 ± 9.34) years; ranged 30–68 years). They were matched against 100 healthy controls, ranging in age from 25–52 years (41.38 ± 7.76 years). The Clinical Laboratory, Medical City, Qassim University collected a total of 200 peripheral blood samples from cases that were clinically evaluated when they visited the Internal Medicine clinic during the study period. The patients were divided into three groups based in the clinical diagnosis; group 1 HTN-only 72 cases (42 male and 30 female); group 2 HTN + T2DM 62 cases (38 male and 24 female). The group 3 of 66 T2DM-only patients (34 males and 32 females). Blood samples were also obtained from the 100 unrelated healthy controls (58 males and 42 females).

The results revealed that the significant differences in age, BMI in all three cases groups compared to controls ($p < 0.0001$, $p < 0.05$) respectively. The SBP, DBP, total cholesterol and LDL were significantly higher in HTN + T2DM as well as HTN only cases in comparison with controls and T2DM groups ($p < 0.0001$). In addition, HBA1C and TG, were significantly higher in cases with HTN + T2DM group and T2DM cases compared to controls and HTN only cases ($p < 0.0001$). However, there was no difference in SBP and DBP between T2DM-only cases and

controls, and HBA1C did not differ between HTN-only cases and controls. Also, the cases groups and controls were homogenous in terms of gender and smoking status groups ($p > 0.05$) (Table 1).

Genetic polymorphisms of $\alpha 2B$ -AR I/D among all subjects

To monitor the genotypic distributions of $\alpha 2B$ -AR I/D gene, HWE test showed significant divergence between the observed and expected frequencies in all cases groups, whereas no deviation was detected in the control group. These findings are attributed to small scale of study participants with eligible inclusion as well as exclusion criteria who visited the Internal Medicine Clinic, Qassim University Hospital during the study period from May 2019 until January 2020 (Table 2).

Our results showed the frequency of I/D genotypes and allele of the $\alpha 2B$ -AR gene in the different groups and subgroups. No significant association was found in the heterozygote ID genotype with all cases groups (HTN-only, $p = 0.7$; HTN with T2DM, $p = 0.9$; and T2DM-only, $p = 0.7$) and subgroups (total cases of HTN, $p = 0.8$; and total cases of T2DM, $p = 0.7$) compared to controls. However, There is a significant association was detected between the homozygote DD genotype in HTN with T2DM group ($p = 0.03$) as well as T2DM-only group ($p = 0.01$) in comparison with controls. Whereas such association was not found for the DD genotype in the HTN-only group ($p = 0.09$). In addition there were significant association between the homozygote DD genotype with total HTN and total T2DM cases (subgroups) ($p = 0.03$, $p = 0.01$) respectively in comparison with controls. In relation to the frequency of the D allele, the association was significant for the HTN with T2DM group ($p = 0.04$) and the T2DM-only group ($p = 0.02$). On the other hand, compared to controls, there was no significant association between D allele and the HTN-only group ($p = 0.2$). While there were significant association between D allele with total HTN and total T2DM cases (subgroups) ($p = 0.05$, $p = 0.01$) respectively in comparison with controls (Figure 1) and (Tables 3 and 4).

From the analysis of the dominant and recessive models, no significant association was detected for II versus DD + ID (dominant model) between the patients and control groups HTN-only ($p = 0.6$); HTN with T2DM ($p = 0.3$) and T2DM-only ($p = 0.3$) as well as total HTN ($p = 0.3$) and total T2DM ($p = 0.2$). However, there were a significant association between II + ID versus DD (recessive model) in the HTN with T2DM group ($p = 0.02$) and T2DM-only group ($p = 0.01$) as well as total HTN ($p = 0.03$) and total T2DM ($p = 0.009$) compared to the controls. No such association was found for the HTN-only group ($p = 0.07$) (Tables 3 and 4).

As regard the genotype and allele internal comparison of cases groups with each other, no significant difference was demonstrated in both genotype and allele frequencies between HTN versus HTN with T2DM cases ($p = 0.81$, $p = 0.4$) respectively. Also, no significant difference was detected in both genotype and allele frequencies between T2DM versus HTN with T2DM cases ($p = 0.9$, $p = 0.85$) respectively (Table 5).

Table 1. Demographic and clinical data of all participants.

