ISSN 2518-1432 (Online); ISSN 2227-4782 (Print) https://doi.org/10.21856/j-PEP.2022.3.06

INVESTIGATION OF 2548G>A LEPTINE GENE POLYMORPHIC VARIANT IMPACT ON RISK OF NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS*

T. V. Tyzhnenko [1,2], K. V. Misyura [1], N. O. Kravchun [3], M. Yu. Gorshunska [4], A. K. Pochernyaev [1,5], N. S. Krasova [1], A. I. Gladkih [1], Z. A. Leshchenko [1], G. V. Fedorova [6], O. O. Plohotnichenko [1], O. B. Hromakovska [1], A. O. Kolesnikova [1], E. Jansen [7], Yu. I. Karachentsev [1,8], V. V. Poltorak [1]

[1] SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine», Kharkiv, Ukraine;
[2] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine;
[3] Multidisciplinary medical center «Life Park», Kharkiv, Ukraine;
[4] Kharkiv Medical Academy of postgraduate education of the Ministry of health of Ukraine, Kharkiv, Ukraine;
[5] Poltava Scientific Research Forensic Center of the Ministry of Internal Affairs of Ukraine, Poltava, Ukraine;
[6] University of South Bohemia in České Budějovice, Vodňany, Czech Republic;
[7] National Institute for Public Health and the Environment, Bilthoven, The Netherlands;
[8] Kharkiv National Medical University, Kharkiv, Ukraine
tyzhnenko@ukr.net

One of the most important problems of modern society is the spread of «diseases of civilization»: obesity, atherosclerosis, diabetes mellitus (DM), non-alcoholic fatty liver disease (NAFLD) and high mortality associated with their complications. It is generally accepted that NAFLD is a component of the metabolic syndrome and is often combined with obesity, type 2 DM, atherogenic dyslipidemia, and others [1]. It is known that NAFLD is a global problem in the field of health care [2].

The pathogenesis of NAFLD includes a number of factors, and several interrelated mechanisms, such as insulin resistance, lipotoxicity, imbalance of inflammatory mediators, endotoxinemia, and others [3]. Chronic inflamma-

* The work was carried out within the frameworks of the fundamental research of the State Institute «V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine»: «To investigate the role of adipocynes in development of cardiovascular complications in patients with type 2 diabetes mellitus with non-alcoholic fatty liver disease» (state registration number — 0116U007262) and within the framework of the agreement on joint work with the National Institute for Public Health and the Environment in Bilthoven, the Netherlands (agreement b/n RIVM dated October 18, 2008).

The institution that finances the study is the National Academy of Medical Sciences of Ukraine.

The authors assume responsibility for the published work.

The authors guarantee absence of competing interests and their own financial interest when carrying out the research and writing the article.

The manuscript was received by the editorial staff 01.08.2022.

tion, fibrosis, and cell death are the main drivers of NAFLD progression [4]. The etiology of NAFLD remains largely unclear but is likely multifactorial. In the development of fatty infiltration of the liver of non-alcoholic origin, exogenous factors, hormonal disorders, and genetic factors are important [5, 6]. The NAFLD pathogenesis remains incompletely understood, a multihit model has been proposed that provide accommodations causal factors from a variety of sources, including adipose and intestinal proinflammatory stimuli acting on the liver simultaneously [7].

Obesity is now considered a systemic inflammation in which adipose tissue and its hormones play crucial role [8]. Adipose tissue functions as an endocrine organ, secretes certain cytokines called «adipocytokines», such as adiponectin, leptin, tumor necrosis factor-α, and so on [9].

Leptin, the first described and the most studied adipokine, can significantly affect liver metabolism according to a number of studies. Under normal conditions, leptin inhibits glucose production in the liver and lipogenesis, thereby ensuring the restoration of insulin sensitivity and preventing the development of steatosis [10]. It has been established that leptin has pro-inflammatory properties, and that hyperleptinemia due to leptin resistance can contribute to the development of chronic inflammation; future studies should reveal the role of leptin resistance in the pathogenesis of metabolic diseases [11].

The unequal prevalence of the disease in representatives of different ethnic groups and the different rates of its progression in individuals with the same or similar risk factors and environmental conditions indicate the pathogenetic role of hereditary factors [12, 13].

However, the molecular mechanisms underlying the development and progression of NAFLD are poorly understood at present. In recent years, the genetic determinants of NAFLD have been unrevealed using genome-wide association studies. These studies have identified leptin (*LEP*) gene variants. To understand the nature of the link between the gene and metabolic state of the organism, *LEP* single nucleotide polymorphisms (*SNPs*) are investigated, among others. Among *LEP* gene polymorphisms is *rs7799039*. This *LEP* gene polymorphism is associated with the replacement of adenine by guanine at position 2548 in the promoter [14]. This leads to a change in the activity of leptin and affects the perception of the feeling of satiety, reducing the control of the central nervous system over appetite. Polymorphism of the *LEP* gene may be associated with reduced leptin secretion and the development of obesity, with homozygous mutations of the *LEP* gene predisposing to the development of obesity at an early age [15, 16]. The clinical significance of this polymorphism is well documented as it was associated with higher risk of several diseases including malnutrition, inflammation syndrome, obesity and metabolic syndrome. In addition, *rs7799039* was associated with blood lipid levels among the Southern Chilean population, dyslipidemia in patients using atypical antipsychotic agents and lipid profile modulation induced by soluble fiber intake [17-22].

Hereditary factors and differences in some ethnic groups suggest that genetic factors may play an important role in determining the phenotypic presentation and overall risk of developing NAFLD [23].

Identification of biochemical and genetic risk factors for the development of NAFLD will allow diagnosing the disease at the preclinical stage, improving differential diagnosis, forming high-risk NAFLD groups, followed by adequate preventive measures to maintain health and increase life expectancy in patients with diabetes mellitus complicated by NAFLD.

