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THE ROLE OF THE IRS1 GENE (rs2943640) IN THE COMORBID COURSE OF TYPE 2 DIABETES MELLITUS, OBESITY AND ARTERIAL HYPERTENSION

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Abstract

Type 2 diabetes mellitus (T2DM) affects more than 7 million people, resulting in 2.8 million hospitalizations and more than 300,000 deaths reported annually. Current scientific data indicate that among the world's population, arterial hypertension (AH) and type 2 diabetes mellitus (T2DM) after obesity are among the leading factors of cardiovascular risk.

Aim of research: was to establish the prevalence of the IRS1 gene (rs2943640) polymorphism in patients with T2DM in combination with obesity and arterial hypertension/

Material and research methods. The study involved 33 type-2 diabetic patients hospitalized to the Endocrinology Department of Ternopil University Hospital (Ternopil, Ukraine) in 2019-2020 and 10 healthy individuals.

Inclusion criteria: clinical, laboratory and instrumental signs of T2DM, AH and obesity. Exclusion criteria from the study: signs of clinically significant neurological, mental, renal, hepatic, immune, gastrointestinal, urogenital disorder; injuries of the musculoskeletal system, skin, sense organs, endocrine system (except T2DM); or uncontrolled hematologic diseases; acute pancreatitis, unstable or life-threatening heart disease; patients with malignant neoplasms who have not been in complete remission for at least 5 years, medication (drug) dependence, and alcohol dependence.

T2DM diagnoses were confirmed according to the 2019 Recommendations of the American Diabetes Association (ADA). The diagnosis of arterial hypertension (Stage I) was made according to 2018 ESC/ESH Guidelines for the management of arterial hypertension. Systolic (140-159 mmHg) and/or diastolic (90-99 mmHg) blood pressure were considered as the presence of Stage I AH. Left ventricular hypertrophy was confirmed by an electrocardiogram.

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (QIAamp Blood DNA Mini Kit, QIAGEN, Germany). The IRS-1 gene rs2943640 C>A polymorphism was genotyped using the TaqMan real-time PCR method (Applied Biosystems, Foster City, CA, USA).

Statistical analysis of the data was performed using the software STATISTICA 7.0.

Results. The frequency distribution of the IRS1 gene (rs2943640) polymorphisms and the assessment of compliance with the Hardy – Weinberg equilibrium were performed in the experimental and control groups. It was found that the frequencies of the genotype responsible for C/A polymorphism of the IRS1 gene at T2DM, T2DM with obesity and in the combined course of T2DM with obesity and arterial hypertension did not deviate from the Hardy – Weinberg equilibrium ($p > 0.05$), while in the control group the selected sample did not correspond to the general population.

The corresponding frequencies for the genotypes of the IRS1 gene were as follows: 66.7% for C/A and 33.3% for A/A in the experimental group 1; 42.9% for C/C, 57.1% for C/A in group 2; 47.1% for C/C, 29.4% for C/A and 23.5% for A/A in group 3 and 100.0% for C/A in the control group.

Analysis of allele frequencies for the IRS1 gene in patients with T2DM and comorbidity showed that in patients with T2DM the A allele prevailed (2.0 times), while in patients with T2DM + obesity and T2DM + obesity + arterial hypertension – the C allele. It should be noted that the C allele and the A allele were equally present in the control group.

Analysis of the odds ratio for IRS1 gene genotypes (rs2943640) in patients with T2DM, T2DM and obesity showed no statistically significant relationship between factor (presence of C or A alleles) and disease onset ($p > 0.05$). At the same time, the significant influence of the C/A genotype of the IRS1 gene on the development of T2DM combined with obesity and arterial hypertension ($p < 0.05$) was established. This is confirmed by a significant difference in the dominant model of inheritance of the IRS1 gene only in the group with the combination of T2DM with obesity and arterial hypertension compared with the control group (reliability coefficient for the chi-square $p < 0.001$).

