The role of genetic polymorphism of β3-adrenergic receptor in the susceptibility to diabetes and its related disorders: a case–control study on Egyptian population

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Background
The β3-adrenergic receptor (β3-AR) is mainly expressed in adipose tissue and plays an important role in lipid metabolism and metabolic rate by mediating lipolysis and thermogenesis. It has been suggested that the Trp64Arg (T→C) polymorphism in the β_3-AR gene affects fat accumulation and/or impairment of lipid and carbohydrate metabolism.
Objective
The aim of this study was to investigate whether common polymorphism (Trp64Arg) of β_3-AR gene has a role in the apparent susceptibility to type 2 diabetes mellitus (DM) and its related disorders in the Egyptian population.
Patients and methods
One hundred and thirty five healthy controls and 123 individuals with type 2 DM were enrolled in the study. The β_3-AR Trp64Arg polymorphism was identified using restriction fragment length polymorphism PCR of peripheral blood DNA samples. Analysis of data was performed using SPSS program 11.
Results
Allele frequency for C was 23.2% in the diabetic group compared with 12.2% in the control group. The carriers of XC genotype (TC and CC) were at high risk of developing type 2 DM (odds ratio=2.8; 95% confidence interval=1.6–4.9) when compared with the carrier of TT genotype. Furthermore, they were at much higher risk of developing its related disorders such as central obesity, dyslipidemia, and hypertension (odds ratio=2.8; 1.8, 1.5, 2.2, and 2.7 for BMI, waist–hip ratio, triglycerides, high-density lipoprotein, and hypertension, respectively).
Conclusion
The prevalence of Arg64 allele of the Trp64Arg polymorphism in the β_3-AR gene is a risk factor for type 2 DM and its related disorders in the Egyptian population.

Keywords:
Keywords, β3-adrenergic receptor polymorphism, dyslipidemia, type 2 diabetes mellitus

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Introduction

In a region such as Egypt, economic development has led to lifestyle alterations characterized by increases in energy and fat intake and reduction in physical activity. These changes have been associated with a dramatic increase in the prevalence of diabetes and related disorders. Obesity, in particular visceral obesity, appears to be an important link between these disorders [1–4].

The β_3-adrenergic receptor (β_3-AR) gene maps to the short arm of chromosome 8; it codes for β_3-ARs, which is characterized by the presence of seven hydrophobic regions, corresponding presumably to seven transmembrane domains [5,6]. It has an extracellular glycated N-terminal domain and intracellular C-terminal domain. The extracellular domain of β_3-AR binds the endogenous catecholamines, transfers the signals to the interior of the cells through the stimulatory guanine nucleotide-binding protein (Gs), and regulates basal metabolic rate [5,7]. Evidence that β_3-AR is expressed in visceral fat makes it a prime candidate for regulation of lipolysis in white adipose tissue and insulin sensitivity in humans [5,8,9]. This receptor, by stimulating the uncoupling protein-1, alters respiration coupling and dissipates oxidation-derived energy as heat in brown adipose tissue [5,9,10]. In rodent, an important mechanism for maintaining constant body weight involves catecholamine-stimulated thermogenesis in brown adipose tissue and skeletal muscle [5,11]. Furthermore, the

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treatment of mice with a selective β_3-AR agonist led to increased lipolysis and increased insulin levels [5,12].

In humans, visceral obesity is associated with the enhanced sensitivity of visceral fat to catecholamine-induced lipolysis and delivery of free fatty acid into the portal circulation [5,13], with subsequent synthesis and release of very low-density lipoprotein from the liver [5,14].

A common polymorphism in the first intracellular loop has been found in the human β_3-AR gene that involves the change in tryptophan (Trp) (TGG) at position 64 to Arg (CGG). Several studies reported an association between the C allele and a marked decrease in β3-AR function [15,16]. Hence, polymorphism has been associated with obesity through its effect on the energy expenditure of fat tissue [17–22]. Genetic polymorphism of the β_3-AR gene has been proposed as a potential modifying factor in the etiology of type 2 diabetes mellitus (DM) [5,18,23,24], its early onset [5,18,20,23,24], and the development of features of the insulin resistance syndrome [5,18,23,25].

