ISSN 1022-7954, Russian Journal of Genetics, 2015, Vol. 51, No. 7, pp. 711–714. © Pleiades Publishing, Inc., 2015.
Original Russian Text © O.V. Kochetova, T.V. Viktorova, O.E. Mustafina, A.A. Karpov, E.K. Khusnutdinova, 2015, published in Genetika, 2015, Vol. 51, No. 7, pp. 830–834.

SHORT COMMUNICATIONS

Genetic Association of *ADRA2A* and *ADRB3* Genes with Metabolic Syndrome among the Tatars

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Received August 7, 2014; in final form, September 24, 2014

Abstract—An association study was performed for genetic polymorphisms in *ADRB3* (rs4994) and *ADRA2A* (rs1800544, rs553668) genes to estimate their effect on quantitative parameters, including glucose, insulin, and HOMA-IR index in women from the Tatar population of Russia. It has been shown that *CT* and *CC* are associated with metabolic syndrome and increased insulin. It was shown that *ADRA2A* (rs1800544) gene polymorphism was associated with high levels of insulin and an increased HOMA-IR index in *GG*- and *GC*-genotype carriers. Polymorphic locus rs553668 of gene *ADRA2A* is associated with increased glucose in *CT*- and *TT*-genotype carriers.

DOI: 10.1134/S1022795415070066

Metabolic syndrome (MS) represents a complex of pathologic states with high social significance that increases every year. MS is observed in every fourth patient among middle age individuals [1, 2]. The high frequency of MS and its role in the predisposition to such comorbid states as type II diabetes mellitus, arterial hypertension, dyslipidemia, and atherosclerosis determine the urgency and significance of the study.

Predisposing genetic factors and their interaction with environmental conditions appear to be significant among all of the etiologic factors. The study of the genes involved in catecholaminergic system regulation is considered as the main task in the determination of the main units of MS. The polymorphism of genes encoding for the adrenergic receptor may play a significant role in the regulation of energy expense and lipolysis. Two classes of adrenergic receptors exist: α and β. An increased expression of type α adrenergic receptors results in decreased insulin secretion from the pancreatic islands, which appears to be an important unit in the pathogenesis of metabolic abnormalities. β-Receptors are G-coupled transmembrane receptors located in brown and white adipose tissue [3]. Decreased ADRB3 activity was shown to cause oxidation suppression and increased fat accumulation in white adipose tissue as a result of adenylate cyclase activation and enhancement of lipolysis in the white adipose tissue, as well as warmth production in brown adipose tissue, which might provide obesity development [3].

The data concerning the role of *ADRB3* rs4994 and *ADRA2A* rs1800544, rs553668 gene polymorphisms in MS development appear to be contradictory. Several studies conducted in Asians and Caucasians revealed associations of *ADRB3*, *ADRA2A* gene polymorphisms with MS, type II diabetes mellitus, obesity, and hyperinsulinemia [4–12]. However, other researchers failed to observe these associations [13, 14]. The published differences may be explained by unstudied gene–gene interactions, as well as by the unique evolutionary diversity in ethnic-specific differences in the distribution of rare allele frequencies of adrenergic receptor genes.

Considering the mentioned findings, the study of the effect of adrenergic receptor gene polymorphisms in metabolic abnormalities in ethnic Tatars appears to be important.

The goal of the present study was to perform an analysis of the genotype, allele, and haplotype frequency distributions of *ADRA2A* rs1800544 (–1252G>C) and rs553668 (427A>G) and *ADRB3* rs4994 (c.190T>C, Trp64Arg) gene polymorphisms in women with MS and in a control group.

DNA samples obtained from 280 women from the Tatar population from Bashkortostan Republic were used in the present study. The ethnic background was determined via questionnaire.

The criteria for the inclusion of patients with metabolic syndrome were the following: type II diabetes mellitus, arterial hypertension, body mass index (BMI) >30 kg/m^2 and/or waist-to-hip ratio (WHR) > 0.85, triglycerides level >2.2 mmol/L, and level of high-density lipoproteins (HDL) cholesterol < 1.0 mmol/L [15]. Abdominal obesity (waist more than 88 cm in women) criteria were the following: high triglycerides level (≥150 mg/dL), low level of high density cholesterol (<39 mg/dL), arterial hypertension ≥130/85 mm Hg, abnormalities in carbohy-

Table 1. Genotype and allele frequency distributions of *ADRA2A* and *ADRB3* genes in women with metabolic syndrome and healthy individuals

