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Adiponectin gene single-nucleotide polymorphisms in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease

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Abstract. ***Background.*** *It is generally believed that environmental and genetic factors interact with the formation of nonalcoholic fatty liver disease (NAFLD) phenotype and determine its progression. Both NAFLD and type 2 diabetes (T2D) are heterogeneous diseases with common pathogenic pathways. Adiponectin is an adipokine, which increases the sensitivity of hepatocytes and muscle to insulin, modulates energy homeostasis, glucose/lipid metabolism, and inflammatory response. A number of significant adiponectin gene polymorphisms are known in this area. The purpose of the study was to evaluate the possible association between two adiponectin gene (ADIPOQ) variants, +276 G/T (rs1501299) and –11391 G/A (rs17300539), and susceptibility to NAFLD in T2D patients of Ukrainian population.* ***Materials and methods.*** *Case-control study included a total of 155 persons with T2D (males/females: 77/78, age 54.55 ± 0.73 years, T2D duration 6.66 ± 0.49 years, body mass index 32.20 ± 0.43 kg/m², waist/hip circumference 0.98 ± 0.01 m, HbA_{1c} 7.26 ± 0.11 %) for biochemical characteristics (lipid profile, non-esterified fatty acids (NEFA), insulin, total adiponectin, etc.), including 90 T2D patients with NAFLD, 245 — with rs1501299 genotyping, 155 — with rs17300539 genotyping, and 51 sex and age-matched control subjects. The +276 G/T and –11391 G/A were determined by polymerase chain reaction — restriction fragment length polymorphism method with endonucleases Mva1269I (BsmI) and MspI (HpaII). Insulin resistance (IR) was assessed using homeostasis model assessment (HOMA) algorithm and as adipose IR (Adipo-IR, NEFA×insulin). Unpaired Student's t test, χ^2 test and Spearman's rank order were used. To predict the probabilities of genetic risk in NAFLD, the odds ratio (OR) and 95% confidence interval (CI) were calculated.* ***Results.*** *T2D patients were characterized by overweight and obesity, which were more significant in the presence of NAFLD ($p < 0.01$). It was accompanied by an increase in HOMA-IR ($p < 0.05$) and triglycerides ($p < 0.001$) levels. We found that Adipo-IR was higher in patients with T2D as compared to the controls ($p < 0.001$), and this index was significantly increased in T2D patients with NAFLD in contrast to obesity-matched persons without NAFLD (190.18 ± 22.15 vs 133.32 ± 13.58 mmol/L·pmol/L, $p < 0.02$), with negative correlation between Adipo-IR and adiponectin level in T2D patients with NAFLD only ($r_s = –0.350$, $p = 0.021$). Stratification of non-NAFLD patients by +276G/T genotype suggests the prevalence of GT- and TT-genotypes. Thus, the rs1501299 G-allele increased the risk of NAFLD in comparison with T-allele (OR = 4.44, 95% CI = 2.89–6.81, $p < 0.05$). We also found a significant difference in the frequency of –11391G/A between T2D and control groups, but not between the patients with and without NAFLD. We observed that the haplotype of GT/GG had been more common in T2D with NAFLD, and twice less often detected in patients without hepatic disease (33 and 16.49 %, respectively, $p < 0.05$).*
Conclusions. *We can recommend Adipo-IR index as a predictive marker for the NAFLD development and the indicator for therapy success in T2D patients. We established new genetic markers (rs1501299 G-allele, rs17300539 and rs1501299 GG/GG and GT/GG haplotypes, respectively) for the risk of NAFLD development in T2D patients.*

Keywords: *adiponectin gene; single nucleotide polymorphism; genotype frequencies; type 2 diabetes mellitus; nonalcoholic fatty liver disease; insulin resistance*

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is closely related with visceral obesity and insulin resistance (IR) [1], which are early and powerful determinants of type 2 diabetes mellitus (T2D). Although NAFLD is not one of the defining criteria for metabolic (IR) syndrome, it is a common hepatic manifestation [2]. On the other hand, L. Amedeo et al. (2015) are thought, that the conventional paradigm of NAFLD representing the "hepatic manifestation of the metabolic syndrome" is outdated [1]. However, NAFLD is usually associated with obesity or IR and thus can be regarded as sign of the metabolic syndrome. It should be looked for in patients with obesity, diabetes or lipid disturbances [3]. Dyslipidemia, IR, increased production of proinflammatory cytokines, low adiponectin, high PAI-1 levels, hypertension, and hyperglycemia are main factors that lead to NAFLD, further aggravate the course of NAFLD, and accelerate the progress of atherosclerosis and the development of cardiovascular disease (CVD) [4]. The high prevalence of T2D, about 2 % of the world's population [5], the specificity of clinical manifestations and the life quality of these patients make the research of this disease and its complications especially urgent. On the other hand, obesity, hyperglycemia, T2D and hypertriglyceridemia are known as risk factors for the NAFLD development [6–8]. It is generally believed that environmental and genetic factors interact to produce NAFLD phenotype and determine its progression [9]. Both NAFLD and T2D are heterogeneous diseases. Common pathways involved in the pathogenesis of NAFLD and T2D include IR, atherogenic dyslipidemia, subclinical inflammation, oxidative stress, CVD, chronic kidney disease, and obesity [10].