The current study **aimed** to investigate the association of polymorphisms in *LEP* (*rs7799039, −2548G/A*) gene with non-alcoholic fatty liver disease in type 2 diabetes patients' in Ukrainian population.

MATERIALS AND METHODS

Case-control study included a total of 61 unrelated patients with a diagnosis of type 2 DM (age 56.40 ± 0.62 yrs, diabetes duration 7.72 ± 0.45 yrs, BMI 32.20 ± 0.43 kg/m^2, WHR 1,00 ± 0,01, HbA_{1c} 7.80 ± 0.19 %) and 51 sex and age-match control subjects. The data were

collected through a standard questionnaire. All patients were interviewed regarding a full medical history (including age, sex, occupation, duration of diabetes, mode and duration of treatment, presence of any associated illness, surgical history, personal history of smoking/alcohol/drug abuse, dietary habit and family history of diabetes). All cases and controls signed an informed consent for clinical, biochemical and genetic studies. The protocols were approved by the institutional review board (IRB) of SI «V. Danilevsky Institute for Endocrine Pathology Problems of NAMS of Ukraine». The cases were clinically and biochemically confirmed as type 2 DM. Diagnosis NAFLD were verified following the recommendations of the American Gastroenterological Association (AGA) and the American Association for the Study of liver disease (AASLD) based on the clinical course of the disease, lipid and carbohydrate metabolism, activity of alaninaminotransferase (ALT), aspartataminotransferase (AST), ratio ALT/AST and sonographic examination [24]. Genotyping of the type 2 DM patients was carried out by the method of polymerase chain reaction and restriction fragment length polymorphism using primers (*LEP2548G>A:* forward: tcccatgagaactattcttcttttg; reverse: atatggctccctttgcccgacc) and *HhaI* endonuclease. Restriction products were analyzed by electrophoresis in a 2 % agarose gel. pUC19 DNA hydrolyzed by *MspI* endonuclease was used as a molecular weight marker. Total leptine concentration, insulin resistance (HOMA-IR) and insulin sensibility (QUICKI), lipid profile were determined. Statistical analysis was performed using parametric and nonparametric methods. To compare the indices with normal distribution Student's t-test was used and for comparison variables with abnormal distribution Mann–Whitney's U-test was used. The data are presented as mean ± SEM. All statistical tests were two tailed and a probability (p) value of 5% or less was considered statistically significant [25].

RESULTS AND THEIR DISCUSSION

In patients with type 2 DM in the presence of NAFLD compared to patients with type 2 DM without NAFLD, the features of dyslipidemia with a significant increase in the content of triglycerides (2.49 ± 0,15 vs. 3.53 ± 0.21 mmol/L, $p < 0.001$), LDL cholesterol (3.52 ± 0.08 vs. 3.76 ± 0.10 mmol/L, $p < 0.1$), and a decrease in the content of HDL cholesterol (1.26 ± 0.05 vs. 1.08 ± 0.02 mmol/L, $p < 0.001$) were found.

Despite the association of NAFLD with obesity and insulin resistance (IR), the pathogenesis of the disease is not well understood, as are the genetic factors predisposing to its development and progression. Obesity and impaired insulin action in adipocytes lead to failure to suppress lipolysis, adipocyte stress, macrophage recruitment and infiltration of adipose tissue with subsequent release of proinflammatory adipocytokines, principally tumor necrosis factor (TNF), interleukin (IL) 6, monocyte chemoattractant protein 1, resistin, and plasminogen activator inhibitor 1. These adipocytokines contribute to the disruption of insulin signaling through nuclear factor-κB (NF-κB) and c-Jun N-terminal kinase (JNK), creating a vicious circle in adipose tissue and the formation of IR in other insulin-sensitive tissue, while the level of protective adipokines (reducing IR), such as adiponectin, decreases in patients with NAFLD [26]. Serum adiponectin levels are usually decreased in NAFLD, while leptin levels are elevated, contributing to a profibrotic environment in fibrosis. The relationship between carbohydrate metabolism, reflected by blood glucose level, and serum leptin content was studied. In our study, to determine the role of leptin in the risk of developing NAFLD, we measured the levels of this adipocytokine in circulation in patients with type 2 DM in the presence and absence of fatty liver damage. It is known that leptin is secreted in proportion to the white fat mass and is present in circulation either in free form or bound to proteins. Its level in circulation and adipose tissue depends on the amount of adipose tissue and the state of energy balance. The level of circulating leptin reflects energy reserves in the body andacute changes in energy consumption [27]. Leptin plays a crucial role in regulating energy homeostasis, glucose and lipid metabolism, reproduction and neuroendo-

crine function [28, 29]. There is also emerging evidence that leptin is involved in cognitive and immune function and bone metabolism [30].

Stratification of the diabetic population in the presence and absence of NAFLD showed a more pronounced increase in circulating leptin levels in the presence of NAFLD (84.73 ± 13.80 vs. 52.57 ± 6.86 μg/L, respectively), ($p < 0.01$), which justifies the expediency of using this indicator for further use as a diagnostic parameter of the complication mentioned above. Our data are consistent with the study of Rotundo L. et al. (2018), which examined the relationship between leptin levels and NAFLD and found that higher leptin levels were associated with increased severity of hepatic steatosis based on ultrasound findings and the NAFLD fibrosis score. This finding remained significant after adjusting for known demographic variables only in classic NAFLD, suggesting that the role of leptin in the pathogenesis of NAFLD may depend on BMI [31].