Gene/polymorphism	Chromosome	Genotypes 11/12/22	MS $N = 116$	Control $N = 164$	χ^2	p
ADRA2A rs1800544	10	*CC/CG/GG*	66/49/1 (56.90/42.24/0.86)	113/49/2 (68.90/29.88/1.22)	4.580	0.101
		C/G	181/51 (78.02/21.98)	275/53 (83.84/16.16)	2.675	0.102
ADRA2A rs553668	10	*CC/CT/TT*	79/36/1 (68.10/31.03/23.82)	116/46/2 (70.73/28.05/1.22)	0.355	0.837
		C/T	194/38 83.62/16.38	278/50 84.76/15.24	0.060	0.806
ADRB3 rs4994	8	*TT/CT/CC*	76/33/7 (65.52/28.45/6.03)	127/37/0 (77.44/22.56/0)	12.170	0.002
		T/C	185/47 (79.74/20.26)	291/37 88.72/11.28	7.90	0.005

N—number of individuals; χ^2—Pearson criteria, *p*—level of statistical significance.

drate metabolism (a glucose level on an empty stomach ≥110 mg/dL). All women gave written informed consent for participation in the study.

An assessment of biochemical (cholesterol (CS), CS-LDL, CS-HDL, glucose, and HOMA-IR index) and anthropometric parameters was performed.

DNA was isolated from peripheral blood leukocytes via phenol-chloroform extraction. *ADRA2A* rs1800544 and rs553668 and *ADRB3* rs4994 gene polymorphisms were analyzed via polymerase chain reaction (PCR) followed by cleavage with restriction endonucleases *Msp*I and *Dra*I (Sibenzyme, Russia). The oligonucleotide primers and identification of allele polymorphisms were described previously [16]. Statistical analysis was conducted with MS Office Excel 2003 and STATISTICA v. 6.0. [17]. A comparison of parameters from clinic-experimental analysis of women with obesity and the control group was performed with the Kruskal–Wallis test. Linkage disequilibrium and the Hardy–Weinberg equilibrium were calculated via Haploview 4.2 [18].

The estimation of biochemical parameters in blood revealed that the content of total cholesterol comprised 5.89 ± 0.72 mmol/L in women with MS and 4.51 ± 0.32 mmol/L in the control group, respectively ($p < 0.001$). The level of CS-LDL was 3.17 ± 0.19 and 2.43 ± 0.19 mmol/L, respectively ($p = 0.019$). The level of CS-HDL was higher in the control group (1.52 ± 0.11 vs. 1.01 ± 0.12 mmol/L, $p = 0.019$). The glucose level in MS women was 5.27 ± 1.28 mmol/L, which was significantly higher than that in control group (4.3 ± 0.87 mmol/L, $p = 0.02$). The assessment of anthropometric parameters demonstrated statistically significant differences in height ($p = 0.008$), weight ($p < 0.001$), and BMI ($p < 0.001$) between women with MS and the control group. All women with MS were characterized by type II diabetes mellitus. The control group and patients with MS showed no difference in the mean age (54.14 ± 6.91 vs. 52.92 ± 7.22 years).

The genotype and allele frequency distributions corresponded to the Hardy–Weinberg equilibrium in patients and control groups, except for *ADRA2A* rs1800544 in patients.

Analysis of the allele and genotype frequency distributions in *ADRA2A* gene polymorphisms revealed no statistically significant differences between the patients and control group (Table 1). Analysis of the genotype frequency distributions of *ADRB3* rs4994 showed an association of *CT-CC* genotypes (OR = 1.8 (CI 95% 1.06–3.07), $p = 0.039$) and *C* allele with MS risk (OR = 1.99 (CI 95% 1.25–3.19), $p = 0.005$). Logistical regression demonstrated an association of *ADRB3* rs4994 with MS in an additive model (OR = 2.56 (CI 95% 1.54–4.26), $p = 0.0002$).

We conducted an analysis of linkage disequilibrium among *ADRA2A* gene markers. The standardized value of linkage disequilibrium coefficient between two loci, rs1800544 and rs553668, was $D' = 0.52$ ($r = 0.42$; $p = 0.0001$) in MS patients and $D' = 0.70$ ($r = 0.66$; $p = 0.0001$) in the control group. The distribution of *ADRA2A* gene haplotypes demonstrated statistically significant differences between women with MS and healthy individuals ($p = 0.02$). It was demonstrated

that *ADRA2A* gene GC haplotype was associated with MS risk. The frequency of the GC haplotype was 0.1168 in the patient group and 0.0487 in the control group (OR = 2.67 (CI 95% 1.08–6.60), $p = 0.01$). Logistic regression also reported an association of GC haplotype with MS risk (OR = 2.86 (CI 95% 1.41–5.80), $p = 0.0038$).