Recent studies have pointed role of adipose tissue as an active endocrine organ that can secrete a number of specific adipokines (including chemokines, cytokines and hormones) which can affect energy balance and endocrine mechanisms responsible for regulating appetite, lipid metabolism, blood pressure, insulin sensitivity, and systemic inflammation. An important role in this system plays adiponectin [5]. Adiponectin is an adipose tissue-specific plasma protein produced in growing adipocytes, prevents the formation of IR syndrome, increases the sensitivity of hepatocytes and muscle to insulin, possesses a protective effect on the vascular wall, modulates insulin sensitivity, energy homeostasis, glucose and lipid metabolism, and anti-inflammatory responses in the vascular system [11, 12]. However, as soon as fat tissue increases in volume, adiponectin concentration is reduced [13], and the low level of this hormone is combined with the further development of obesity-related disturbances [14].

Adiponectin shows protective properties in alcoholic and nonalcoholic fatty liver disease [15]. In liver, adiponectin attenuates IR by increasing of insulin sensitivity [16]. At present time, however, the molecular mechanisms underlying the development and the progression of NAFLD are poorly understood. To clarify the nature of the link between the gene and metabolic state of the organism, *ADIPOQ* SNPs are investigated, among others [17]. Adiponectin is encoded by *ARM1* (*ADIPOQ*, *ACDC*) gene, located on the long arm of chromosome 3 locus at 3q27 [18, 19], which covers 16 kb and contains three exons and two introns. The results of genome wide study of the locus 3q27 identified its relationship with susceptibility to diabetes [20, 21]: it is associated with T2D and metabolic syndrome in Japanese [21], USA [22], French (Caucasoids) [19] and Italian [23] populations. We have previously identified association of adiponectin gene (*ADIPOQ*) SNP +276 G/T and paraoxonase-1 gene (*PON-1*) SNP Q192R with the risk of T2D development in Ukrainian population [24, 25].

The purpose of the present study was to evaluate the possible association between two adiponectin gene variants, +276 G/T (rs1501299) and –11391 G/A (rs17300539), and susceptibility to NAFLD in type 2 diabetic subjects of Ukrainian population.

Materials and methods

Case-control study included a total of 155 patients with a diagnosis of T2D (M/F: 77/78, age 54.55 ± 0.73, T2D duration 6.66 ± 0.49 yrs, body mass index (BMI) 32.20 ± 0.43 kg/m^2, waist-to-hip ratio (WHR) 0.98 ± 0.01, HbA_{1c} 7.26 ± 0.11 %) including 90 T2Ds with NAFLD and 51 sex and age-match control subjects (C) for biochemical characteristics, 245 T2Ds and 103 C for rs1501299 genotyping, 155 T2Ds and 51 C for rs17300539 genotyping. The data were collected through a standard questionnaire. All patients were interviewed regarding a full medical history that included 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. Controls were individuals with no clinically significant abnormal physical findings. None of the controls had any personal history of diabetes at the time of blood donation, which was ascertained with a questionnaire completed by each healthy volunteer. All cases and controls signed an informed consent for clinical, biochemical and genetic studies and the protocols were approved by the institutional review board of SI "V. Danilevsky Institute of Endocrine Pathology Problems of NAMS of Ukraine".

Cases and controls from study were all Caucasoids and residents of Kharkiv region (Ukraine). The cases were clinically and biochemically confirmed as T2D. The diagnosis NAFLD was verified in accordance with the recommendations of the American Gastroenterological Association (AGA) and the American Association for the Study of liver disease based on the clinical course of the disease, lipid and carbohydrate metabolism, activity of alaninaminotransferase (ALT), aspartataminotransferase (AST), ratio ALT/AST and sonographic examination [26]. Nutritional status was assessed by measuring weight, height and abdominal circumference using well established techniques.

In all the *ADIPOQ* SNP +276 G/T (rs1501299) and *ADIPOQ* SNP –11391 G/A (rs17300539) were determined by polymerase chain reaction (PCR) — restriction fragment length polymorphism method (RFLP) with endonucleases *Mva1269I* (*BsmI*) and *MspI (HpaII*). Primers for *ADIPOQ* SNP +276G/T were as follows: Forward (ADIPOQ276F GGCCTCTTTCATCACAGACC)

and Reverse (ADIPOQ276R AGATGCAGCAAAGCCAAAGT). Primers for *ADIPOQ* SNP –11391G/A were as follows: Forward GTTGGTGCTGGCATCCTAAG and Reverse GCCTGGAGAACTGGAAGCTG. Each reaction was verified on 2 % agarose gel. As molecular weight marker was used DNA pUC19, hydrolyzed by endonuclease *MspI* [27].