Aim

The aim of this study was to investigate whether common polymorphism (Trp64Arg) of β_3-AR gene has a role in the apparent susceptibility to type 2 DM and its related disorders in the Egyptian population.

Patients and methods

Patients

The control group (group I) consisted of 135 healthy individuals (65 male and 70 female) with a mean age of 48.3±8.2 years. The case group (group II) consisted of 123 individuals with type 2 diabetes (61 male and 62 female) with a mean age of 47.5±7.4 years. The study was conducted in the Internal Medicine Department in Ain Shams University Hospitals. All participants signed an informed consent form to participate in the study. Approval was obtained from Ain Shams University, Faculty of Medicine, Research Ethics Committee (FWA00017858).

People with type 2 DM were subjected to full history taking, thorough physical examination, and laboratory tests. Weight, height, and waist and hip circumferences were measured. BMI and waist–hip ratio (WHR) were calculated. Hypertension was defined as blood pressure more than or equal to 140/90 mmHg or current use of antihypertensive medication.

Anthropometric measurements

Individuals with BMI at least 25 kg/m^2 were considered positive for overweight/obesity as defined by WHO; those with waist circumference (WC) more than 80 cm for women and more than 94 cm for men or with WHR more than 0.85 for women and more than 1 for men were considered positive for abdominal obesity [26].

Laboratory tests

Lipid profile [total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, and triglycerides (TG)] was evaluated using routine enzymatic tests (Diasys Kits; DiaSys Diagnostic Systems GmbH, Holzheim, Germany) [27,28].

DNA extraction

DNA was extracted from white blood cells using a salting-out method [29]. Red blood cell lysis was performed using red cell lysis buffer (20 mmol/l Tris-HCl, pH 7.6) followed by centrifugation. Nuclei lysis was carried out using cell lysis buffer [10 mmol/l Tris-HCl, pH 8.0; 1 mmol/l EDTA, pH 8.0; 0.1% (w/v) SDS] and proteinase K (20 mg/ml) followed by centrifugation. Protein was precipitated using protein precipitation solution (60 ml of 5 mol/l potassium acetate, 11.5 ml of glacial acetic acid, and 28.5 ml of water) followed by centrifugation. Finally, DNA was precipitated using isopropanol and then ethanol 70% followed by centrifugation after each. Pellet of DNA was dried in air and rehydrated in TE buffer (pH 7.6) and stored in −20°C. The DNA purity and concentration were determined by means of spectrophotometer measurement of absorbance at 260 and 280 nm.

Polymorphism detection

Gene was amplified using PCR on 96-well Amp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, California, USA). Primer sequences, PCR conditions, and restriction enzyme digestion were as given below. Oligonucleotides were synthesized using the following primers (Promega Corporation, Madison, WI, USA): the forward primer was 5′-CGCCCAATACCGCCAACAC-3′ and reverse primer was 5′-CCACCAGGAGTCCCATCACC-3′. PCR was carried out in a 50-µl reaction volume containing 100 ng of genomic DNA, 0.4 mmol/l of each primer, 0.2 mmol/l dNTPs, 2 mmol/l $MgCl_2$ in 10% PCR buffer, and 1 U of DNA polymerase (Promega). PCR involved an initial 5-min denaturation at 94°C, 35 cycles of denaturing at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for

10 min. Aliquots of 5 µl of the PCR products were digested with 5 U of BstOI (Promega) at 60°C for 2 h. Restriction fragment length polymorphism products were analyzed on 3% agarose gel and stained with ethidium bromide [29]. The TT genotype gave five bands (99, 62, 30, 12, and7 bp), TC gave six bands (161, 99, 62, 30, 12, and 7 bp), and CC gave four bands (161, 30, 12, and 7 bp). The 30, 12, and 7 bp were very small to be resolved on the gel (Fig. 1).

Statistical analysis

Genotype and allele frequency were calculated by allele counting as described by Emery [30]. Genotype distribution was investigated in relation to Hardy–Weinberg equilibrium. Three allele groups were considered for β_3-AR polymorphism (TT, TC, and CC). The TC and CC were combined together in one group, XC genotype. Statistical analysis was performed with SPSS software, version 11 (SPSS Inc., Chicago, Illinois, USA). Difference in genotype prevalence and association between the case and the control group was assessed using the χ^2-test, correlation coefficient, odds ratio (OR), and 95% confidence interval (CI) to describe the strength of association. Mean serum levels for lipids and lipid fractions were compared between different allele groups using Student's *t*-test and the Mann–Whitney *U*-test for nonparametric data. *P* value less than 0.05 was considered significant.