The association analysis of quantitative biochemical and hormonal parameters with *ADRA2A* and *ADRB3* gene polymorphisms in patients is shown in Table 2. According to published data, *ADRA2A* and *ADRB3* gene polymorphisms were associated with glucose and insulin concentrations in blood [3]. Therefore, we conducted an analysis of genotype distributions of *ADRA2A* rs1800544 and rs553668 and *ADRB3* rs4994 gene polymorphisms with respect to these parameters. A higher glucose level was established among women with MS bearing *ADRB3* rs4994 *CT* and *CC* genotypes compared to *TT* genotype carriers (Table 2). However, no association was detected between insulin parameters and the HOMA-IR index or *ADRB3* gene polymorphisms (Table 2). The published data concerning the association of *ADRB3* rs4994 locus with glucose tolerance and insulin sensitivity remain contradictory. Hence, Christiansen et al. [19] demonstrated hyperglycemia and a decreased insulin level in the blood of dizygotic twins bearing the *CT* genotype (aged 65–70 years). At the same time, the differences failed to be confirmed in patients of younger age with type II diabetes and dyslipidemia [13–20].

Analysis of genotype frequency distributions of *ADRA2A* rs1800544 gene polymorphism in MS women revealed higher insulin levels and HOMA-IR index in *GG* and *GC* genotype carriers compared to those with the *CC* genotype ($p = 0.0006$ and $p = 0.005$, respectively) (Table 2). No statistically significant differences were reported for the distributions of genotype frequencies of rs1800544 with respect to the glucose level.

Analysis of the genotype frequency distribution of *ADRA2A* rs553668 gene polymorphism detected that individuals with *CT* and *TT* genotypes possessed higher glucose levels as compared to *CC* genotype carriers ($p = 0.0114$). No association between the insulin level, HOMA-IR index, or *ADRA2A* rs553668 polymorphism was revealed. The obtained findings are congruent with published data [21–24].

The present study revealed no association for the blood lipid spectrum and various polymorphic variants of *ADRA2A* and *ADRB3* genes (data not shown).

The peformed association analysis of adrenergic receptor genes with the development of a complex of pathologic states characteristic for metabolic syndrome in women from Tatar population confirmed the role of *ADRA2A* and *ADRB3* gene polymorphisms in this process.

Table 2. Association analysis of biochemical and hormonal parameters with various *ADRA2A* and *ADRB3* gene polymorphisms in patients with metabolic syndrome

Parameter	Genotype $N = 116$	Mean value (standard deviation)	p (Kruskal–Wallis test)
ADRB3 rs4994			
Glucose, mmol/L	*TT* (76)	4.67 (0.69)	**0.007**
	CT (33)	5.42 (1.34)	
	CC (7)	4.97 (0.88)	
Insulin, μU/mL	*TT* (76)	10.18 (7.40)	0.098
	CT (33)	10.52 (7.27)	
	CC (7)	15.44 (9.34)	
HOMA-IR index	*TT* (76)	2.42 (1.47)	0.24
	CT (33)	2.18 (1.60)	
	CC (7)	3.87 (1.07)	
ADRA2A rs1800544			
Glucose, mmol/L	*CC* (66)	5.32 (1.29)	0.67
	GC (49)	5.31 (1.21)	
	GG (1)	4.7 (0.70)	
Insulin, μU/mL	*CC* (66)	9.72 (7.12)	**0.0006**
	GC (49)	13.96 (8.06)	
	GG (1)	17.30 (9.80)	
HOMA-IR index	*CC* (66)	2.23 (1.85)	**0.005**
	GC (49)	3.45 (2.42)	
	GG (1)	3.38 (2.82)	
ADRA2A rs 553668			
Glucose, mmol/L	*CC* (79)	4.94 (0.78)	**0.0114**
	CT (36)	5.41 (1.27)	
	TT (1)	6.9 (0)	
Insulin, μU/mL	*CC* (79)	11.68	0.232
	CT (36)	11.06	
	TT (1)	26 (0)	
HOMA-IR index	*CC* (79)	2.90 (2.14)	0.158
	CT (36)	2.35 (2.14)	
	TT (1)	3.69	

Statistically significant differences according to Kruskal–Wallis test ($p < 0.05$) are shown in bold.

ACKNOWLEDGMENTS

This study was partly supported by the Russian Foundation for Humanities (project no. 13-06-00101) and Russian Foundation for Basic Research (project nos. 13-04-00287a and 14-06-97003 region).

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Translated by A. Kazantseva