One of the causes of leptin resistance may be the *G2548A* mutation in the leptin gene

Table 1

Clinical and laboratory characteristics of examined patients with type 2 diabetes mellitus – carriers of different genotypes for the G2548A polymorphism of the LEP gene

Parameter	Genotypes		
	GG	AA	AG
Duration of diabetes, years	5.42 ± 1.05	9.05 ± 2.64	5.19 ± 0.79
Age at the time of examination, years	55.71 ± 2.26	59.25 ± 2.43	51.88 ± 1.57
Age at the onset of the disease, years	50.29 ± 2.10	50.29 ± 3.13	46.85 ± 1.55
BMI, kg/m²	32.79 ± 1.79	34.83 ± 2.53	32.20 ± 0.80
Waist-to-Hip Ratio	0.96 ± 0.02	0.97 ± 0.05	0.98 ± 0.02
Systolic pressure, mmHg	144.29 ± 3.17	145.00 ± 5.00	141.71 ± 6.55
Diastolic pressure, mmHg	89.29 ± 1.70	90.00 ± 0.01	89.71 ± 4.56
Fasting glycemia, mmol/L	9.05 ± 0.64	8.73 ± 0.55	8.97 ± 0.53
HbA1c, %	6.92 ± 0.32	7.15 ± 0.36	7.12 ± 0.26
Insulin, pmol/L	130.00 ± 18.81	110.62 ± 22.07	136.59 ± 16.64
HOMA-IR, units	8.42 ± 1.29	6.41 ± 1.14	8.18 ± 1.11
QUICKI, units	0.46 ± 0.01	0.47 ± 0.02	0.47 ± 0.01
HOMA-BCF, units	83.19 ± 16.24	75.18 ± 18.13	152.06 ± 45.26
Total cholesterol mmol/L	5.71 ± 0.35	5.30 ± 0.60	5.82 ± 0.27
HDL cholesterol, mmol/L	1.08 ± 0.06	1.03 ± 0.07	1.02 ± 0.05
LDL cholesterol, mmol/L	3.56 ± 0.31	2.93 ± 0.61	3.56 ± 0.23
Triglycerides, mmol/L	2.95 ± 0.26	2.15 ± 0.38	3.67 ± 0.66
Atherogenicity index	4.55 ± 0.49	3.94 ± 0.54	5.19 ± 0.89
ß-lipoproteins, units	89.47 ± 7.05	77.83 ± 12.21	102.50 ± 11.30
FFA, mmol/L	1.28 ± 0.15	1.32 ± 0.20	1.12 ± 0.09
Adiponectin, mg/L	5.58 ± 0.59	5.50 ± 0.45	4.97 ± 0.51
Leptin, μg/L	73.54 ± 18.61	58.62 ± 16.61	61.59 ± 9.92
AST, mmol/Lh	0.72 ± 0.08	0.58 ± 0.08	0.72 ± 0.05
ALT, mmol/Lh	1.20 ± 0.16	0.92 ± 0.12	1.01 ± 0.08

Notes:
BMI — body mass index; HOMA-IR — Homeostatic Model Assessment for Insulin Resistance; QUICKI — quantitative insulin sensitivity check index; HOMA-BCF — Homeostatic Model Assessment for beta-cell function; HDL — high-density lipoprotein; LDL — low-density lipoprotein; FFA — free fatty acids; AST — aspartataminotransferase; ALT — alaninaminotransferase.

Table 2

Anthropometric, laboratory, and instrumental indicators in patients with type 2 diabetes in the presence and absence of non-alcoholic fatty liver disease in carriers of the GG genotype by the G2548A polymorphism of the LEP gene

Parameter	Type 2 DM patients with NAFLD, ($\bar{X} \pm s\bar{X}$)	Type 2 DM patients without NAFLD, ($\bar{X} \pm s\bar{X}$)	U	P
Age, years	56.50 ± 3.50	53.50 ± 4.47	5.00	0.74
Duration of diabetes, years	2.00 ± 1.00	6.33 ± 1.23	0.50	0.07
Weight, kg	112.50 ± 35.50	84.67 ± 3.72	4.00	0.50
BMI, kg/m^2	43.00 ± 13.00	29.75 ± 1.60	3.50	0.40
Waist-to-hip Ratio	0.95 ± 0.015	0.93 ± 0.005	1.00	0.16
Leptin, μg/L	159.15 ± 11.15	53.75 ± 12.80	0.00	**0.05**
Glycemia, mmol/L	10.23 ± 0.98	6.45 ± 0.05	0.00	**0.05**
HbA1c, %	5.48 ± 0.625	7.22 ± 0.64	0.50	0.07
Insulin, pmol/L	203.19	104.80 ± 12.29	0.00	1.00
HOMA-IR, units (1 measurement in the group with NAFLD)	9.02	7.32 ± 0.97	0.00	1.00
QUICKI, units (1 measurement in the group with NAFLD)	0.43	0.46 ± 0.01	0.00	1.00
Total cholesterol, mmol/L	5.72 ± 1.175	6.16 ± 0.48	4.00	0.50
HDL cholesterol, mmol/L	1.14 ± 0.165	1.09 ± 0.14	6.00	1.00
LDL cholesterol, mmol/L	3.35 ± 0.91	3.91 ± 0.37	4.00	0.51
VLDL cholesterol, mmol/L	1.09 ± 0.57	1.34 ± 0.20	4.00	0.70
Atherogenicity index	4.19 ± 1.91	5.27 ± 1.06	3.00	0.44
ß-lipoproteins, units	78.00 ± 34.00	89.50 ± 12.20	5.00	0.74
Triglycerides, mmol/L	2.47 ± 0.965	2.995 ± 0.37	5.00	0.74
FFA, mmol/L	1.06 ± 0.04	1.26 ± 0.29	6.00	1.00

Notes:
BMI — body mass index; HOMA-IR — Homeostatic Model Assessment for Insulin Resistance; QUICKI — quantitative insulin sensitivity check index; HDL — high-density lipoprotein; LDL — low-density lipoprotein; VLDL — very low-density lipoprotein; FFA — free fatty acids; U —Mann–Whitney U-test; p — level of significance when comparing groups of patients.