Conclusion. The presence of the C allele of the IRS1 gene (rs2943640) in both homozygous and heterozygous states may increase the risk of comorbid course of T2DM, obesity and arterial hypertension.

Key words: type 2 diabetes mellitus; overweight/obesity; arterial hypertension comorbidity and IRS1 gene

РОЛЬ ГЕНА IRS1 (rs2943640) В КОМОРБИДНОМ ТЕЧЕНИИ САХАРНОГО ДИАБЕТА 2 ТИПА, ОЖИРЕНИЯ И АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИИ

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Резюме

Сахарный диабет 2 типа (СД2) поражает более 7 миллионов человек, ежегодно регистрируется 2,8 миллиона госпитализаций и более 300 000 смертей. Современные научные данные показывают, что среди населения мира артериальная гипертензия (АГ) и СД2 после ожирения являются одними из ведущих факторов риска сердечно-сосудистых заболеваний.

Цель исследования: установить распространенность полиморфизма гена IRS1 (rs2943640) у пациентов с СД2 в сочетании с ожирением и артериальной гипертензией.

Материал и методы исследования. В исследовании приняли участие 33 пациента с диабетом 2 типа, госпитализированных в отделение эндокринологии Тернопольской университетской больницы (Тернополь, Украина) в 2019-2020 гг. и 10 здоровых человек.

Критерии включения: клинические, лабораторные и инструментальные признаки СД2, АГ и ожирения. Критерии исключения из исследования: признаки клинически значимого неврологического, психического, почечного, печеночного, иммунного, желудочно-кишечного, урогенитального расстройства; травмы опорно-двигательного аппарата, кожи, органов чувств, эндокринной системы (кроме СД2); или неконтролируемые гематологические заболевания; острый панкреатит, нестабильное или опасное для жизни заболевание сердца; пациенты со злокачественными новообразованиями, не находящиеся в полной ремиссии не менее 5 лет, лекарственная (лекарственная) зависимость и алкогольная зависимость.

Диагноз СД2 подтвержден в соответствии с Рекомендациями Американской диабетической ассоциации (ADA) 2019 г. Диагноз артериальной гипертензии (стадия I) был поставлен в соответствии с рекомендациями ESC / ESH 2018 по лечению артериальной гипертензии. Систолическое (140–159 мм рт. ст.) и / или диастолическое (90–99 мм рт. Ст.) артериальное давление считалось наличием АГ I стадии. Гипертрофия левого желудочка подтверждена электрокардиограммой.

Геномную ДНК выделяли из лейкоцитов периферической крови с использованием коммерчески доступного набора для выделения ДНК (QIAamp Blood DNA Mini Kit, QIAGEN, Германия). Полиморфизм rs2943640 C> A гена IRS-1 был генотипирован с использованием метода ПЦР в реальном времени TaqMan (Applied Biosystems, Foster City, CA, USA).

Статистический анализ данных проводился с помощью программы STATISTICA 7.0.

Результаты. Частотное распределение полиморфизмов гена IRS1 (rs2943640) и оценку соответствия равновесию Харди - Вайнберга проводили в опытной и контрольной группах. Установлено, что частоты генотипа, ответственного за полиморфизм С / А гена IRS1 при СД2, СД2 с ожирением и при сочетанном течении СД2 с ожирением и артериальной гипертензией, не отклоняются от равновесия Харди - Вайнберга ($p > 0,05$), тогда как в контрольной группе выбранная выборка не соответствовала генеральной совокупности.

Соответствующие частоты для генотипов гена IRS1 были следующими: 66,7% для С / А и 33,3% для А / А в экспериментальной группе 1; 42,9% для С / С, 57,1% для С / А в группе 2; 47,1% для С / С, 29,4% для С / А и 23,5% для А / А в группе 3 и 100,0% для С / А в контрольной группе.

