Association of polymorphism genes *LPL*, *ADRB2*, *AGT* and *AGTR1* with risk of hyperinsulinism and insulin resistance in the Kazakh population

AIZHAN SHAKHANOVA[1], NURLAN AUKENOV[2], ALMA NURTAZINA[1], MERUYERT MASSABAYEVA[1], DMITRIY BABENKO[3], MADINA ADIYEVA[1] and NURLAN SHAIMARDONOV[1]

[1]Department of Propaedeutic of Internal Diseases, Semey Medical University, Semey, East Kazakhstan Region F17G0D3; [2]Department of Health and Human Resources, Ministry of Health of the Republic of Kazakhstan, Nur-Sultan, East Kazakhstan Region Z05K5K8; [3]Scientific and Research Center, Karaganda Medical University, Karaganda, East Kazakhstan Region M01K6T3, Kazakhstan

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Abstract. Hyperinsulinism and insulin resistance are closely associated with several common diseases including type 2 of diabetes, cardiovascular diseases, and metabolic syndrome. The present study aimed to determine the association between hyperinsulinism, insulin resistance and the polymorphism of genes, including *angiotensin II receptor type 1* (*AGTR1*), *angiotensinogen* (*AGT*), *β2-adrenoreceptor* (*ADRB2*) and *lipoprotein lipase* (*LPL*), in the Kazakh population. The design of the current research was a case-control study, involving 460 subjects (age range, 18-65 years). For every subject, plasma glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, apolipoprotein B and apolipoprotein A1 were examined. Moreover, reverse transcription-quantitative PCR was conducted to detect the polymorphism genes *LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C*. Hyperinsulinism was considered when the insulin level was elevated >24.9 IU/ml. The homeostasis model assessment insulin resistance (HOMA-IR) was used to evaluate insulin resistance. The subjects were divided into hyperinsulinism (17 men and 24 women) and normal level insulin (214 men and 205 women) groups, which were also split into insulin resistance group (HOMA-IR >2.7; 80 men and 105 women) and those without insulin resistance group (151 men and 124 women). The results suggested that *LPL Ser447Ter* (*rs328*) allele G was associated with a lower risk of hyperinsulinism (P=0.037). Furthermore, polymorphisms of genes *ADRB2 Gln27Glu (rs1042714), AGT Thr174Met (rs4762)* and *AGTR1 A1166C (rs5186)* were not associated with hyperinsulinism and insulin resistance in the Kazakh population. No interaction was identified between *LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C*. Therefore, the results indicated that haplotype combinations were not associated with insulin resistance.

Correspondence to: Dr Aizhan Shakhanova, Department of Propaedeutic of Internal Diseases, Semey Medical University, 103 Abay Kunanbayev Street, Semey, East Kazakhstan Region F17G0D3, Kazakhstan
E-mail: aizhanshakhanova@gmail.com

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Introduction

Metabolic syndrome is defined as a cluster of abdominal obesity, insulin resistance, dyslipidemia and arterial hypertension (1). Insulin resistance in obese individuals appears as a result of long-term nutrient excess and is manifested by an increase in the flow of fatty acids, nutrient overload, adipose tissue hypoxia, endoplasmic reticulum stress, cytokine secretion in adiposities, chronic inflammation of the tissues and a genetic predisposition (2). Furthermore, insulin resistance mechanisms may differ between patients and populations (2).

Lipoprotein lipase (LPL) is known to be involved in several diseases, such as dyslipidemia, type 2 diabetes and hypertension (3). *LPL* gene polymorphisms affect the lipolytic function of LPL, and are considered valuable candidate genes for diabetes and hypertension (4). Insufficient synthesis or dysfunction of LPL leads to reduced hydrolysis of chylomicron and very-low-density lipoprotein (VLDL), and ultimately results in excessive accumulation of lipoproteins in plasma (5). Polymorphisms of *LPL* genes cause dyslipidemia and insulin resistance via various pathophysiological mechanisms (such as hypertriglyceridemia, dysfunction of antioxidation stress in brain and atherosclerosis), as well as serving an indirect role in the etiology of diabetes mellitus and hypertension (5). Although there have been several studies on the effect of *LPL* gene polymorphism, their results vary based on the ethnicity of the participants (6-8).

The β2-adrenoceptor (ADRB2) acts as a lipolytic receptor in human white adipose tissue and skeletal muscle (9).

Polymorphism of *ADRB2* gene is associated with an elevated sympathetic nervous activity, blood pressure, weight gain and insulin resistance.

The renin-angiotensin-aldosterone system (RAS) stimulates hyperglycemia, changes insulin secretion and decreases sensitivity to insulin (10). Obesity is associated with enhanced activity of both systemic and adipose tissue RAS (10). When RAS tissue activity is high, fat accumulates in visceral adipose tissue more intensively compared with in the subcutaneous area (10). Previous studies have reported that activated angiotensin II in skeletal muscle, adipose tissue and the pancreas influences glucose metabolism, which subsequently leads to insulin resistance (11). Evidence suggests that some individuals are predisposed to insulin resistance via activation of the RAS (11). Angiotensinogen (AGT), as a precursor of angiotensin II, is involved in the pathogenesis of metabolic syndrome and diabetes mellitus (10). *AGT* gene polymorphisms, in turn, are associated with hypertension and insulin sensitivity (11). A recent study revealed a relationship between the *angiotensin II receptor type 1* (*AGTR1*) *A1166C* C allele and insulin resistance (12); this study suggested that the effect of *AGTR1 A1166C* on insulin resistance and endothelial dysfunction in non-alcoholic fatty liver disease occurred via modulating the activation of adipokines, chemokine and pro-inflammatory cells (12). Further investigations of the link between RAS and insulin resistance could facilitate the understanding of their multiple mechanisms (11). Given the regulatory role and the possible impact of *LPL Ser447Ter*, *ADRB2 Gln27Glu*, *AGT Thr174Met* and*AGTR1 A1166C* on the development of insulin resistance, it was hypothesized that these polymorphisms may be potential risk factors for insulin resistance.