	HTN without T2DM	HTN with T2DM	T2DM without HTN	Controls	p-Value
	N = 72 (mean ± SD)	N = 62 (mean ± SD)	N = 66 (mean ± SD)	N = 100 (mean ± SD)	
Age	52.32 ± 10.08 ^a	53.03 ± 8.43 ^a	53.45 ± 9.43 ^a	41.38 ± 7.76	<0.0001***
Gender:					
Male (%)	42 (58.3)	38 (61.3)	34 (51.5)	58 (58)	(NS)
Female (%)	30 (41.7)	24 (38.7)	32 (49.5)	42 (42)	
Body mass index (BMI)	29.71 ± 6.29 ^a	30.59 ± 5.94 ^a	29.22 ± 6.76 ^a	24.03 ± 3.27	<0.05*
Smokers (%)	12 (16.7)	15 (24.2)	9 (13.6)	11 (11)	(NS)
Exsmokers (%)	3 (4.2)	2 (3.2)	3 (4.5)	3 (3)	(NS)
Non-Smokers (%)	57 (79.1)	45 (72.6)	54 (81.9)	86 (86)	(NS)
Systolic blood pressure (SBP)	149.4 ± 25.20 ^{a,c}	159.6 ± 21.10 ^{a,b,c}	123.3 ± 10.11 ^b	119.1 ± 11.75	<0.0001***
Diastolic blood pressure (DBP)	92.51 ± 11.70 ^{a,c}	96.29 ± 10.09 ^{a,c}	79.11 ± 6.65 ^b	77.94 ± 6.82	<0.0001***
HBA1C	5.18 ± 0.54 ^c	7.18 ± 1.06 ^{a,b,c}	6.56 ± 0.96 ^{a,b}	5.04 ± 0.40	<0.0001***
Total cholesterol	211.3 ± 55.23 ^{a,c}	217.5 ± 37.34 ^{a,c}	177.5 ± 28.51 ^{a,b}	163.1 ± 34.54	<0.0001***
Triglycerides	188.8 ± 83.89 ^{a,c}	260.4 ± 82.72 ^{a,b}	241.1 ± 75.58 ^{a,b}	111.7 ± 24.72	<0.0001***
High density lipoprotein (HDL)	39.85 ± 7.12 ^a	37.65 ± 7.08 ^a	40.09 ± 2.37 ^a	49.57 ± 4.682	<0.0001***
Low density lipoprotein (LDL)	124.6 ± 28.68 ^{a,c}	127.0 ± 25.55 ^{a,c}	104.9 ± 23.40 ^{a,b}	84.47 ± 12.73	<0.0001***

One way a nova with post hoc Tukey's test and Chi-Square for comparing all pair groups with each other.

HBA1C: glycated haemoglobin; HTN: hypertension; NS: non-significant; SD: standard deviation; T2DM: type 2 diabetes mellitus.

^aSignificant compared to controls.

^bSignificant to HTN.

^cSignificant to DM.

p Value is significant if <0.05.

Table 2. Hardy-Weinberg equilibrium tests for the investigated $\alpha 2B$ -AR I/D polymorphisms among all cases groups and control.

Genotype	Observed	Expected	χ^2	p Value
HTN without T2DM				
II	53	48.3	13.7	$p^{***} = 0.0005$
ID	12	21.3		
DD	7	2.3		
HTN with T2DM				
II	43	37.9	14.2	$p^{***} = 0.0005$
ID	11	21.4		
DD	8	2.9		
T2DM only				
II	46	23.6	21.3	$p^{***} = 0.00001$
ID	10	31.7		
DD	10	10.6		
Controls				
II	77	75.69	1.3	$p = 0.13$
ID	20	22.62		
DD	3	1.69		

D: deletion; HTN: hypertension; I: insertion; p Value: non-significant; p^{***} : very high significant; T2DM: type 2 diabetes mellitus; χ^2 : Chi-Square.

Discussion

The factors responsible for causing the chronic non-infectious diseases, HTN and T2DM, are multiple and of genetic and environmental origins. The prevalence of these diseases in developing and developed nations is high, particularly among older people.³² There is abundant evidence that indicates the presence of a relationship between HTN and T2DM. The WHO estimates that 10% of the world's adult population has diabetes. In Saudi population the percentage of cases affected by DM is reported to be 25%,³³ potentially even as high as 30%.^{32,34} This high prevalence emphasises the importance of determining the influence and prevalence of the polymorphic alpha-2 beta-adrenergic receptor gene in cases with HTN and T2DM among Saudi population in Qassim region. The results of the current study may help to identify the predisposing risk factor for HTN and T2DM diseases and effective management strategies.