(*LEP*) in patients with NAFLD. In connection with this, we studied the *G2548A* polymorphism of the *LEP* gene in patients with type 2 DM complicated by NAFLD.

Measured biochemical and hormonal parameters in patients with type 2 DM, in the presence and absence of NAFLD, stratificated by genotypes according to the *G2548A* polymorphism of the *LEP* gene, are presented in Tables 1–4.

The levels of total leptin did not differ in carriers of different genotypes of the leptin gene in patients with type 2 DM regardless of liver damage (see Table 1). Taking into account the comparable degree of insufficient glycemic control and lipid profile in the above-mentioned groups of patients we can suggest the determining contribution of gluco- and lipotoxicity to the formation of hypoleptinemia, which, however, does not exclude the modulating effect of the single nucleotide polymorphism *G2548A* of the *LEP* gene on the biological effects of the hormone.

We analyzed leptin levels depending on the genotypes of the polymorphic locus *G2548A* of the *LEP* gene (Tables 2 - 4). It turned out that in patients with type 2 DM with NAFLD who are homozygous *AA* variant carriers, the leptin

Table 3

Anthropometric, laboratory and instrumental indicators in patients with type 2 diabetes in the presence and absence of non-alcoholic fatty liver disease in carriers of the AA genotype by the G2548A polymorphism of the LEP gene

Parameter	Type 2 DM patients with NAFLD, ($\bar{X} \pm s\bar{X}$)	Type 2 DM patients without NAFLD, ($\bar{X} \pm s\bar{X}$)	U	P
Age, years	55.50 ± 9.50	61.00 ± 1.47	4.00	1.00
Duration of diabetes, years	3.67 ± 3.34	11.75 ± 3.90	1.00	0.16
Weight, kg (1 measurement in the group with NAFLD)	122.00	78.67 ± 10.93	0.00	1.00
BMI, kg/m^2	38.50 ± 2.50	33.00 ± 3.40	2.00	0.35
Waist-to-hip Ratio	1.05 ± 0.05	0.91 ± 0.06	1.00	0.25
Leptin, μg/L	69.40 ± 45.10	53.23 ± 17.94	3.00	0.64
Glycemia, mmol/L	8.71 ± 2.13	8.47 ± 0.42	4.00	1.00
HbA1c, %	7.26 ± 0.92	6.87 ± 0.47	3.00	0.64
Insulin, pmol/L	86.86 ± 23.40	131.89 ± 35.38	2.00	0.35
HOMA-IR, units	4.90 ± 0.13	7.52 ± 1.88	2.00	0.35
QUICKI, units	0.49 ± 0.002	0.46 ± 0.03	2.00	0.35
Total cholesterol, mmol/L	5.91 ± 1.09	4.31 ± 0.22	0.00	0.06
HDL cholesterol, mmol/L	1.06 ± 0.06	1.01 ± 0.16	2.00	1.00
LDL cholesterol, mmol/L	3.43 ± 1.29	2.43 ± 0.31	1.00	0.44
VLDL cholesterol, mmol/L	1.42 ± 0.26	0.80 ± 0.47	1.00	0.44
Atherogenicity index	4.56 ± 0.74	3.32 ± 0.68	1.00	0.44
β-lipoproteins, units (1 measurement in the group with NAFLD)	88.00	64.75 ± 12.61	0.00	1.00
Triglycerides, mmol/L	3.16 ± 0.59	1.63 ± 0.45	1.00	0.16
FFA, mmol/L	1.33 ± 0.075	1.39 ± 0.37	4.00	1.00

Notes:
BMI — body mass index; HOMA-IR — Homeostatic Model Assessment for Insulin Resistance; QUICKI — quantitative insulin sensitivity check index; HDL — high-density lipoprotein; LDL — low-density lipoprotein; VLDL — very low-density lipoprotein; FFA — free fatty acids; U — Mann–Whitney U-test; p — level of significance when comparing groups of patients.

plasma level is probably lower ($p < 0.05$) than when the *G* allele is present in the homozygous ($p < 0.01$) or heterozygous variants ($p > 0.05$). It should be noted that we observed the highest level of leptin in carriers of the *GG* genotype of the *G2548A* polymorphic locus of the *LEP* gene ($p < 0.001$). At the same time, the leptin content in the circulation did not differ in the group without liver damage. Thus, it can be assumed that the *G* allele is associated with increased levels of leptin in the blood of type 2 DM patients with NAFLD.

In our study, in *A* allele carriers of the *G2548A* polymorphic variant of the *LEP* gene type 2 DM patients with NAFLD lower leptin levels were determined in comparison with *GG* genotype carriers of the *G2548A* polymorphic variant of the *LEP* gene, which is consistent with the results of the study by Samir Ben Ali et al. (2009), conducted on obese patients [32]. Similar data were obtained by Constantin A. et al. (2010), who performed an analysis of association with quantitative signs of the metabolic syndrome and showed that homozygous carriers of the *LEP -2548G* allele had significantly higher leptin levels (17.2 ± 6.6 vs. 13.2 ± 4.9 μg/L, $p = 0.011$) [33]. While the study of Badnaran Dagdan et al. (2018), conducted

Table 4

Anthropometric, laboratory, and instrumental indicators in patients with type 2 diabetes in the presence and absence of non-alcoholic fatty liver disease in carriers of the AG genotype by the G2548A polymorphism of the LEP gene