The Kazakh population is the indigenous population of the Republic of Kazakhstan, which is located between Europe and Asia. The geographic location of the Republic of Kazakhstan for numerous centuries has contributed to the formation of a specific gene pool, which is different from the genetic pools of other populations (13). A recent study reported that the Kazakh population was anthropologically similar to the Mongoloid ethnicity, and they have an intermediate position between the Caucasian and Asian populations (13). Moreover, allelic frequency distributions of 22 autosomal short tandem repeats in the Kazakh population differ from Han, Xinjiang, Kyrgyz and Xibe populations, but are similar to the Uyghurs (14). To enrich the database of population genetics of the Kazakh group and consider the association with the pathogenesis of insulin resistance, the present study examined *LPL Ser447Ter*, *ADRB2 Gln27Glu*, *AGT Thr174Met* and *AGTR1 A1166C*.

The current study aimed to investigate the association between *LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C,* and the level of insulin and insulin resistance in the Kazakh population.

Materials and methods

Subjects. In total, 460 subjects of Kazakh ethnicity (231 men; 229 women; age range, 18-65 years) living in Semey, Kazakhstan, were recruited. Samples were collected in the Primary Health Care Centers of Semey City, between January 2018 and December 2019. All subjects had no history of cancer, cardiovascular or renal failure, mental diseases, pregnancy or lactation.

Out of all the participants, 17 men (3.69%) and 24 women (5.21%) had a high level of insulin, while 214 men (46.52%) and 205 women (44.56%) had a healthy insulin level. Insulin resistance [The homeostasis model assessment insulin resistance (HOMA-IR)>2.7] was observed in 80 men (17.39%) and 105 women (22.82%). The mean ages for subjects with hyperinsulinism and a healthy level of insulin were 46±10.8 and 48±11.8 years, respectively. In total, 83 men (18.04%) and 102 women (22.17%) had a healthy weight, 107 men (23.26%) and 86 women (18.69%) were overweight, and 41 men (8.92%) and 41 women (8.92%) were obese. All participants signed a written informed consent form to participate in this research, which was performed in accordance with the guidelines set out in the Helsinki Declaration of the World Medical Organization 1964. The Ethics Committee of the Semey Medical University approved the research protocol (Protocol no. 11 from 27.09.2017).

Phenotype measurements. The systolic and diastolic arterial blood pressure, height, weight, BMI and waist circumference of the subjects were evaluated. Arterial blood pressure was measured twice after 5 min of rest with the subjects seated. BMI was defined as the weight (kg)/height (m^2). The fasting venous blood samples (10 ml) were collected in the morning.

Single-nucleotide polymorphism (SNP) selection. SNPs were selected using the data from the 1,000 Genomes Browser project (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes) and The Text-mined Hypertension, Obesity, and Diabetes candidate gene database (T-HOD;bws.iis.sinica.edu.tw/THOD). The 1,000 Genomes Browser project provides an extensive international foundation of polymorphisms of the human genome for research of links between genotype and phenotype (15,16). T-HOD contain all the polymorphisms of the genes that are studied for hypertension, obesity and diabetes mellitus (17). Considering the pathogenesis of insulin resistance, the following candidate genes were selected: *LPL Ser447Ter*, *ADRB2 Gln27Glu*, *AGT Thr174Met* and *AGTR1 A1166C*.

Genotyping. The blood of all subjects was collected into vacuum tubes with K2/K3 EDTA (10 ml). For genetic research, four genes, *LPL Ser447Ter*, *ADRB2 Gln27Glu*, *AGT Thr174Met* and *AGTR1 A1166C*, were examined. Genomic DNA was isolated from blood samples (40 ng) using the ready-made commercial GeneJET Mini kit (Thermo Fisher Scientific, Inc.). Fluorometer Qubit 4 (Thermo Fisher Scientific, Inc.) was used to evaluate DNA concentration, and the extracted DNA was frozen and stored at -20˚C.

All 460 blood samples were genotyped via quantitative PCR (qPCR) (18) using CFX 96 system (Bio-Rad Laboratories, Inc.) with mixed primers and TaqMan samples. The sequences of the primers used were: *LPL Ser447Ter (rs328)* forward, 5'-AATAAGAAGTCAGGCTGGTGA-3' and reverse, 5'-TTATTCTTCAGTCCGACCACT-3'; *ADRB2 Gln27Glu (rs1042714)* forward, 5'-CGTCACGCAGGAAAGGGACGA-3' and reverse 5'-GCAGTGCGTCCTTTCCCTGCT-3'; *AGT Thr174Met (rs4762)* forward, 5'-GCCCACCACCGTGGACAGCAG-3' and reverse,

Table I. Description for the four single-nucleotide polymorphisms.

Gene	Chromosome	Major/minor alleles
LPL Ser447Ter	8:19962213	C/G
ADRB2 Gln27Glu	5:148826910	C/G
AGT Thr174Met	1:230,702,523-230,714,122	G/A
AGTR1 A1166C	3:148742201	A/C

AGTR1, angiotensin II receptor type 1; *AGT*, angiotensinogen; *ADRB2*, β2-adrenoreceptor; *LPL*, lipoprotein lipase.

Table II. Association between insulin levels and insulin resistance with clinical characteristics.