Results

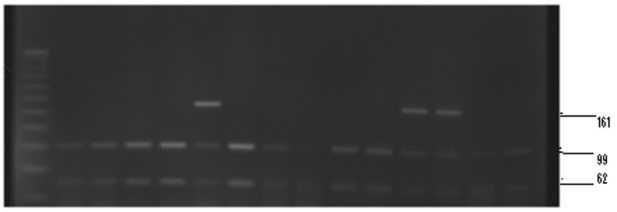
The frequency of the T and C alleles in our pooled sample was analyzed; it was 82.6 and 17.4%, respectively. The frequency of genotypes TT, TC, and CC was 69.8, 25.6, and 4.7%, respectively (Table 1). The genotypes were found to be within Hardy–Weinberg equilibrium.

The frequencies of different genotypes among the studied groups are shown in Table 2. Individuals with XC genotype were at much higher risk of developing DM. OR was 2.8 (OR=2.8, 95% CI=1.6–4.9) when compared with the TT genotype.

Diabetes-related traits, including obesity and dyslipidemia, were measured (Table 3). Participants with XC genotype had higher mean BMI (27.97±3.9 vs. 25.7±4.8, $P<0.001$) and high mean WC (99.5±10.5 vs. 94.9±13.8, $P<0.001$) when compared with carriers of TT genotype. Similarly, there was a tendency of XC genotype to be associated with a significantly high mean serum level of TG ($P<0.01$) and a low mean level of HDL ($P<0.01$) when compared with TT individuals.

All metabolic disorders associated with DM were analyzed and assessed using χ^2-test and OR (Tables 4 and 5). It was clear that individuals with XC were at high risk of developing obesity as measured using BMI, WC, and WHR. Moreover, they were at risk of developing dyslipidemia. The risk for hypertension was estimated to be 2.7. The TT genotype was used as a reference in the previous comparisons.

Figure 1



Beta 3 Adrenergic receptor polymorphim. Restriction fragment length polymorphism on 3% agarose gel. Lane 1, DNA marker. Lane 2, 3, 4, 5, 7, 8, 9, 10, 11, 14 and 15 (TT) genotype, Lane 6, 12 and 13 (TC) genotype.

Table 1 Frequency of different β3-adrenergic receptor genotypes and alleles among the studied groups

Genotypes	Control (*n*=135) (%)	Cases (*n*=123) (%)	Pooled sample (*N*=258) (%)
TT	80.0	58.5	69.8
TC	15.6	36.6	25.6
CC	4.4	4.9	4.7
Allele			
T	87.8	76.8	82.6
C	12.2	23.2	17.4

Table 2 Frequency and odds ratios of β3-adrenergic receptor genotype among the studied groups

Genotypes	Control (*n*=135) [*n* (%)]	Cases (*n*=123) [*n* (%)]	OR	95% CI	χ^2
TT	108 (80.0)	72 (58.5)	2.8	1.6–4.9	14.1*
TC and CC	27 (20.0)	51 (41.5)			

CI, confidence interval; OR, odds ratio. *$P<0.05$.

Table 3 Different parameters of lipid profile and anthropometric measurements among carriers of different genotypes of β3-adrenergic receptor polymorphism

Lipid parameters	TT	TC and CC
BMI (mean±SD)	25.7±4.8	27.97±3.99**
WC (mean±SD)	94.9±13.8	99.5±10.5**
TG (mean) (mg/dl)	118.8	150.1*
TC (mean±SD) (mg/dl)	189.2±43.9	188.4±50.3
HDL-C (mean±SD) (mg/dl)	40.1±9.4	36.1±10.9*
LDL-C (mean±SD) (mg/dl)	122.5±41.9	118.2±51.4

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides; WC, waist circumference. *$P<0.01$. **$P<0.001$.