Parameter	Type 2 DM patients with NAFLD, ($\bar{X} \pm s\bar{X}$)	Type 2 DM patients without NAFLD, ($\bar{X} \pm s\bar{X}$)	U	P
Age, years	52.00 ± 1.98	52.00 ± 3.49	38.00	0.57
Duration of diabetes, years	6.90 ± 1.06	4.22 ± 1.87	22.00	0.06
Weight, kg	104.39 ± 6.71	90.94 ± 5.61	20.50	0.08
BMI, kg/m^2	34.68 ± 1.69	30.00 ± 1.19	19.50	0.06
Waist-to-hip Ratio	1.04 ± 0.03	0.98 ± 0.01	21.50	0.29
Leptin, μg/L	74.69 ± 17.81	56.69 ± 9.67	38.00	0.57
Glycemia, mmol/L	10.21 ± 1.01	8.54 ± 1.24	29.50	0.21
HbA1c, %	7.44 ± 0.39	7.19 ± 0.55	32.00	0.70
Insulin, pmol/L	163.13 ± 34.74	159.32 ± 49.82	36.00	0.46
HOMA-IR, units	12.27 ± 3.16	7.67 ± 1.96	28.00	0.17
QUICKI, units	0.44 ± 0.02	0.48 ± 0.03	28.00	0.17
Total cholesterol, mmol/L	5.74 ± 0.51	5.75 ± 0.59	44.00	0.93
HDL cholesterol, mmol/L	1.04 ± 0.15	1.06 ± 0.06	21.00	1.00
LDL cholesterol, mmol/L	4.08 ± 0.61	3.42 ± 0.32	10.00	0.45
VLDL cholesterol, mmol/L	1.27 ± 0.17	1.00 ± 0.18	5.00	0.21
Atherogenicity index	7.68 ± 3.11	4.10 ± 0.39	12.00	0.37
ß-lipoproteins, units	118.50 ± 21.56	79.22 ± 10.79	22.50	0.19
Triglycerides, mmol/L	4.68 ± 1.09	2.00 ± 0.24	17.00	**0.02**
FFA, mmol/L	1.10 ± 0.13	0.96 ± 0.15	37.00	0.51

Notes:
BMI — body mass index; HOMA-IR — Homeostatic Model Assessment for Insulin Resistance; QUICKI — quantitative insulin sensitivity check index; HDL — high-density lipoprotein; LDL — low-density lipoprotein; VLDL — very low-density lipoprotein; FFA — free fatty acids; U — Mann–Whitney U-test; p — level of significance when comparing groups of patients.

on a Mongolian population with metabolic syndrome, have completely opposite results, according to which carriers of the *A* allele of the *G2548A* polymorphism of the *LEP* gene have an increased concentration of leptin in the circulation [34].

There are other data on the association of the SNP of the *LEP* gene: *-2548 (2453) G > A* with increased leptin levels and the risk of obesity. According to Hoffstedt et al. (2002), *AA* genotype carriers had higher serum leptin levels than *GA/GG* genotype carriers. The secretion of leptin by adipose tissue with the *AA* genotype increased 2 times, and the level of leptin mRNA was 60% higher. Thus, the polymorphism (*-2548G > A*) of the leptin gene at the transcriptional level affects leptin expression [35]. The EPIC-Heidelberg study (2002) revealed the role of the homozygous genotype of the *LEP*: *-2548 AA* gene in the development of obesity. The polymorphism correlated with the level of serum leptin and leptin mRNA [36].

Differences between fasting insulin, HOMA-IR, QUICKI and other biochemical indicators among genotypes for the *G2548A* polymorphism of the *LEP* gene were insignificant in our study (p > 0.05), which correlates with the results obtained by Nawfal Hussein Amhe et al. (2022) [37]. Whereas, Essa M. Sabi et al. (2022), showed, that participants with minor *AA* genotype had significantly higher blood glucose levels (6.8 ± 0.55 vs. 5.8 ± 0.30 mmol/L;

p < 0.04) and HOMA-IR (4.1 ± 0.84 vs. 2.6 ± 0.67; p = 0.03) against those carrying major *GG* genotype. Participants with heterozygous *GA* genotype had significantly higher serum leptin levels against those carrying major *GG* genotype (40.0 ± 2.6 vs. 29.6 ± 2.6 μg/L; p = 0.04) [38].

In our study, there was no difference in BMI between men and women carriers of different genotypes for the *G-2458A* polymorphic variant of the *LEP* gene. Although the literature data on the relationship between this polymorphism and the the metabolic syndrome development are ambiguous.

The association of the *LEP G-2548A* polymorphism and increased BMI was found in overweight Europeans [39] and in Taiwanese aborigines with extreme obesity [40]. In addition, common variants located in the 5 region of the *LEP* gene, including *G-2548A*, have been associated with increased BMI in men [41]. Mei Yang et al. (2016) analyzed the characteristics of metabolic indicators according to the genotypes of the polymorphic variant *G2548A* of the *LEP* gene. A significant difference was shown between the *LEP G2548A* polymorphism and BMI (F = 4.48, P = 0.01). And the *2548GA* genotype had a significantly higher BMI than the *2548GG* (21.17 ± 3.05 vs. 20.15 ± 2.54 kg/m^2), although the BMI between the *2548AA* and *2548GG* genotypes was similar [42]. On the other hand, other studies failed to demonstrate an association between these polymorphisms and obesity or increased BMI [43, 44].

According to Hamilton M. Hinuy et al. (2008), analysis of the *LEP G-2458A* polymorphism showed that women carrying the *–2548G* allele (*GG* genotype) had a four-fold increased risk of obesity (OR: 4.11, 95 % CI: 1.06-15.90), than carriers of *–2548A* alleles (p = 0.041) [45].