	Insulin, IU/ml			Insulin resistance		
Characteristic	≤24.9	>24.9	P-value	HOMA-IR ≤2.7	HOMA-IR >2.7	P-value
Age, years	46 (39-56)	48 (39-56)	0.98	46 (38-55)	48 (40-56.5)	0.24
Sex[b]	419	41	0.25	275	185	0.023
Male	214	17		151	80	
Female	205	24		124	105	
BMI, kg/m^{2}[a]	26.17 (23.53-29.02)	24.09 (21.9-28.72)	0.067	26.12 (23.61-28.73)	26.12 (22.81-29.1)	0.94
Glucose, mmol/l[a]	5.59 (4.51-6.42)	5.6 (4.72-6.26)	0.86	5.2 (4.25-6.01)	6.06 (5.21-6.89)	0.0001
Total cholesterol, mmol/l[a]	1.8 (1.29-2.53)	1.72 (1.24-2.17)	0.98	3.51 (2.52-4.59)	3.31 (2.46-4.45)	0.21
Triglycerides, mmol/l[a]	1.18 (0.85-1.68)	1.74 (1.26-2.17)	0.0001	1.13 (082-1.56)	1.49 (0.95-2.02)	0.0001
HDL, mmol/l[a]	0.98 (0.8-1.18)	1.06 (0.88-1.2)	0.24	0.95 (0.69-1.44)	0.9 (0.7-1.46)	0.91
LDL, mmol/l[a]	1.83 (1.3-2.53)	1.76 (1.3-2.2)	0.2	1.86 (1.38-2.61)	1.77 (1.18-2.37)	0.03
Apolipoprotein B, g/l[a]	0.98 (0.8-1.17)	1.04 (0.86-1.19)	0.43	0.96 (0.78-1.14)	1.03 (0.84-1.21)	0.013
Apolipoprotein A1, g/l[a]	1.5 (1.32-1.72)	1.47 (1.27-1.62)	0.26	1.51 (1.33-1.73)	1.48 (1.29-1.64)	0.18

[a]Mann-Whitney test; [b]χ^2 test. Data are presented as the median (Q1-Q3) for age, BMI, glucose, total cholesterol, triglycerides, HDL, LDL, apolipoprotein B and apolipoprotein A1. Data are presented as n for sex. P<0.05 was considered to indicate a statistically significant difference. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

5'-CGGGTGGTGGCACCTGTCGTC-3'; and *AGTR1 A1166C (rs5186)* forward, 5'-CCAAATGAGCATTAGCTACTT-3' and reverse, 5'-GTTTACTCGTAATCGATGAA-3'. In total, 20 μl TaqMan Genotyping MasterMix reagent (Synthol, Inc. or Lytech, Inc. dependent on SNP being assessed) and 10 ng DNA were used as a template with a final volume of 25 μl in 96-well plates. The amplification program included the pre-denaturation step at 95˚C for 3 min, followed by 48 cycles at 95˚C for 10 sec and 60˚C for 40 sec for three SNPs [reagents for *ADRB2 Gln27Glu (rs1042714), AGT Thr174Met (rs4762)* and *AGTR1 A1166C (rs5186)* were manufactured by Synthol, Inc.]. For SNP *LPL Ser447Ter (rs328)* the amplification program included, the pre-denaturation step of 93˚C for 1 min, followed by 35 cycles at 93˚C for 10 sec, 64˚C for 10 sec and 72˚C for 20 sec [reagent for *LPL Ser447Ter (rs328)* was manufactured by Lytech, Inc.]. The description for the four single-nucleotide polymorphismsis presented in Table I.

Laboratory tests. Glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein (HDL), LDL, apolipoprotein B and apolipoprotein A1 were measured on Cobas 8000 analyzers (Roche Diagnostics GmbH) according to manufacturer's instructions. Reference values were based on the manufacturer's protocol: Glucose 3.89-5.83 mmol/l; insulin 2.6-24.9 IU/ml; total cholesterol 2.9-5.2 mmol/l; HDL 0.78-2.2 mmol/l, and LDL 2.33-5.31 mmol/l; triglycerides 1.7-2.25 mmol/l; apolipoprotein A1 1.04-2.02 g/l (for men) and 1.08-2.25 g/l (for women); and apolipoprotein B 0.66-1.33 g/l (for men) and 0.6-1.17 g/l (for women). HOMA-IR was used to determine insulin sensitivity and calculated as fasting plasma glucose (mmol/l) x fasting insulin (mU/l)/22.5. Therefore, in the current research, the dichotomous classification of insulin resistance was conducted at HOMA-IR >2.7, in addition to the evaluation of HOMA-IR as a continuous variable.

Statistical analysis. All variables were examined to determine whether they were normally distributed. Non-parametric tests were used for non-normally distributed data, including the Mann-Whitney test for comparison between two groups and Kruskal-Wallis in co-dominant, dominant and recessive models for differences in phenotypic variables for genotype (19). The post-hoc Dunn's test was performed after Kruskal-Wallis test or a Mann-Whitney U test for non-normally distributed quantitative variables (20). The χ^2 test was used for comparing

Table III. Genotype and allele frequencies of four single-nucleotide polymorphism associations with the response to insulin.

A, *LPL Ser447Ter*

Model	Genotype	Insulin ≤24.9, n	Insulin >24.9, n	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	327	27	1.0	0.084	278.1	294.7
	C/G	77	9	0.72 (0.33-1.6)			
	G/G	15	5	0.26 (0.009-0.79)			
Dominant	C/C	327	27	1.0	0.11	278.5	290.9
	C/G-G/G	92	14	0.56 (0.28-1.12)			
Recessive	C/C-C/G	404	36	1.0	0.037	276.8	289.2
	G/G	15	5	0.28 (0.1-0.84)			
Overdominant	C/C-G/G	342	32	1.0	0.62	280.8	293.2
	C/G	77	9	0.82 (0.37-1.79)			
Log-additive				0.57 (0.35-0.94)	0.037	276.7	289.1

B, *ADRB2 Gln27Glu*

Model	Genotype	Insulin ≤24.9, n	Insulin >24.9, n	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	204	23	1.0	0.58	282	298.5
	C/G	177	14	1.44 (0.72-2.89)			
	G/G	38	4	1.13 (0.37-3.48)			
Dominant	C/C	204	23	1.0	0.34	280.2	292.6
	C/G-G/G	215	18	1.37 (0.72-2.62)			
Recessive	C/C-C/G	381	37	1.0	0.96	281.1	293.5
	G/G	38	4	0.97 (0.33-2.88)			
Overdominant	C/C-G/G	242	27	1.0	0.31	280.1	292.5
	C/G	177	14	1.41 (0.72-2.77)			
Log-additive				1.2 (0.72-2)	0.47	280.6	293