Table 4 Frequency and odds ratio of anthropometric measurements among carriers of different genotypes of β3-adrenergic receptor polymorphism

Anthropometric parameters	TT (*n*=180) [*n* (%)]	TC and CC (*n*=78) [*n* (%)]	OR	95% CI	χ^2
BMI<25 kg/m^2	99 (80.5)	24 (19.5)	2.8	1.6–4.8	12.8**
BMI≥25 kg/m^2	81 (60.0)	54 (40.0)			
WC (normal)	69 (74.2)	24 (25.8)	1.4	0.8–2.5	1.4
WC (abnormal)	111 (67.3)	54 (32.7)			
WHR (normal)	81 (77.1)	24 (22.9)	1.8	1.1–3.3	4.6*
WHR (abnormal)	99 (64.7)	54 (35.3)			

CI, confidence interval; OR, odds ratio; WC, waist circumference; WHR, waist–hip ratio. *P<0.05. **P<0.01.

Table 5 Frequency and odds ratio of different parameters of lipid profile among carriers of different genotypes of β3-adrenergic receptor polymorphism

Parameters	TT (*n*=180) [*n* (%)]	TC and CC (*n*=75) [*n* (%)]	OR	95% CI	χ^2
TG<150 mg/dl	117 (73.6)	42 (26.4)	1.5	0.8–2.5	1.8
TG≥150 mg/dl	63 (65.6)	33 (34.4)			
TC<200 mg/dl	111 (71.2)	45 (28.8)	1.1	0.6–1.9	0.6
TC≥200 mg/dl	69 (69.7)	27 (21.4)			
HDL-C>40 mg/dl	81 (62.8)	48 (37.2)	2.2	1.2–3.8	7.6*
HDL-C≤40 mg/dl	52 (38.2)	84 (61.8)			
LDL-C<160 mg/dl	153 (70.8)	63 (29.2)	1.1	0.5–2.3	0.04
LDL-C≥160 mg/dl	27 (69.2)	12 (30.8)			

CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OR, odds ratio; TC, total cholesterol; TG, triglycerides. *P<0.05.

Discussion

Sympathetic stimulation of the adipocytes acts in part upon the β_3-AR, both in mediating the lipolysis activation at subcutaneous and visceral sites [18] and in the utilizations of intracellular fatty acids in the uncoupled respiration of the thermogenic mitochondria that depends on the uncoupling proteins [31].

To evaluate the biochemical consequences of the Trp64Arg substitution, this change was introduced in the β_3-AR gene using site-directed mutagenesis, and it was studied in transfected cells. No change was observed in the binding or adenylyl cyclase activation properties of the Arg64 of β_3-AR expressed in CHO or HEK293 cells [32,33]. However, the absolute amounts of accumulated cyclic AMP were lower in the cells transfected with the polymorphic β_3-AR than in the wild type receptors [32]. This may result in lower activation of hormone-sensitive lipase and hence reduce lipolysis [34]. It was found that the Arg64 form deteriorates lipolysis induced by β_3-AR agonist in human omental adipocytes [35]. It has been suggested that impaired lipolysis could promote insulin resistance and hyperinsulinism, presumably through enlargement of visceral adipose cells [13].

The overall Arg64 allelic frequency in our pooled sample was 17.4%. Worldwide screening studies have revealed that Arg64 form is actually present in all populations of the world, except for the people of the Pacific island of Nauru [34]. In Pima Indians, the allelic frequency of the variant exceeds 30% [23], and it reaches 19–20% in Japanese [19,24,25,36–40]. In majority of other populations, the allele frequency is 4.9–13% [18,20,23,41–43].

In the present study, carriers of Arg64 allele were at a significantly three-fold increased risk of developing type 2 DM compared with carrier of Trp64 allele (OR=2.8, 95% CI=1.6–4.9). The relative contribution of the β_3-AR polymorphism to the etiology of type 2 DM is still a matter of debate. Knowler [44] reported that C allele was a risk factor for DM (OR=1.71, 95% CI=1.01–2.89). In meta-analysis, the association was significant [24,45], leading to the view of a modest, but nevertheless important contribution of the Trp64Arg β_3-AR genotype to the polygenic disorders of insulin resistance and type 2 DM [24]. However, in some other studies the association of the β_3-AR genotype with the prevalence of type 2 DM failed to reach statistical significance [18,46,47]. Moreover, a consistent association with younger age at onset of diabetes (mean difference in age at onset −1.9 years, 95% CI=−2.8 to −1.0) has been reported in several studies [18,23–25,45,47–54]. It is likely that Arg64-associated phenotypic alteration might play a role as a promotive factor in an individual genetically predisposed to type 2 DM, resulting in acceleration of the development of DM [45]. In our study Arg64 polymorphism was associated with early onset of DM,

although not significant. However, individuals with type 2 DM are often asymptomatic initially and the age of diagnosis may not accurately reflect the age of onset of the diabetes.