Our study showed that women carriers of the *GG* genotype with the *G-2458A* polymorphic variant of the *LEP* gene have a 3.4 times higher leptin level than men carriers of the same genotype (p < 0.03). An association between the *LEP-2548G* allele and elevated plasma leptin concentrations found in our study corresponds to data of Le Stunff C. (2000), who has also described the same phenomenon in obese and diabetic individuals from European and Asian populations [46, 47]. This allele was associated with plasma leptin levels by interacting with obesity and gender in healthy individuals from Greece [48]. On the other hand, the *-2458AA* genotype was associated with increased plasma leptin in obese subjects and in French men [49]. Menezes C. A. et al. (2022) found that patients with leptin *2458AA* and *2458AG* genotypes of *LEP* gene had higher serum leptin concentrations when compared to the *GG* haplotype [50].

These inconsistencies in results may arise from different genetic origins or environmental conditions of the studied populations, sample population, various criteria for diagnosing patients, different target populations, and different sampling time. Also, different results may be due to the interaction of the *G-2548A* polymorphism with other leptin and/or leptin receptor gene variants, as well as other variables such as sex, sample size, and population or the model used in the genetic analyses.

CONCLUSIONS

1. An average leptin levels are elevated in patients with NAFLD. In patients with type 2 diabetes complicated by NAFLD, the level of leptin is 84.73 μg/L against 52.57 μg/L in the group without complications.
2. The levels of total leptin did not differ in carriers of different genotypes of the leptin gene in patients with type 2 diabetes regardless of liver damage. According to our data, a statistically significant contribution of the single nucleotide polymorphism *G-2548A* of the *LEP* gene to the formation of the predisposition to the development of type 2 diabetes mellitus has not been determined.
3. In our study in *GG* carriers genotype of the *G2548A LEP* gene polymorphic locus type 2 diabetes patients with NAFLD the highest level of leptin was observed (159.15 μg/L), compared to other genotypes. At the same time, the circulation leptin levels in the group without liver damage did not differ for all genotypes. Thus, it can be assumed that the G allele is associated with increased leptin levels in the blood of patients with NAFLD.
4. Our study showed that women with type 2 diabetes mellitus carrying the *GG* genotype with the *G-2458A* polymorphic variant of the *LEP* gene have 3.4 times higher leptin

levels than men carrying the same genotype ($p < 0.03$).

5. Therefore, it is necessary to continue work to identify the functional role of the studied *LEP* gene polymorphism and its possible associations with indicators of hormonal and metabolic components of insulin resistance in the development of non-alcoholic fatty liver disease in the presence of type 2 diabetes under the conditions of increasing the sample.
6. Prospects for further research should also be based on the study of the role of other candidate genes in the development of NAFLD and their relationship with various metabolic parameters, which will allow clarifying the role of genes in the formation of NAFLD in the Ukrainian population.

REFERENCES

1. Pouwels S, Sakran N, Graham Y, et al. *BMC Endocr Disord* 2022;22(1): 63.
2. Vernon G, Baranova A, Younossi ZM. *Aliment Pharmacol Ther* 2011;34: 274-285.
3. Baltieri L, Chaim EA, Chaim FM. *Arq Gastroenterol* 2018;55: 247-251.
4. Schuppan D, Surabattula R, Wang XY. *J Hepatol* 2018; 68: 238-250.
5. Wong T, Wong RJ, Gish RG. *Gastroenterol Hepatol (NY)* 2019;15(2): 83-89.
6. Marchisello S, Di Pino A, Scicali R, et al. *Int J Mol Sci* 2019;20(8): 1948.
7. Yu J, Marsh S, Hu J, Feng W, Wu C. *Gastroenterol Res Pract.* 2016;2016:2862173.
8. Greenberg AS, Obin MS. *Am J Clin Nutr* 2006;83(2): 461S-465S.
9. Estep JM, Baranova A, Hossain N, et al. *Obes Surg* 2009;19: 617-624.
10. Moon HS, Dalamaga M, Kim SY, et al. *Endocr Rev* 2013;34: 377-412.
11. Wryblewski A, Strycharz J, Srwiderska E. *Nutrients* 2019;11: 1872.
12. Lee S, Kuk JL, Boesch C, Arslanian S. *Appl Physiol Nutr Metab* 2017;42(8): 829-833.
13. Maskarinec G, Garber AK, Wong MC, et al. *Obes Sci Pract* 2021;7: 53-62.
14. Mammes O, Betoulle D, Aubert R, et al. *Diabetes* 1998; 47: 487-489.
15. Ortega-Azorín C, Coltell O, Asensio EM, et al. *Nutrients* 2019;11: 2751.
16. Sabi EM, Bin Dahman LS, Mohammed AK, et al. *Medicina (Kaunas)* 2022;58(3): 346.
17. Sharma R, Agrawal S, Saxena A, et al. *Genes Nutr* 2013;8(6): 611-621.
18. Zayani N, Omezzine A, Boumaiza I, et al. *J Clin Lab Anal* 2017;31(6).
19. Alnry A, Gad H, Hegazy G, Shaker O. *Turk J Med Sci* 2016;46(5): 1335-1340.
20. Manriquez V, Aviles J, Salazar L, et al. *Mol Diagn Ther* 2018;22(1): 101-113.
21. Crescenti A, Sola R, Valls RM, et al. *Genes Nutr* 2013; 8(1): 127-136.
22. Gregoor JG, van der Weide J, Loovers HM, et al. *Psychiatr Genet* 2010;20(6): 311-316.
23. Szanto KB, Li J, Cordero P, Oben JA. *Diab Metab Syndr Obes* 2019;12: 357-367.
24. Chalasani N, Younossi Z, Lavine JE, et al. *Hepatology* 2012;55(6): 2005-2023.
25. Armitage P, Berry G. Statistical methods in medical research. 4rd ed., *Blackwell Scientific Publications*, 2001: 826 p.
26. Chen Z, Yu R, Xiong Y, et al. *Lipids Health Dis* 2017; 16(1): 203.
27. Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. *Ann Intern Med* 2010;152: 93-100.
28. Moon HS, Dalamaga M, Kim SY, et al. Endocr Rev 2013;34: 377-412.
29. Mantzoros CS, Magkos F, Brinkoetter M, et al. *Am J Physiol Endocrinol Metab* 2011;301: E567-E584.
30. Dalamaga M, Chou SH, Shields K, et al. *Cell Metab* 2013;18: 29-42.
31. Rotundo L, Persaud A, Feurdean M, et al. *Clin Mol Hepatol* 2018;24(4): 392-401.
32. Ben Ali S, Kallel A, Ftouhi B, et al. *Clin Biochem* 2009;42(7-8): 584-588.
33. Constantin A. et al. *Biochem Biophys Res Commun* 2010;391: 282-286.
34. Dagdan B, Chuluun -Erdene A, Sengeragchaa O, et al. *Med Sci (Basel)* 2018;7(1): E3.
35. Hoffstedt J, Eriksson P, Mottagui-Tabar S, Arner P. *Horm Metab Res* 2002;34(7): 355-359.
36. Nieters A, Becker N, Linseisen J. *Eur J Nutr* 2002;41(5): 210-221.
37. Nawfal Hussein Amhe, Fawzi Hassan Zayr Al-Fahdawi, Ali Gheisarzadeh, A.A Hatamnia *PJMHS* 2022;16 (04): 927-929
38. Sabi EM, Bin Dahman LS, Mohammed AK, et al. *Medicina* 2022;58: 346.
39. Mammes O, Betoulle D, Aubert R, et al. *Ann Hum Genet* 2000;64: 391-394.