C, *AGT Thr174Met*

Model	Genotype	Insulin ≤24.9, n	Insulin >24.9, n	OR (95% CI)	P-value	AIC	BIC
Codominant	G/G	339	36	1.0	0.22	280	296.6
	G/A	76	4	2.18 (0.75-6.35)			
	A/A	4	1	0.41 (0.04-3.8)			
Dominant	G/G	339	36	1.0	0.2	279.5	291.8
	G/A-A/A	80	5	1.82 (0.69-4.81)			
Recessive	G/G-G/A	415	40	1.0	0.43	280.5	292.9
	A/A	4	1	0.37 (0.04-3.41)			
Overdominant	G/G-A/A	343	37	1.0	0.11	278.6	290.9
	G/A	76	4	2.21 (0.76-6.43)			
Log-additive				1.49 (0.62-3.54)	0.35	280.2	292.6

D, *AGTR1 A1166C*

Model	Genotype	Insulin ≤24.9, n	Insulin >24.9, n	OR (95% CI)	P-value	AIC	BIC
Codominant	A/A	335	34	1.0	0.78	282.6	299.1
	A/C	74	6	1.37 (0.55-3.43)			
	C/C	10	1	1.14 (0.14-9.23)			
Dominant	A/A	335	34	1.0	0.49	280.6	293
	A/C-C/C	84	7	1.34 (0.57-3.17)			
Recessive	A/A-A/C	409	40	1.0	0.95	281.1	293.5
	C/C	10	1	1.07 (0.13-8.62)			

Table III. Continued.

D, *AGTR1 A1166C*

Model	Genotype	Insulin ≤24.9, n	Insulin >24.9, n	OR (95% CI)	P-value	AIC	BIC
Overdominant	A/A-C/C	345	35	1.0	0.49	280.6	293
	A/C	74	6	1.37 (0.55-3.41)			
Log-additive				1.25 (0.6-2.6)	0.55	280.7	293.1

Adjusted by sex. *AGTR1*, angiotensin II receptor type 1; *AGT*, angiotensinogen; *ADRB2*, β2-adrenoreceptor; *LPL*, lipoprotein lipase; OR, odds ratio. AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion.

differences in allele frequencies of SNPs between subjects with hyperinsulinism (defined using the dichotomous qualitative trait of insulin ≤24.9 and insulin >24.9), those with insulin resistance (defined using the dichotomous qualitative trait of HOMA-IR ≤2.7 and HOMA-IR >2.7) and control subjects. P<0.05 was considered to indicate a statistically significant difference for a single test. After Bonferroni's correction for multiple testing, frequencies were considered statistically significant if P<0.0125. All statistical analyses were performed using SPSS version 20 (IBM Corp.) and SNPStat version 2.2.1 (snpstats.net/start.htm).

Results

Comparisons of the subjects and their general information. The present study genotyped all 460 subjects, and the clinical and biochemical characteristics of these subjects are presented in Table II. After adjustments for age and sex, no significant difference was observed in the levels of glucose, total cholesterol, HDL, LDL, apolipoprotein B and apolipoprotein A1, except for triglycerides(P=0.0001), between hyperinsulinism and normal insulin level groups. However, the levels of glucose (P=0.01), triglycerides (P=0.0001), LDL (P=0.03) and apolipoprotein B (P=0.01), were significantly higher in the insulin-resistant group compared with the non-insulin resistant group. It was also found that insulin resistance was more prevalent in women compared with men (P=0.023).

Genotype and allele analysis of genes polymorphism of LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met and AGTR1 A1166C. All variants examined in this study, except *LPL Ser447Ter*, were not deviated from the Hardy-Weinberg equilibrium. The distribution of the frequency of the genotypes for the SNPs of *LPL Ser447Ter* were C/C (77%), C/G (18.7%) and G/G (4.3%). The frequencies of the C/C, C/G, and G/G genotypes of *ADRB2 Gln27Glu*were 49.3, 41.5, and 9.1%, respectively. The frequencies of the G/G, G/A and A/A genotypes of *AGT Thr174Met* were 81.5, 17.4, and 1.1%, respectively. The frequencies of the A/A, A/C, and C/C genotypes of *AGTR1 A1166C* were 80.2, 17.4, and 2.4%, respectively (data not shown). When analyzing the linkage between SNPs and insulin, it was found that risk of hyperinsulinism was significantly associated with *LPL Ser447Ter* (P=0.025) and odds ratio; 95% confidence interval [ORs; (95% CI)]= for C/G and G/G genotypes were 0.706 (0.319-1.563) and 0.248 (0.084-0.733), respectively (Table SI). Moreover, the association between SNPs and the risk of insulin resistance was not significant (Table SII).

Logistic regression between the level of insulin, HOMA-IR and gene polymorphism of LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met and AGTR1 A1166C. Logistic regression analysis demonstrated that risk of hyperinsulinism was significantly lower in carriers of *LPL Ser447Ter* G allele compared with those with C/C genotype [C/G+G/G vs. C/C; adjusted OR (95% CI)=0.57 (0.35-0.94); P=0.037; Table III). It was found that only the recessive model of *LPL Ser447Ter* polymorphism (C/C+C/G vs. G/G) was associated with a lower risk of hyperinsulinism.

Logistic regression analysis identified that the risk of insulin resistance was not significantly associated with SNPs (Table IV).