Анализ частот аллелей гена IRS1 у пациентов с СД2 и сопутствующей патологией показал, что у пациентов с СД2 преобладает аллель А (2,0 раза), а у пациентов с СД2 + ожирением и СД2 + ожирением + артериальной гипертензией - аллель С. Следует отметить, что аллель С и аллель А одинаково присутствовали в контрольной группе.

Анализ отношения шансов для генотипов гена IRS1 (rs2943640) у пациентов с СД2, СД2 и ожирением не показал статистически значимой связи между фактором (наличие аллелей С или А) и началом заболевания ($p > 0,05$). При этом установлено достоверное влияние генотипа С / А гена IRS1 на развитие СД2, сочетающегося с ожирением и артериальной гипертензией ($p < 0,05$). Это подтверждается достоверным

различием доминантной модели наследования гена *IRS1* только в группе с сочетанием СД2 с ожирением и артериальной гипертензией по сравнению с контрольной группой (коэффициент достоверности хи-квадрат $p < 0,001$).

Вывод. Наличие аллеля С гена *IRS1* (rs2943640) как в гомозиготном, так и в гетерозиготном состояниях может повышать риск коморбидного течения СД2, ожирения и артериальной гипертензии.

Ключевые слова: сахарный диабет 2 типа; избыточный вес / ожирение; коморбидность; артериальная гипертензия и ген *IRS1*

Introduction

Type 2 diabetes mellitus (T2DM) affects more than 7 million people, resulting in 2.8 million hospitalizations and more than 300,000 deaths reported annually [1]. Current scientific data indicate that among the world's population, arterial hypertension (AH) and type 2 diabetes mellitus (T2DM) after obesity are among the leading factors of cardiovascular risk [2]. Studies have shown that improved glycemic control can reduce the progression and onset of microvascular complications [3]. Studies also report that impaired glucose metabolism in humans is likely to be associated with an increased risk of arterial hypertension and complications of cardiovascular disease (CVD) [4]. Arterial hypertension is diagnosed in about 50% of patients with T2DM, and the risk of arterial hypertension developing in patients with T2DM is much higher [5], because T2DM increases the stiffness of peripheral and central arteries, which leads to increased blood pressure [4]. The American Diabetes Association and the European Diabetes Association have shown that adequate management of CVD risk factors, including control of high blood pressure and treatment of obesity, may be a priority for patients with T2DM due to the high risk of morbidity and mortality from CVD [6].

The association between arterial hypertension, insulin resistance and, consequently, hyperinsulinemia is well documented [7]. In general, scientific evidence suggests the existence of a common genetic pathway for the development of essential hypertension and insulin resistance, a concept that is also confirmed by the detection of altered glucose metabolism in normotensive descendants of hypertensives [8]. The general genetic background of insulin resistance and hypertension is further confirmed by the detection of specific genetic abnormalities in people with a combination of insulin resistance, obesity, dyslipidemia and hypertension [9].

The aim of this study was to establish the prevalence of the IRS1 gene (rs2943640) polymorphism in patients with T2DM in combination with obesity and arterial hypertension.

Material and research methods. The study involved 33 type-2 diabetic patients hospitalized to the Endocrinology Department of Ternopil University Hospital (Ternopil, Ukraine) in 2019-2020 and 10 healthy individuals. The distribution of individuals to study groups is presented in table 1.

Table 1

The distribution of individuals to study groups

№	Groups	n	%
1	T2DM	9	27,3
2	T2DM+Obesity	7	21,2
3	T2DM+Obesity+Arterial Hypertension	17	51,5
4	Control	10	100

Inclusion criteria: clinical, laboratory and instrumental signs of T2DM, AH and obesity.

Exclusion criteria from the study: signs of clinically significant neurological, mental, renal, hepatic, immune, gastrointestinal, urogenital disorder; injuries of the musculoskeletal system, skin, sense organs, endocrine system (except T2DM); or uncontrolled hematologic diseases; acute pancreatitis, unstable or life-threatening heart disease; patients with malignant neoplasms who have not been in complete remission for at least 5 years, medication (drug) dependence, and alcohol dependence.