40. Wang TN, Huang MC, Chang WT, et al. *Obesity (Silver Spring)* 2006;14: 183-187.

41. Jiang Y, Wilk JB, Borecki I, et al. *Am J Hum Genet* 2004;75: 220-230.

42. Yang M, Peng S, Li W, et al. *Scientific Reports* 2016;6: 23948.

43. Mattevi VS, Zembrzuski VM, Hutz MH. *Int J Obes Rel Metab Res* 2002;26: 1179-1185.

44. Portoles O, Sorli JV, Frances F, et al. *Eur J Epidemiol* 2006;21: 605-612.

45. Hinuy HM, Hirata MH, Forti N, et al. *Arq Bras Endocrinol Metab* 2008;52(4): 1-6.

46. Le Stunff C, Le Bihan C, Schork NJ, Bougneres P. *Diabetes* 2000;49: 2196-2200.

47. Ren W, Zhang SH, Wu J, Ni YX. *Chin Med J (Engl)* 2004;117: 558-561.

48. Yiannakouris N, Melistas L, Yannakoulia M, et al. *Hormones (Athens)* 2003;2: 229-236.

49. Mammes O, Betoulle D, Aubert R, et al. *Ann Hum Genet* 2000;64: 391-394.

50. Menezes CA, Alves Junior ER, Costa GNO, et al. *Rev Paul Pediatr* 2022;40: e2021030.

INVESTIGATION OF 2548G > A LEPTINE GENE POLYMORPHIC VARIANT IMPACT ON RISK OF NONALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

T. V. Tyzhnenko [1,2], K. V. Misyura [1], N. O. Kravchun [3], M. Yu. Gorshunska [4], A. K. Pochernyaev [1,5], N. S. Krasova [1], A. I. Gladkih [1], Z. A. Leshchenko [1], G. V. Fedorova [6], O. O. Plohotnichenko [1], O. B. Hromakovska [1], A. O. Kolesnikova [1], E. Jansen [7], Yu. I. Karachentsev [1,8], V. V. Poltorak [1]

[1] SI «V. Danilevsky Institute for Endocrine Pathology Problems of the NAMS of Ukraine», Kharkiv, Ukraine;
[2] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine;
[3] Multidisciplinary medical center «Life Park», Kharkiv, Ukraine;
[4] Kharkiv Medical Academy of postgraduate education of the Ministry of health of Ukraine, Kharkiv, Ukraine;
[5] Poltava Scientific Research Forensic Center of the Ministry of Internal Affairs of Ukraine, Poltava, Ukraine;
[6] University of South Bohemia in České Budějovice, Vodňany, Czech Republic;
[7] National Institute for Public Health and the Environment, Bilthoven, The Netherlands;
[8] Kharkiv National Medical University, Kharkiv, Ukraine
tyzhnenko@ukr.net

Background. It is known that single nucleotide polymorphisms (SNPs) in adipokine genes can influence the development of pathological conditions associated with obesity, type 2 diabetes mellitus (T2D), non-alcoholic fatty liver disease (NAFLD) and their complications. In this study, we **aimed** to investigate the link between common *-2548G > A* (*rs7799039*) promoter variant of the human leptin gene (*LEP*) with leptin levels in type 2 diabetes patients with non-alcoholic fatty liver diseasese.

Materials and methods. 61 patients with T2D aged from 28 to 80 years old (34 men / 27 women, age 56.40 ± 0.62 yrs, diabetes duration 7.72 ± 0.45 yrs, BMI 32.20 ± 0.43 kg/m^2, WHR 1,00 ± 0,01, HbA_{1c} 7.80 ± 0.19 %) with varying degrees of glycemic control and overweight, without renal insufficiency and 51 sex and age-match control subjects were examined. Genotyping according to SNP *LEP 2548G > A* was performed using the polymerase chain reaction method with appropriate primers and HhaI endonuclease.