Multiple-SNP analysis. Multiple-SNP analysis was measured between four SNPs for gene-gene interactions; the D-value between *LPL Ser447Ter (rs328)* and *ADRB2 Gln27Glu (rs1042714)* was 0.0311 (P=0.56), and the D-value between *LPL Ser447Ter (rs328)* and *AGT Thr174Met (rs4762)* was 0.0045 (P=0.91). Moreover, the D-value between *LPL Ser447Ter (rs328)* and *AGTR1 A1166C (rs5186)* was 0.3201 (P=0.17), while the D-value between *ADRB2 Gln27Glu (rs1042714)* and *AGT Thr174Met (rs4762)* was 0.0172 (P=0.79). It was also demonstrated that the D-value between *ADRB2 Gln27Glu (rs1042714)* and *AGTR1 A1166C (rs5186)* was 0.00004 (P=0.99), and that between *AGT Thr174Met (rs4762)* and *AGTR1 A1166C (rs5186)* was 0.076 (P=0.03). A significant association was identified only between the gene-gene interactions, *AGT Thr174Met (rs4762)* and *AGTR1 A1166C (rs5186)* (data not shown).

Haplotypes containing the four SNPs were not associated with the risk of hyperinsulinism and the risk of insulin resistance (Tables V and VI).

Discussion

Yang *et al* (21) reported that *LPL* mutations lead to LPL enzyme deficiency, and that an elevated triglycerides level triggers insulin resistance. In the current study, the level of triglycerides was associated with the risk of insulin resistance

Table IV. Genotype and allele frequencies of four single-nucleotide polymorphism associations with response HOMA-IR.

A, *LPL Ser447Ter*

Model	Genotype	HOMA-IR ≤2.7, n	HOMA-IR >2.7, n	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	219	135	1.0			
	C/G	48	38	0.8 (0.5-1.29)	0.18	618.5	635
	G/G	8	12	0.45 (0.18-1.14)			
Dominant	C/C	219	135	1.0	0.14	617.8	630.2
	C/G-G/G	56	50	0.72 (0.46-1.12)			
Recessive	C/C-C/G	267	173	1.0	0.1	617.3	629.7
	G/G	8	12	0.47 (0.19-1.18)			
Overdominant	C/C-G/G	227	147	1.0	0.47	619.4	631.8
	C/G	48	38	0.84 (0.52-1.35)			
Log-additive				0.73 (0.52-1.03)	0.076	616.8	629.2

B, *ADRB2 Gln27Glu*

Model	Genotype	HOMA-IR ≤2.7, n	HOMA-IR >2.7, n	OR (95% CI)	P-value	AIC	BIC
Codominant	C/C	137	90	1.0			
	C/G	114	77	0.98 (0.66-1.46)	0.98	621.9	638.4
	G/G	24	18	0.94 (0.48-1.83)			
Dominant	C/C	137	90	1.0	0.89	619.9	632.3
	C/G-G/G	138	95	0.97 (0.67-1.42)			
Recessive	C/C	251	167	1.0	0.86	619.9	632.3
	C/G-G/G	24	18	0.94 (0.49-1.8)			
Overdominant	C/C-G/G	161	108	1.0	0.97	619.9	632.3
	C/G	114	77	0.99 (0.68-1.45)			
Log-additive				0.97 (0.73-1.3)	0.85	619.9	632.3

C, *AGT Thr174Met*

Model	Genotype	HOMA-IR ≤2.7, n	HOMA-IR >2.7, n	OR (95% CI)	P-value	AIC	BIC
Codominant	G/G	227	148	1.0			
	G/A	44	36	0.86 (0.52-1.4)	0.54	620.7	637.2
	A/A	4	1	2.55 (0.28-23.26)			
Dominant	G/G	227	148	1.0	0.69	619.8	632.2
	G/A-A/A	48	37	0.91 (0.56-1.47)			
Recessive	G/G-G/A	271	184	1.0	0.35	619.1	631.5
	A/A	4	1	2.62 (0.29-23.87)			
Overdominant	G/G-A/A	231	149	1.0	0.52	619.5	631.9
	G/A	44	36	0.85 (0.52-1.39)			
Log-additive				0.97 (0.62-1.51)	0.88	619.9	632.3

D, *AGTR1 A1166C*

Model	Genotype	HOMA-IR ≤2.7, n	HOMA-IR >2.7, n	OR (95% CI)	P-value	AIC	BIC
Codominant	A/A	218	151	1.0	0.27	619.3	635.8
	A/C	52	28	1.44 (0.86-2.42)			
	C/C	5	6	0.66 (0.2-2.22)			
Dominant	A/A	218	151	1.0	0.28	618.7	631.1
	A/C-C/C	57	34	1.31 (0.81-2.12)			
Recessive	A/A-A/C	270	179	1.0	0.42	619.3	631.7
	C/C	5	6	0.61 (0.18- 2.05)			

Table IV. Continued.

D, *AGTR1 A1166C*

Model	Genotype	HOMA-IR ≤2.7, n	HOMA-IR >2.7, n	OR (95% CI)	P-value	AIC	BIC
Overdominant	A/A-C/C	223	157	1.0	0.14	617.8	630.1
	A/C	52	28	1.47 (0.88-2.45)			
Log-additive				1.15 (0.76-1.72)	0.51	619.5	631.9

Adjusted by sex. HOMA-IR, homeostasis model assessment insulin resistance; *AGTR1*, angiotensin II receptor type 1; *AGT*, angiotensinogen; *ADRB2*, β2-adrenoreceptor; *LPL*, lipoprotein lipase; OR, odds ratio; AIC, Akaike's Information Criterion; BIC, Bayesian Information Criterion.

Table V. Haplotype association with response levels of insulin.

Haplotype				Frequency			
LPL Ser447Ter	*ADRB2 Gln27Glu*	*AGT Thr174Met*	*AGTR1 A1166C*	Case[a]	Control[a]	OR (95% CI)	P-value
C	C	G	A	0.4548	0.4889	1.0	
C	G	G	A	0.1429	0.2138	1.37 (0.672.83)	0.39
G	C	G	A	0.1579	0.0721	0.49 (0.23-1.04)	0.064
G	C	G	C	0.0726	0.0553	1.33 (0.38-4.66)	0.65
C	C	A	A	0.0462	0.0581	0.77 (0.28-2.11)	0.61
G	G	G	A	0.0736	0.0326	0.61 (0.2-1.89)	0.39
C	G	G	C	0.0249	0.0269	1.2 (0.21-6.79)	0.84
C	G	A	A	0.0269	0.0127	0.54 (0.11-2.74)	0.46
Rare haplotypes				0.00004	0.0394		0.0001

[a]Control insulin levels ≤24.9, case insulin levels >24.9. Adjusted by sex. Global haplotype association P-value, 0.12. *AGTR1*, angiotensin II receptor type 1; *AGT*, angiotensinogen; *ADRB2*, β2-adrenoreceptor; LPL, lipoprotein lipase; OR, odds ratio.