T2DM diagnoses were confirmed according to the 2019 Recommendations of the American Diabetes Association (ADA) [10]. The diagnosis criteria use the level of glycated haemoglobin (HbA1c) ($\geq 6.5\%$), which was determined using an automatic biochemical analyzer COBAS 6000 (Roche Hitachi, Germany) and plasma glucose level, which was determined on an automatic biochemical analyzer BAS INTEGRA® 400 (Roche Diagnostics) using a standard set.

The diagnosis of arterial hypertension (Stage I) was made according to 2018 ESC/ESH Guidelines for the management of arterial hypertension [11]. Systolic (140-159 mmHg) and/or diastolic (90-99 mmHg) blood pressure were considered as the presence of Stage I AH. Left ventricular hypertrophy was confirmed by an electrocardiogram.

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available DNA isolation kit (QIAamp Blood DNA Mini Kit, QIAGEN, Germany). The IRS-1 gene rs2943640 C>A polymorphism was genotyped using the TaqMan real-time PCR method

(Applied Biosystems, Foster City, CA, USA). Quality control was performed with eight negative control and positive control samples in each 96-well plate. In addition, approximately 10% of the samples were randomly selected for further quality control, and the concordance rate was 100%. Amplification of the 25-bp IRS-1 sequence including rs2943640 was performed by using PCR with 5'-GAAATGAGAGGAACCCTTCTAACTA-3' as the forward primer and 5'-AGGAACTCTTCTAACTATTAGCCC-3' as the reverse primer. Two alleles of the rs2943640 IRS-1 polymorphism were detected (C and A).

The ethical principles included in the Declaration of Human Rights adopted in Helsinki, in 1975, and revised in 2008, were fully respected in our study. The enrolled subjects participated in this study voluntarily, completed and signed a written informed consent. Study protocol was approved by the Ethics Committee of I. Horbachevsky Ternopil National Medical University.

Statistical analysis of the data was performed using the software STATISTICA 7.0. Hardy-Weinberg equation was applied to verify the conformity of genotype distribution in the sample to expected distribution in general population. Observed and expected frequencies, calculated using the formula $p^2 + 2pq + q^2 = 1$ (Hardy-Weinberg equation), were compared using Pearson's chi-squared test (χ^2). Significance values of $p > 0.05$ were calculated assuming the null hypothesis of the sample equality, namely the correspondence of frequencies distribution in the selected sample to general population. Comparative analysis of frequency tables was performed using Pearson's chi-squared test and two-tailed p-value for Fisher's exact test (pF - in cases where the values of expected frequencies of individual indicators did not exceed 5). To assess effect of a factor (the presence of a certain genotype or allele) on the occurrence of disease, the odds ratio (OR), its 95% confidence interval (CI) and significance coefficient p-value were determined.

Findings and discussion

The frequency distribution of the IRS1 gene (rs2943640) polymorphisms and the assessment of compliance with the Hardy – Weinberg equilibrium were performed in the experimental and control groups. It was found that the frequencies of the genotype responsible for C/A polymorphism of the IRS1 gene at T2DM, T2DM with obesity and in the combined course of T2DM with obesity and arterial hypertension did not deviate from the Hardy – Weinberg equilibrium ($p > 0.05$), while in the control group the selected sample did not correspond to the general population (Table 2).

The corresponding frequencies for the genotypes of the IRS1 gene were as follows: 66.7% for C/A and 33.3% for A/A in the experimental group 1; 42.9% for C/C, 57.1% for

C/A in group 2; 47.1% for C/C, 29.4% for C/A and 23.5% for A/A in group 3 and 100.0% for C/A in the control group (Table 2).