The role of polymorphic β_3-AR in the pathogenesis of insulin resistance in muscle can be explained by the following: increased deposits of abdominal fat could provide more free fatty acids for the synthesis of very-low-density lipoprotein in the liver, which could result in changes in the fatty acid composition of skeletal muscle membranes [14] or higher concentration of TG in muscle. In rats, the accumulation of TG in muscle is related to the impaired action of insulin [55]. Visceral obesity is also associated with decreased uptake of free fatty acids by muscle and with insulin resistance in skeletal muscle, in particular impaired synthesis of insulin-stimulated glycogen [56]. Insulin resistance is a major predictor of type 2 DM [57].

In the present study, all phenotypic characteristics of insulin resistance were analyzed. Analysis revealed that carriers of the XC genotype were at a relatively higher risk of developing both central and abdominal obesity manifested by the significant increase in their mean BMI and WC when compared with carriers of TT genotype (OR=2.8, 95% CI=1.6–4.8, mean BMI=27.97±3.9 vs. 25.7±4.8, $P<0.001$, and OR=1.4, 95% CI=0.8–2.5, mean WC=99.5±10.5 vs. 94.9±13.8, $P<0.001$) for BMI and WC, respectively. Moreover, the relation between the BMI and the different genotypes was dosage related; the mean level of BMI among carriers of different genotypes was 25.7±4.8, 27.8±3.6, and 28.8±5.9 for TT, TC, and CC genotypes, respectively. On analysis of variance the results were significant ($P<0.001$). Similar results were obtained by Thomas and colleagues, in which the mean BMI was 28.8, 27.9, and 26.6 kg/m^2 in CC, CT, and TT, respectively, with a significant increase ($P=0.001$) using analysis of variance [57]. These markers consistently and significantly increased with increasing proportions of the Arg allele. Clement reported that carriers of Arg 64 allele were not only at risk of weight gain but also they resist weight loss [20,37].

In a meta-analysis study by Fujisawa and colleagues, using data from more than 9000 individuals, a significant association of the Trp64Arg polymorphism with BMI was found (mean difference in BMI=0.3, 95% CI=0.13–0.47 kg/m^2) [45]. In addition, the association among the diverse population groups exhibited a relatively similar strength despite the different genetic (including distribution of β_3-AR genotypes) and environmental backgrounds. Therefore, the β_3-AR locus has been shown for the first time to be a genetic factor associated with body weight in a universal manner [45]. Moreover, Tchernof *et al.* [58] confirmed the previous finding. However, in a study on the association of Arg64 with BMI and body weight, it was found to be significant in men not in women. Body weight was 11.8 kg more in homozygous CC ($P<0.001$) and BMI was 3.7 kg/m^2 greater in CC ($P=0.001$) than in homozygous TT [16]. Some studies failed to replicate the association of Arg64 with obesity [42,59–61].

In the present study, the Arg allele was associated with atherogenic lipid profile manifested by significantly high mean level of TG and a low mean level of HDL-C ($P<0.01$). Carrier of XC genotype was at a significantly higher risk of having low HDL-C (OR=2.2, 95% CI=1.2–3.8) when compared with carriers of TT genotype. In another study, Arg64 allele was associated with a significant decrease in HDL and a significant increase in TG, whereas the increase in TC and low-density lipoprotein was nonsignificant [62,63]. However, some studies failed to replicate our results [16,64].

In general, as understanding the genetic and environmental parameters that contribute to diabetes may lead to earlier identification of individuals at risk and to more effective interventions to prevent the development or progression of the disease and its complications. It is recommended to focus more studies on the genetic susceptibility of the different ethnic groups to diabetes and its complications.

Conclusion

The prevalence of Arg 64 allele of the Trp64Arg polymorphism in the β_3-AR gene is a risk factor for type 2 DM and its related disorders in the Egyptian population.

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Conflicts of interest

There are no conflicts of interest.

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