Table VI. Haplotype association with response homeostasis model assessment insulin resistance.

Haplotype				Frequency			
LPL Ser447Ter	*ADRB2 Gln27Glu*	*AGT Thr174Met*	*AGTR1 A1166C*	Case	Control	OR (95% CI)	P-value
C	C	G	A	0.4626	0.5037	1.0	
C	G	G	A	0.1938	0.2128	1.01 (0.69-1.48)	0.97
G	C	G	A	0.0985	0.0657	0.75 (0.4-1.41)	0.37
C	C	G	C	0.0603	0.0564	0.99 (0.5-1.97)	0.98
C	C	A	A	0.0585	0.0542	0.95 (0.47-1.91)	0.89
G	G	G	A	0.0549	0.0256	0.52 (0.2-1.33)	0.17
C	G	G	C	0.0227	0.029	1.3 (0.47-3.63)	0.62
C	G	A	A	0.0208	0.0132	0.76 (0.2-2.86)	0.69
Rare haplotypes				0.061	0.385	1.12 (0.48-26)	0.8

Adjusted by sex. Global haplotype association P-value, 0.72. *AGTR1*, angiotensin II receptor type 1; *AGT*, angiotensinogen; *ADRB2*, β2-adrenoreceptor; LPL, lipoprotein lipase; OR, odds ratio; Control, HOMA-IR ≤2.7; case, HOMA-IR >2.7.

and a higher level of insulin. It was also found that a higher risk of hyperinsulinism was associated with *LPL Ser447Ter*-C allele but not with the G/G genotype. Moreover, a lower risk of hyperinsulinism was associated with the *LPL Ser447Ter*-G allele. Thus, the impact of gene-gene interaction on the risk of hyperinsulinism requires further investigation. In a previous

study that examined the effects of *LPL Ser447Ter* and *Hind III* gene polymorphisms on factors affecting metabolic syndrome in a northern population of Iran, *LPL Ser447Ter* gene polymorphisms were observed to be associated with a reduced risk of developing low HDL and the risk factors for incidence of metabolic syndrome only in men (22). Vishram *et al* (23) also reported a lower prevalence of metabolic syndrome in carriers of *LPL 447Ter* variant and a significant difference between *447Ter* and *Ser447Ser* variants, as well as identified a higher concentration of insulin in women. The present results are consistent with these aforementioned findings. However, the current study was focused only on the relationship between *LPL Ser447Ter* and insulin level.

It has been reported that SNP *ADRB2 Gln27Glu* affects the levels of triglycerides and HDL (24). A previous study has also shown that *ADRB2 Gln27Glu* homozygous variant was associated with type 2 diabetes mellitus, and this was a risk factor that possibly influences the accumulation of visceral fat and the development of type 2 diabetes mellitus in men but not in women (25). Wang *et al* (26) revealed that the *ADRB2 Gln27Glu* variant exhibited markedly elevated norepinephrine level, while *ADRB2 Thr54Thr* was indicative of insulin resistance. The present results suggested that *ADRB2 Gln27Glu* alleles were not associated with a significantly increased risk of hyperinsulinism and insulin resistance.

Kalupahana and Moustaid-Moussa (27) discovered the role of RAS polymorphisms in affecting insulin sensitivity and protecting against alimentary obesity and insulin resistance. According to the results of a study by Hsiao *et al* (28), *AGT*, *angiotensin I converting enzyme* and *cytochrome P450* family 11 subfamily B member 2 polymorphisms were associated with high fasting, postprandial glucose and insulin levels. In their meta-analysis, Liu and Wang (29) analyzed six case-control studies conducted on different populations, and concluded that *AGT T174M* increased the risk of diabetic nephropathy in the Asian community. Furthermore, Moussa *et al* (30) evaluated the association of RAS polymorphisms with diabetic nephropathy in a Tunisian population, and their results demonstrated an increased risk of diabetic nephropathy linked to *AGT rs4762*C> T, *AGT rs699*A> G and *AGTR1 rs5186*A> C. In contrast, another study conducted in Slovenia, did not identify an association between *AGT rs699, rs4762* and diabetic nephropathy (31). While Underwood and Adler (11) reported that the *AGT* gene polymorphisms were associated with hypertension and insulin sensitivity, the present study did not identify an association between polymorphisms of the *AGT* gene and insulin resistance. Moreover, no association was found between the haplotype containing *AGT Thr174Met* alleles and an elevated risk of hyperinsulinism and the risk of insulin resistance.

Procopciuc *et al* (32) suggested that the A/A genotype of the *AGT Thr174Met* and the C/C genotype of the *AGTR1* could be a risk factor for central obesity and dyslipidemia in hypertensives. Moreover, Goulart *et al* (33) reported that candidate genes associated with cardiovascular disease represent potential risk factors for the metabolic syndrome, and discovered that *AGTR1 (rs5186)* was associated with decreased risk of metabolic syndrome in postmenopausal women. Similarly, Herrera *et al* (34) revealed a higher risk of metabolic syndrome in male carriers of the *AGTR1 (rs5186)* A/A genotype. Furthermore, Akasaka *et al* (35) observed that genetic variants of RAS were involved in insulin sensitivity, and reported that the *AGTR1 A1166C (rs5186)* may affect insulin resistance. The present study demonstrated the opposite results, and found no significant gene-gene interaction between *LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C*.