The results of this study demonstrated a significant difference among the cases and control groups in relation to age, BMI, SBP, DBP and lipid profiles. Furthermore, the SBP, DBP, total cholesterol and LDL were significantly higher in HTN + T2DM group and HTN cases in comparison with T2DM group. HBA1C and TG, were significantly elevated in HTN + T2DM and T2DM cases compared to HTN cases.

There were two exceptions, being the level of DBP in the T2DM-only group, and the level of HBA1C in the HTN-only groups. In both cases the levels did not differ significantly compared to controls. Similarly, in relation to gender and smoking habits, no significant differences were found between the groups. These results

Table 3. Frequency of $\alpha 2B$ -AR I/D genotypes and alleles among all cases groups compared to control.

Genotype	HTN without T2DM	Control	p [OR (95% CI)]
	N (%) 72	N (%) 100	
II	53 (73.6)	77 (77)	Reference 1.0
ID	12 (16.7)	20 (20)	$p = 0.7$ [0.9 (0.4–1.9)]
DD	7 (9.7)	3 (3)	$p = 0.09$ [3.3 (0.8–13.7)]
Allele			
I	118 (81.9)	174 (88.0)	Reference 1.0
D	26 (18.1)	26 (12.0)	$p = 0.2$ [1.4 (0.8–2.7)]
Statistics			
II vs DD + ID (dominant model)	53 vs 19	77 vs 23	$p = 0.6$ [0.8 (0.4–1.6)]
II + ID vs DD (recessive model)	65 vs 7	97 vs 3	$p = 0.07$ [0.3 (0.1–1.1)]
Genotype	HTN with T2DM	Control	p [OR (95% CI)]
	N (%) 62	N (%) 100	
II	43 (69.4)	77 (77)	Reference 1.0
ID	11 (17.7)	20 (20)	$p = 0.9$ [1.0 (0.4–2.0)]
DD	8 (12.9)	3 (3)	$p^* = 0.03$ [4.8 (1.2–18.9)]
Allele			
I	97 (78.0)	174 (88.0)	Reference 1.0
D	27 (22.0)	26 (12.0)	$p^* = 0.04$ [1.8 (1.0–3.4)]
Statistics			
II vs DD + ID (dominant model)	43 vs 19	77 vs 23	$p = 0.3$ [0.7 (0.3–1.3)]
II + ID vs DD (recessive model)	54 vs 8	97 vs 3	$p^* = 0.02$ [0.2 (0.1–0.8)]
Genotype	T2DM without HTN	Control	p [OR (95% CI)]
	N (%) 66	N (%) 100	
II	46 (69.8)	77 (77)	Reference 1.0
ID	10 (15.1)	20 (20)	$p = 0.7$ [0.8 (0.4–1.9)]
DD	10 (15.1)	3 (3)	$p^{**} = 0.01$ [5.5 (1.5–21.3)]
Allele			
I	102 (77.3)	174 (88.0)	Reference 1.0
D	30 (22.7)	26 (12.0)	$p^* = 0.02$ [2.0 (1.1–3.5)]
Statistics			
II vs DD + ID (dominant model)	46 vs 20	77 vs 23	$p = 0.3$ [0.6 (0.3–1.4)]
II + ID vs DD (recessive model)	56 vs 10	97 vs 3	$p^{**} = 0.01$ [0.2 (0.1–0.7)]

95% CI: confidence interval; D: deletion; HTN: hypertension; I: insertion; OR: odds ratio; T2DM: type 2 diabetes mellitus; p Value: non-significant.

p^* significant <0.05. p^{**} high significant.

Table 4. Frequency of $\alpha 2B$ -AR I/D genotypes and alleles among cases subgroups compared to control.

Genotype	Total HTN N (%) 134	Control N (%) 100	p [OR (95% CI)]
II	96 (71.7)	77 (77)	Reference 1.0
ID	23 (17.1)	20 (20)	p = 0.8 [0.9 (0.4–1.8)]
DD	15 (11.2)	3 (3)	p* = 0.03 [4.0 (1.1–14.3)]
Allele			
I	215 (80)	174 (88.0)	Reference 1.0
D	53 (20)	26 (12.0)	p* = 0.05 [1.6 (1.0–2.7)]
Statistics			
II vs ID + DD (dominant model)	96 vs 38	77 vs 23	p = 0.3 [0.7 (0.4–1.3)]
II + ID vs DD (recessive model)	119 vs 15	97 vs 3	p* = 0.03 [0.2 (0.1–0.8)]
Genotype	Total T2DM N (%) 128	Control N (%) 100	p [OR (95% CI)]
II	89 (69.5)	77 (77)	Reference 1.0
ID	21 (16.4)	20 (20)	p = 0.7 [0.9 (0.4–1.8)]
DD	18 (14.1)	3 (3)	p** = 0.01 [5.1 (1.4–18.2)]
Allele			
I	199 (77.7)	174 (88.0)	Reference 1.0
D	57 (22.3)	26 (12.0)	p** = 0.01 [1.9 (1.1–3.1)]
Statistics			
II vs ID + DD (dominant model)	89 vs 39	77 vs 23	p = 0.2 [0.6 (0.3–1.2)]
II + ID vs DD (recessive model)	110 vs 18	97 vs 3	p** = 0.009 [0.1 (0.05–0.6)]