Results. In our study of T2DM patients with NAFLD compared to T2D patients without NAFLD features of dyslipidemia i.e. significant increase in triglycerides ($p < 0,001$), LDL cholesterol ($p < 0,1$), lower HDL cholesterol ($p < 0.001$) were found. Stratification of the diabetic patients in the presence and absence of NAFLD showed more pronounced increase in circulating leptin levels in the presence of NAFLD (84.73 ± 13.80 vs. 52.57 ± 6.86 μg/L, respectively), ($p < 0.01$), which justifies the feasibility of using this indicator for further needs as a diagnostic parameter of the above complication. In our study in *GG* carriers genotype of the *G2548A LEP* gene polymorphic locus type 2 diabetes patients with NAFLD the highest level of leptin was observed (159.15 μg/L), compared to other genotypes. Thus, it can be assumed that the *G* allele is associated with increased leptin levels in the blood of patients with NAFLD. This study showed that women with type 2 diabetes mellitus carrying the *GG* genotype with the *G-2458A* polymorphic variant of the LEP gene have 3.4 times higher leptin levels than men carrying the same genotype ($p < 0.03$).

Conclusions. The data obtained regarding the *2548G > A* polymorphic variant of the LEP gene can be used as a basis for personalized prevention and the formation of risk groups for the development of NAFLD.

Keywords: type 2 diabetes mellitus, non-alcoholic fatty liver disease, leptin, single nucleotide polymorphisms.

ДОСЛІДЖЕННЯ ВПЛИВУ ПОЛІМОРФНОГО ВАРІАНТУ 2548G > A ГЕНА ЛЕПТИНУ НА РИЗИК РОЗВИТКУ НЕАЛКОГОЛЬНОЇ ЖИРОВОЇ ХВОРОБИ ПЕЧІНКИ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 2 ТИПУ

Тижненко Т. В.[1,2], Місюра К. В.[1], Кравчун Н. О.[3], Горшунська М. Ю.[4], Почерняєв А. К.[1,5], Красова Н. С.[1], Гладких О. І.[1], Лещенко Ж. А.[1], Федорова Г. В.[6], Плохотніченко О. О.[1], Громаковська О. Б.[1], Колеснікова А. О.[1], Йенсен Е.[7], Караченцев Ю. І.[1,4], Полторак В. В.[1]

[1]ДУ «Інститут проблем ендокринної патології ім. В.Я. Данилевського НАМН України»; м. Харків, Україна;
[2]Харківський національний університет імені В.Н. Каразіна, м. Харків, Україна;
[3]Багатопрофільний медичний центр «Life Park», м. Харків, Україна;
[4]Харківська медична академія післядипломної освіти МОЗ України, м. Харків, Україна;
[5]Полтавський науково-дослідний експертно-криміналістичний центр, м. Полтава, Україна;
[6]Університет Південної Богемії в Ческе-Будейовіце, м. Водняни, Чеська Республіка;
[7]Національний інститут громадського здоров'я та навколишнього середовища, м. Білтховен, Нідерланди;
[8]Харківський національний медичний університет, м. Харків, Україна
tyzhnenko@ukr.net

Вступ. Відомо, що однонуклеотидні поліморфізми (SNP) у генах адипокінів можуть впливати на розвиток патологічних станів, пов'язаних із ожирінням, цукровим діабетом (ЦД) 2 типу, неалкогольною жировою хворобою печінки (НАЖХП) та їх ускладненнями. У цьому дослідженні ми мали на **меті** дослідити зв'язок між варіантом промотора *-2548G > A* (*rs7799039*) гена лептину людини (*LEP*) з рівнями лептину у пацієнтів з цукровим діабетом 2 типу та неалкогольною жировою хворобою печінки.

Матеріали та методи. Було обстежено 61 пацієнта із ЦД 2 типу віком від 28 до 80 років (34 чоловіки / 27 жінок, вік 56,40 ± 0,62 року, тривалість діабету 7,72 ± 0,45 року, ІМТ 32,20 ± 0,43 кг/м^2, ОТ/ОБ 1,00 ± 0,01, HbA1c 7,80 ± 0,19 %) з різним ступенем глікемічного контролю та надмірною вагою, без ниркової недостатності. 51 практично здорову людину розглядали в якості відповідної статево-вікової контрольної групи. Генотипування за SNP *LEP 2548G > A* проводили методом полімеразної ланцюгової реакції з відповідними праймерами та ендонуклеазою HhaI.

Результати. У нашому дослідженні у хворих на ЦД 2 типу з НАЖХП порівняно з хворими на ЦД 2 типу без НАЖХП було виявлено ознаки дисліпідемії, а саме значуще підвищення рівня тригліцеридів ($p < 0,001$), холестерину ЛПНЩ ($p < 0,1$), зниження рівня холестерину ЛПВЩ ($p < 0,001$). Стратифікація хворих на цукровий діабет за наявності та відсутності НАЖХП показала більш виражене підвищення рівня циркулюючого лептину за наявності НАЖХП (84,73 ± 13,80 проти 52,57 ± 6,86 мкг/л відповідно), ($p < 0,01$), що підтверджує доцільність використання даного показника як діагностичного параметра вищевказаного ускладнення. У нашому дослідженні у хворих на ЦД 2 типу з НАЖХП у носіїв генотипу *GG* поліморфного локусу гена *G2548A LEP* спостерігався найвищий рівень лептину (159,15 мкг/л), порівняно з іншими генотипами. Таким чином, можна припустити, що алель G асоціюється з підвищенням рівня лептину в крові пацієнтів з НАЖХП. Це дослідження показало, що жінки з цукровим діабетом 2 типу з генотипом *GG* за поліморфним варіантом *G-2458A* гена *LEP* мають у 3,4 рази вищий рівень лептину, ніж чоловіки з таким же генотипом ($p < 0,03$).

Висновки. Отримані дані щодо поліморфного варіанту *2548G > A* гена LEP можуть бути покладені в основу персоналізованої профілактики та формування груп ризику щодо розвитку НАЖХП.

Ключові слова: цукровий діабет 2 типу, неалкогольна жирова хвороба печінки, лептин, однонуклеотидний поліморфізм.