Wang *et al* (36) compared three populations, including Han, Uygur and Kazakh, in terms of BMI, waist circumference, systolic blood pressure, diastolic blood pressure and HDL. These authors revealed that Kazakh men had the highest BMI, waist circumference, blood pressure and HDL, as well as the lowest triglycerides among the three groups (36). Kazakh men also had the highest insulin sensitivity (36). In the present study, only the level of triglycerides was different between the hyperinsulinism group and the healthy level of insulin group. This finding may be explained by the high consumption of meat and carbohydrates, and low consumption of vegetables and fruits in the Kazakh population, as high-calorie food leads to dyslipidemia (37). In the current research, the levels of glucose, triglycerides, LDL and apolipoprotein B were significantly higher in the resistant group compared with the non-resistance group. Moreover, women had higher insulin resistance compared with men.

There were several limitations to the current research. First, a significant association with hyperinsulinism was only observed for the recessive model and allele frequency *LPL Ser447Ter* gene. The P-value was 0.037 for the genetic predisposition to hyperinsulinism in subjects with a C allele, which was higher compared with the significance level estimated from Bonferroni's correction (P<0.0125). Furthermore, the *LPL Ser447Ter* polymorphism was associated with the dichotomous categorization of insulin groups as a qualitative trait. The present study found no significant association between quintiles of log-transformed HOMA-IR and genotype or allelic frequencies.

In conclusion, the current study suggested that the *LPL Ser447Ter* polymorphism was linked to the risk of hyperinsulinism. In the Kazakh population, *LPL Ser447Ter* allele G was associated with decreased risk of hyperinsulinism, while *ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C* alleles had no association. Moreover, no interaction was identified between *LPL Ser447Ter, ADRB2 Gln27Glu, AGT Thr174Met* and *AGTR1 A1166C,* and haplotype combinations that are not associated with insulin resistance. The present results also suggested that the level of triglycerides was associated with a higher level of insulin and the risk of insulin resistance. However, further investigation is required to confirm these findings in a larger population in prospective longitudinal research.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AS, AN and MM analyzed the data and were responsible for collection of the clinical samples, and were major contributors in the present study. NA, AS, MA, NS and MM made substantial contributions to conception and design of the study. AS, MA and DB contributed to the qPCR analysis. AN and MM wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All participants provided written informed consent to participate in this research (according to the Helsinki Declaration of the World Medical Organization). The Ethics Committee of the Semey medical university approved the research protocol (Protocol no. 11 from 27.09.2017).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Alberti G, Zimmet P, Shaw J and Grundy SM: The IDF consensus worldwide definition of the metabolic syndrome. International Diabetes Federation, Brussels, Belgium, 2006.
2. Schenk S, Saberi M and Olefsky JM: Insulin sensitivity: Modulation by nutrients and inflammation. J Clin Invest 118: 2992-3002, 2008.
3. Beisiegel U: Lipoprotein metabolism. Eur Heart J 19 (Suppl A): A20-A23, 1998.
4. Muñoz-Barrios S, Guzmán-Guzmán IP, José Francisco JF, Salgado-Bernabé AB, Salgado-Goytia L and Parra-Rojas I: Association of the HindIII and S447X polymorphisms in LPL gene with hypertension and type 2 diabetes in Mexican families. Dis Markers 33: 313-320, 2012.
5. Xie C, Wang ZC, Liu XF and Yang MS: The common biological basis for common complex diseases: Evidence from lipoprotein lipase gene. Eur J Hum Genet 18: 3-7, 2010.
6. Blain JF, Aumont N, Théroux L, Dea D and Poirier J: A polymorphism in lipoprotein lipase affects the severity of Alzheimer's disease pathophysiology. Eur J Neurosci 24: 1245-1251, 2006.
7. Socquard E, Durlach A, Clavel C, Nazeyrollas P and Durlach V: Association of HindIII and PvuII genetic polymorphisms of lipoprotein lipase with lipid metabolism and macrovascular events in type 2 diabetic patients. Diabetes Metab 32: 262-269, 2006.
8. Goodarzi MO, Guo X, Taylor KD, Quiñones MJ, Saad MF, Yang H, Hsueh WA and Rotter JI: Lipoprotein lipase is a gene for insulin resistance in Mexican Americans. Diabetes 53: 214-220, 2004.
9. Tellechea ML, Muzzio DO, Iglesias Molli AE, Belli SH, Graffigna MN, Levalle OA, Frechtel GD and Cerrone GE: Association between β2-adrenoceptor (ADRB2) haplotypes and insulin resistance in PCOS. Clin Endocrinol (Oxf) 78: 600-606, 2013.
10. Skov J, Persson F, Frøkiær J and Christiansen JS: Tissue renin-angiotensin systems: A unifying hypothesis of metabolic disease. Front Endocrinol (Lausanne) 5: 23, 2014.
11. Underwood PC and Adler GK: the renin angiotensin aldosterone system and insulin resistance in humans. Curr Hypertens Rep 15: 59-70, 2013.
12. Musso G, Saba F, Cassader M, Paschetta E, De Michieli F, Pinach S, Framarin L, Berrutti M, Leone N, Parente R, *et al*: Angiotensin II type 1 receptor rs5186 gene variant predicts incident NAFLD and associated hypertension: Role of dietary fat-induced pro-inflammatory cell activation. Am J Gastroenterol 114: 607-619, 2019.
13. Sikhayeva N, Talzhanov Y, Iskakova A, Dzharmukhanov J, Nugmanova R, Zholdybayeva E and Ramanculov E: Type 2 diabetes mellitus: Distribution of genetic markers in Kazakh population. Clin Interv Aging 13: 377-388, 2018.
14. Guo Y, Chen C, Jin X, Cui W, Wei Y, Wang H, Kong T, Mu Y and Zhu B: Autosomal DIPs for population genetic structure and differentiation analyses of Chinese Xinjiang Kyrgyz ethnic group. Sci Rep 8: 11054, 2018.
15. Zhuo ZJ, Liu W, Zhang J, Zhu J, Zhang R, Tang J, Yang T, Zou Y, He J and Xia H: Functional polymorphisms at ERCC1/XPF genes confer neuroblastoma risk in Chinese children. EBioMedicine 30: 113-119, 2018.
16. Zhuo ZJ, Zhang R, Zhang J, Zhu J, Yang T, Zou Y, He J and Xia H: Associations between lncRNA MEG3 polymorphisms and neuroblastoma risk in Chinese children. Aging (Albany NY) 10: 481-491, 2018.
17. Dai HJ, Wu JC, Tsai RT, Pan WH and Hsu WL: T-HOD: A literature-based candidate gene database for hypertension, obesity and diabetes. Database 2013: bas061, 2013.
18. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
19. Chan YH: Biostatistics 102 : Quantitative data-parametric & non-parametric tests. Singapore Med J 44: 391-396, 2003.
20. Chan YH: Biostatistics 103: Qualitative data-tests of independence. Singapore Med J 44: 498-503, 2003.
21. Yang T, Pang C, Tsang M, Lam CW, Poon PM, Chan LY, Wu XQ, Tomlinson B and Baum L: Pathogenic mutations of the lipoprotein lipase gene in Chinese patients with hypertriglyceridemic type 2 diabetes. Hum Mutat 21: 453-453, 2003.
22. Alinaghian N, Abdollahi E, Torab M, Khodaparast M, Zamani F and Rahimi-Moghaddam P: Gender-related relation between metabolic syndrome and S447X and HindIII polymorphisms of lipoprotein lipase gene in northern Iran. Gene 706: 13-18, 2019.
23. Vishram JK, Hansen TW, Torp-Pedersen C, Madsbad S, Jørgensen T, Fenger M, Lyngbæk S and Jeppesen J: Relationship between two common lipoprotein lipase variants and the metabolic syndrome and its individual components. Metab Syndr Relat Disord 14: 442-448, 2016.
24. Apalasamy YD, Ming MF, Rampal S, Bulgiba A and Mohamed Z: Gender-dependent association of a β(2)-adrenergic gene variant with obesity parameters in Malaysian Malays. Asia Pacific J Public Heal 27: NP154-NP165, 2015.
25. González Sánchez JL, Proenza AM, Martínez Larrad MT, Ramis JM, Fernández Pérez C, Palou A and Serrano Ríos M: The glutamine 27 glutamic acid polymorphism of the beta2-adrenoceptor gene is associated with abdominal obesity and greater risk of impaired glucose tolerance in men but not in women: A population-based study in Spain. Clin Endocrinol (Oxf) 59: 476-481, 2003.
26. Wang T, Zhang Y, Wang N, Liu Q, Wang Z, Liu B and Niu K: Synergistical action of the β2 adrenoceptor and fatty acid binding protein 2 polymorphisms on the loss of glomerular filtration rate in Chinese patients with type 2 diabetic nephropathy. Int Urol Nephrol 50: 715-723, 2018.
27. Kalupahana NS and Moustaid-Moussa N: The renin-angiotensin system: A link between obesity, inflammation and insulin resistance. Obes Rev 13: 136-149, 2012.
28. Hsiao CF, Sheu WW, Hung YJ, Lin MW, Curb D, Ranadex K, Quertermous T, Chen YM, Chen IY and Wu KD: The effects of the renin-angiotensin-aldosterone system gene polymorphisms on insulin resistance in hypertensive families. J Renin Angiotensin Aldosterone Syst 13: 446-454, 2012.
29. Liu N and Wang Y: Association between angiotensinogen T174M polymorphism and the risk of diabetic nephropathy: A meta-analysis. J Renin Angiotensin Aldosterone Syst 20: 1470320318823927, 2019.
30. Moussa A, Triki S, Hamdouni H, Fekih O, Ajmi M, hajMbarek IB, Koubaa A, Neffati F, Omezzine A, Najjar MF and Bouslama A: Genetic variation in the renin-angiotensin system and diabetic nephropathy in the Tunisian population. Clin Lab 63: 2368-2371, 2017.