95%CI: confidence interval; D: deletion; I: insertion; HTN: hypertension; OR: odds ratio; T2DM: type 2 diabetes mellitus; p Value: non-significant.

p* significant < 0.05.

p** high significant.

come in accordance with previous studies reported that, HTN was more common in older age regardless of geographical, cultural, social and economic factors, and gender.^{35,36} Moreover, other studies reported that, the prevalence of HTN was found to be higher in poorly educated workers and unskilled retirees.³⁷

Primary HTN is a complex disease due to environmental and genetic factors.³⁸ The rich expression of $\alpha 2$ adrenergic receptor gene in the central and peripheral nervous systems is demonstrated. The different categories of $\alpha 2$ adrenergic receptor work antagonistically to modulate blood pressure. Hypertension is induced by the stimulation of $\alpha 2B$ -AR by the sympathetic nervous system, which inhibits $\alpha 2A$ -AR; whereas hypotension arises from the stimulation of $\alpha 2$ -AR inhibiting the sympathetic outflow from the central nervous system.²⁴

Table 5. Genotype and allele internal comparison among the three cases groups (HTN vs HTN with T2DM) and (T2DM vs HTN with T2DM).

Genotype	HTN without T2DM	T2DM without HTN	HTN with T2DM
	N (%) 72	N (%) 66	N (%) 62
II	53 (73.6)	46 (69.8)	43 (69.4)
ID	12 (16.7)	10 (15.1)	11 (17.7)
DD	7 (9.7)	10 (15.1)	8 (12.9)
χ^2 ; <i>p</i> Value for genotype	$\chi^2=0.4$, <i>p</i> =0.81	$\chi^2=0.2$, <i>p</i> =0.9	Reference group
Allele			
I	118 (81.9)	102 (77.3)	97 (78.0)
D	26 (18.1)	30 (22.7)	27 (22.0)
χ^2 ; <i>p</i> Value for allele	$\chi^2=0.5$, <i>p</i> =0.4	$\chi^2=0.03$, <i>p</i> =0.85	Reference group

D: deletion; I: insertion; HTN: hypertension; T2DM: type 2 diabetes mellitus; χ^2 : Pearson-chi-square test. *p* > 0.05: non-significant.

The current study showed no significant association was found in the heterozygote ID genotype with all cases groups (HTN-only; HTN with T2DM and T2DM-only) and subgroups (total HTN and total T2DM cases) compared to controls. However, There is a significant association was detected between the homozygote DD genotype in HTN with T2DM group as well as T2DM only group in comparison with controls. Whereas such association was not detected for the DD genotype in HTN only group. In addition there were significant association between the homozygote DD genotype with total HTN and total T2DM cases (subgroups) in comparison with controls.

In relation to the frequency of the D allele, significant association was detected for the HTN with T2DM and the T2DM-only groups compared to controls. On the other hand, compared to controls, there was no significant association found between D allele and the HTN only group. While there were significant association between D allele with total HTN and total T2DM cases (subgroups) in comparison with controls.

A correlation has been demonstrated between the SNP in the 3' UTR of ADRA2A and the increased expression of α 2A-AR in mRNA and proteins.¹⁸ This same correlation also appears to be responsible for aberrant insulin synthesis when stimulated by glucose, a decline in fasting insulin and increasing the risk of T2DM. In vitro study of the risk allele found that it disrupted the attachment of insulin granules to the beta cell membrane and exocytosis of insulin at a site distal to modulation of calcium ion levels within cells. Other adverse metabolic and vascular effects associated with the deletion allele are the elevated risk of coronary ischaemia, impaired insulin synthesis prior to the onset of diabetes, a decline in the basal metabolic rate and obesity.²⁷