Table 2

Polymorphism of IRS1 genes (rs2943640) according to Hardy-Weinberg equilibrium in patients with T2DM with comorbidity

Genotype		T2DM		T2DM+ Obesity		T2DM+Obesity+Arterial Hypertension		Control	
		Expected	Present	Expected	Present	Expected	Present	Expected	Present
Homozygotes, common	CC	1	0	3,6	3	6,5	8	2,5	0
Heterozygotes	CA	4	6	2,9	4	8,0	5	5	10
Homozygotes, rare	AA	4	3	0,6	0	2,5	4	2,5	0
χ^2, P		$\chi^2=2,25; p>0,05$		$\chi^2=1,12; p>0,05$		$\chi^2=2.42; p>0,05$		$\chi^2=10,00; p<0,01^*$	

Analysis of allele frequencies for the IRS1 gene in patients with T2DM and comorbidity showed that in patients with T2DM the A allele prevailed (2.0 times), while in patients with T2DM + obesity and T2DM + obesity + arterial hypertension – the C allele (Table. 3). It should be noted that the C allele and the A allele were equally present in the control group.

Table 3

The allele frequencies of IRS1 genes (rs2943640) in patients with T2DM with comorbidity

The allele frequencies	T2DM		T2DM+ Obesity		T2DM+Obesity+ Arterial Hypertension		Control	
	n	%	n	%	n	%	n	%
Allele C	6	33,33	10	71,43	21	61,76	10	50,00
Allele A	12	66,67	4	28,57	13	38,24	10	50,00
p_F (patients/control)	$p_F=0,342$		$p_F=0,296$		$p_F=0,569$		-	

Analysis of the odds ratio for alleles of the IRS1 gene (rs2943640) in different study groups showed no statistically significant relationship between factor (presence of C or A alleles) and disease onset ($p > 0.05$) (Table 4).

Analysis of the odds ratio for IRS1 gene genotypes (rs2943640) in patients with T2DM, T2DM and obesity showed no statistically significant relationship between factor (presence of C or A alleles) and disease onset ($p > 0.05$). At the same time, the significant influence of the C/A genotype of the IRS1 gene on the development of T2DM combined with obesity and arterial hypertension ($p < 0.05$) was established (Table 5).

Table 4

The odds ratio for alleles of the IRS1 gene (rs2943640) in patients with T2DM with comorbidity

	Allele C			Allele A		
	OR	95 % CI	p	OR	95 % CI	p
T2DM	0,50	0,13–1,86	>0,05	2,00	0,54–7,45	>0,05
T2DM+Obesity	3,00	0,62–14,47	>0,05	0,33	0,07–1,61	>0,05
T2DM+Obesity+Arterial Hypertension	1,62	0,53–4,93	>0,05	0,62	0,20–1,89	>0,05

Table 5

The odds ratio for genotypes in different study groups of patients with T2DM with comorbidity

Group	CC		CA		AA	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
T2DM	1,11	0,02–61,38	0,09	0,01–2,00	11,31	0,50–256,21
T2DM+Obesity	16,33	0,69–385,89	0,06	0,01–1,45	1,40	0,02–78,80
T2DM+Obesity+Arterial Hypertension	18,79	0,95–371,48	0,02*	0,01–0,42	7,00	0,34–145,03

Notes. * – $p < 0,05$.

This is confirmed by a significant difference in the dominant model of inheritance of the IRS1 gene only in the group with the combination of T2DM with obesity and arterial hypertension compared with the control group (reliability coefficient for the chi-square $p < 0.001$). Thus, the presence of the C allele in both homozygous and heterozygous states may increase the risk of T2DM comorbidity, obesity and arterial hypertension (Table 6).