31. Makuc J, Šeruga M, Završnik M, Cilenšek I and Petrovič D: Angiotensinogen (AGT) gene missense polymorphisms (rs699 and rs4762) and diabetic nephropathy in Caucasians with type 2 diabetes mellitus. Bosn J Basic Med Sci 17: 262-267, 2017.
32. Procopciuc LM, Sitar-Tăut A, Pop D, Sitar-Tăut D-A, Olteanu I and Zdrenghea D: Renin angiotensin system polymorphisms in patients with metabolic syndrome (MetS). Eur J Intern Med 21: 414-418, 2010.
33. Goulart AC, Rexrode KM, Cheng S, Rose L, Buring JE, Ridker PM and Zee RYL: Association of genetic variants with the metabolic syndrome in 20,806 white women: The women's health genome study. Am Heart J 158: 257-262.e1, 2009.
34. Herrera CL, Castillo W, Estrada P, Mancilla B, Reyes G, Saavedra N, Guzmán N, Serón P, Lanas F and Salazar LA: Association of polymorphisms within the Renin-Angiotensin System with metabolic syndrome in a cohort of Chilean subjects. Arch Endocrinol Metab 60: 190-198, 2016.
35. Akasaka H, Katsuya T, Saitoh S, Sugimoto K, Fu Y, Takagi S, Ohnishi H, Rakugi H, Ura N, Shimamoto K and Ogihara T: Effects of angiotensin II type 1 receptor gene polymorphisms on insulin resistance in a Japanese general population: The Tanno-Sobetsu study. Hypertens Res 29: 961-967, 2006.
36. Wang Y, Zhang J, Ma Y, Song X, Li S, Zhan X and Wu L: Different lipid profiles, insulin sensitivity, and insulin resistance among Han, Uygur, and Kazak men with normal glucose tolerance in Xinjiang, China. Lipids Health Dis 17: 209, 2018.
37. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference B, *et al*: 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. Eur Heart J 41: 111-188, 2020.