In its statistical analysis of dominant and recessive models, this study did not detect any significant relationship between II versus DD + ID (dominant model)

in the cases groups in comparison with controls. However, it did find a significant association between II + ID versus DD (recessive model) in the HTN with T2DM and T2DM-only groups as well as total HTN and total T2DM compared to controls. This recessive model-association was not evident in the HTN-only. As regard the genotype and allele comparison of cases groups with each other, no significant difference was detected in both genotype and allele frequencies among cases groups compared to each other. These results echo those of Vasudevan et al.,²⁵ who found the D allele variant of the α 2B-AR gene in Malaysian cases with essential HTN with and without T2DM. Moreover, other study demonstrated a strong association between the DD genotype, the D allele with HTN disease and that this association is more evident in hypertensive patients complicated by diabetes in Egyptian population.²⁴ Also, Siitonen et al.,³⁹ reported that the function of the α 2B-AR receptor appears to be adversely affected by the I/D 12Glu9 polymorphism of α 2B-AR that leads to impairment of first stage insulin secretion and increase the risk of Type 2 diabetes in Finnish patient.

Moreover, the polymorphism affects the agonist-mediated phosphorylation and desensitisation of the receptor. The association between α 2B-AR I/D gene polymorphism and diabetes results from impairment of α 2B-AR desensitisation due to the allelic variant that inhibits insulin secretion. As insulin sensitivity decreases, the requirement for insulin increases, so individuals with an impaired capacity to secrete insulin are predisposed to T2DM.²⁷ An alternative explanation for the association between α 2-AR I/D polymorphism and early-onset diabetes is altered function of the autonomic nervous system that may influence glucose metabolism.²⁸

This study did not detect any significant relationship between HTN disease not complicated with DM and the α 2B-AR (301–303 I/D) gene of cases in the Qassim region; this might be a product of the diverse genetics and environmental factors involved in essential hypertension. Our results were similar to the study conducted in Sweden population demonstrated a weak association between the DD genotype and nondiabetic primary hypertension and a stronger association with early-onset hypertension and the diabetic phenotype seems to add complexity to the phenotype of primary hypertension.³⁸ The possible mechanism for the relation of DD genotype with hypertension was demonstrated in human in vivo studies includes decrease coronary blood flow, and enhance peripheral resistance on epinephrine infusion.²⁹

Meta-analyses of genome-wide association studies have improved our understanding of the genetic foundations of a number of diseases, including diabetes. These SNPs have often been validated through re-sequencing efforts as not just tag SNPs, but as causative SNPs, and so must play a role in disease development or progression.⁴⁰ More than 120 published reports have described associations between SNPs and T2DM. This association involved markers in or around a candidate gene or linkage region that have a potential role in diabetes-related metabolic pathways.⁴¹

Moreover other study revealed a significant association between rs7903146, rs2283228, rs13266634, rs179881 and rs5210 SNPs and gestational diabetes mellitus

(GDM) referred to meta-analysis conducted on T2DM among Indian population.³¹ On the other hands, similar meta-analysis was performed in Japanese population confirmed the linkage between the risk for hypertension and several common SNPs of ATP2B1, CYP17A1, CSK and FGF5 genes.⁴²

Strengths and constraints of the current study

Although the current study is relatively small scale among Qassim region which is a tribalism area, the results confirmed that the DD polymorphism of the α 2B-AR genotype have a significant association with risk for T2DM for the first time in Saudi population in Qassim region. However there are limitation in our study included small number of participants with eligible inclusion as well as exclusion criteria visited the Internal Medicine Clinic, Qassim University Hospital during the study period from May 2019 until January 2020. However, further study with large scale should be covered the most different regions to determine the relationship between the I/D α 2B-AR gene polymorphism with the risk for HTN or T2DM diseases in Saudi population.

Conclusion

This study showed, there was a significant association between the α 2B-AR I/D gene polymorphism and D allele with the risk for T2DM with or without HTN disease among Saudi population in Qassim region. However, no significant association was detected between the same polymorphism and allele with the risk for HTN not complicated by T2DM disease. In future, large scale study involving different regions among Saudi population are recommended to confirm the role of α 2B-AR (301–303 I/D) gene polymorphism or D allele for development of T2DM and HTN diseases.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

Ethical approval for this study entitled 'Alpha-2 beta-adrenergic receptor (301–303 I/D) gene polymorphism in hypertension and type 2 diabetes mellitus diseases among Saudi cases in the Qassim region of Saudi Arabia' was obtained from *The Subcommittee of Health

Research Ethics, Deanship of Scientific Research, Qassim University, KSA (APPROVAL NUMBER/ID (01-05- .*(2019).

Informed consent

Informed consent Written informed consent was obtained from all subjects before the study.

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