Insulin plays a fundamental role in controlling blood sugar levels by stimulating glucose transport through adipocytes and skeletal muscle fibers after IRS activation [12]. Studies of the mechanism of blood sugar control over the past three decades have shown that insulin stimulates the translocation of glucose transporters from the compartments of the intracellular membrane to the plasma membrane, increasing the rate of sugar absorption. Although more than one glucose transporter (GLUT) is expressed in adipocytes and skeletal fibers, published studies show that GLUT4 is the major transporter responsible for glucose uptake in these tissues [13]. Insulin binds and activates insulin receptor tyrosine kinase (IR), culminating in the phosphorylation of IRS1, IRS2, IRS3 and IRS4, which, upon binding to several signaling partners, including phosphoinositide-3-kinase (PI3K), activate the cascade Akt/protein kinase B and protein kinase C- α , play an important role in insulin function [9].

IRS subtypes show tissue-specific distribution and different signaling pathways, with IRS1 mediating the effect of insulin on glucose uptake in adipocytes and skeletal muscles.

Table 6

Dominant and recessive models of IRS1 gene (rs2943640) inheritance at T2DM in combination with obesity and arterial hypertension

Genotypes	T2DM+Obesity+ Arterial Hypertension	Control	pF	OR	95 % CI	p
	%	%				
dominant model						
CC	47,06	0	<0,012*	18,79	0,95–371,48	0,054
CA+AA	52,94	100,00		0,05	0,002–0,1,05	0,054
recessive model						
CC+CA	76,47	100,00	0,264	0,14	0,01–2,96	>0,05
AA	23,53	0		7,00	0,34–145,03	>0,05

Note. * – statistically significant differences.

The interaction between insulin resistance and hypertension can be seen either as the effect of two independent processes or as a reflection of a causal relationship (insulin resistance as a cause of hypertension). In an unreasonable association, both insulin resistance and hypertension can be two independent consequences of the same cellular disorder, namely an increase of intracellular free calcium, which can lead to both vasoconstriction and insulin dysfunction [14]. In addition, insulin resistance can be considered as a molecular marker of multiple metabolic abnormalities that are often associated with hypertension. On the other hand, hyperinsulinemia can be considered as the main cause of arterial hypertension through several mechanisms: increased sodium reabsorption in the renal tubules, activation of the sympathetic nervous system and changes of vascular resistance due to increased calcium concentration in smooth muscle cells [15].

However, hyperinsulinemia, which is present in T2DM, obesity, or metabolic syndrome, can lead to hypertension. Although published results may suggest that the contributing role of hyperinsulinemia in the development of hypertension is predominantly associated with insulin resistance, it remains unclear whether the effects of insulin can lead to hypertension at the absence of insulin resistance. Studies involving slim and obese individuals have shown that insulin-induced vasodilation due to transmission of PI3K signals is impaired in individuals with insulin resistance [14, 15]. In obese patients with insulin resistance, urinary sodium excretion was reduced by insulin, indicating the ability of insulin to stimulate

salt absorption [16]. This confirms the significant role of insulin-stimulated salt reabsorption and vasodilation disorders in the pathogenesis of arterial hypertension at insulin resistance.

Studies by Nakamura and colleagues using sodium bicarbonate cotransporter (NBCe1) activity as a marker for salt uptake in the proximal tubules have shown that insulin stimulates NBCe1 activity in control rats via IRS2 [12]. In addition, they noted that stimulation of NBCe1 by insulin (mediated by IRS2) persists, while stimulation of glucose uptake in adipocytes (mediated by IRS1) is inhibited. The results obtained by Hevko and Marushchak indicate an association between the IRS1 gene (rs2943640) polymorphism and lipid profile abnormalities in patients with mono T2DM and the combined course of T2DM with obesity [17].

Conclusion. The presence of the C allele of the IRS1 gene (rs2943640) in both homozygous and heterozygous states may increase the risk of comorbid course of T2DM, obesity and arterial hypertension.

Prospects for further research. In the future, it is planned to analyze the prevalence of the AGT gene polymorphism in patients with T2DM in combination with obesity and arterial hypertension.

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