Serum lipid metabolism parameters (triglycerides (TG), non-esterified fatty acids (NEFA), total and lipoprotein profile cholesterol) and ALT/AST were measured spectrophotometrically. There were estimated plasma total adiponectin (Biovendor, Chezh Republic) and insulin (DRG, Germany) by ELISA. IR was assessed using homeostasis model assessment (HOMA-IR) algorithm [28] and as adipose IR (Adipo-IR, NEFA × insulin) [29]. Unpaired Student's t test, χ^2-test and Spearman's rank order were used. Data are expressed as mean ± SEM. To predict the probabilities of genetic risk in NAFLD the odds ratio (OR) and 95% confidence interval (CI) were calculated [30].

Results

Comparing with control subjects T2D patients were characterized by overweight and obesity, which were more pronounced at the presence of NAFLD ($p < 0.01$). It was accompanied by more pronounced increase in HOMA-IR ($p < 0.05$), TG ($p < 0.001$), and LDL ($p < 0.1$) levels. The adiponectin level in women with T2D and NAFLD was 5.92 ± 0.66 mg/ml (10.3–14.5 mg/ml in controls), and in men it was 4.71 ± 0.54 mg/ml (7.0–10.5 mg/ml in controls); non-NAFLD women with T2D had 4.32 ± 0.50 mg/ml, and male — 4.09 ± 0.51 mg/ml. In both groups, adiponectin levels were significantly lower ($p < 0.001$) than in control individuals (15.23 ± 0.63 mg/ml).

We found, that Adipo-IR was higher in T2D (164.32 ± 13.23 mmol/L·pmol/L) as compared to controls (62.35 ± 9.36 mmol/L·pmol/L, $p < 0.001$), and this index was significantly increased in T2D patients with NAFLD as compared to non-NAFLD diabetic subjects (190.18 ± 22.15 *vs* 133.32 ± 13.58 mmol/L·pmol/L, $p < 0.02$). Interestingly, there was a correlation between Adipo-IR and adiponectin level in T2D patients with NAFLD only ($r_s = -0.350$, $p = 0.021$; $r_s = 0.051$, $p = 0.762$ in non-NAFLD diabetics).

The genotype and allele frequencies of *ADIPOQ* SNP +276G/T and –11391G/A are shown in Table 1. For SNP +276G/T 155 patients with T2D without NAFLD and 90 T2D patients with NAFLD were genotyped. Stratification of non-NAFLD patients by +276G/T genotype suggests the prevalence of heterozygotes GT and homozygotes TT compared with GG homozygotes. Thus, the rs1501299 G-allele increased the risk of NAFLD in comparison with T-allele (OR = 4.44, 95% CI = 2.89–6.81, $p < 0.05$).

The frequencies of alleles among healthy persons for polymorphic variants *ADIPOQ* +276G/T were $p_G = 0.45$, $p_T = 0.55$. The frequencies of alleles for polymorphic variants *ADIPOQ* +276G/T among T2D patients without NAFLD were $p_G = 0.42$, $p_T = 0.58$; among the patients with T2D complicated NAFLD were $p_G = 0.41$, $p_T = 0.59$. No statistically significant differences in allele frequencies between studied groups were found ($p > 0.05$).

We also found that there was a significant difference in frequency of *ADIPOQ* SNP -11391G/A between T2D patients and control subjects, but not between the patients with and without NAFLD.

The *ADIPOQ* SNP –11391G/A in controls followed Hardy-Weinberg equilibrium (HWE) ($\chi^2 = 0.078$, $p = 0.962$) but in case of +276G/T, it was out of HWE ($\chi^2 = 17.114$, $p < 0.001$). The most prevalent genotype for the +276G/T SNP was GT in both the non-NAFLD T2D patients (45.2 %) and T2D individuals with NAFLD (41.1 %). The most prevalent genotype of the –11391G/A SNP was GG in both the T2D patients with NAFLD (75.6 %) and without NAFLD (74.5 %). As shown in Table 1, genotype frequencies of +276G/T

Table 1. Distribution of genotypes for polymorphic variants +276G/T and –11391G/A of adiponectin gene in type 2 diabetes patients with or without NAFLD

Gene SNP	Genotype	The actual and the theoretically expected distribution of genotypes in patients											
		Control subjects				Patients with NAFLD				Patients without NAFLD			
		actual		theoretically expected		actual		theoretically expected		actual		theoretically expected	
		n	%	n	%	n	%	n	%	n	%	n	%
ADIPOQ +276G/T	TT	17	17.3	31	30.1	18	20.0	15	16.7	55	35.5	52	33.5
	GT	79	75.5	51	49.5	37	41.1	44	48.8	70	45.2	76	49.1
	GG	7	7.3	21	20.4	35	38.9	32	35.5	30	19.4	27	17.4
		$\chi^2 = 17.114$, $\chi_{ст} = 3.84$, $p < 0.001$				$\chi^2 = 0.964$, $\chi_{ст} = 3.84$, $p < 0.618$				$\chi^2 = 0.362$, $\chi_{ст} = 3.84$, $p < 0.05$			
ADIPOQ –11391G/A	GG	43	84.3	42	82.4	102	75.6	100	74.1	38	74.5	39	76.5
	GA	7	13.7	8	15.7	29	21.5	32	23.7	13	25.5	11	21.5
	AA	1	2.0	1	2.0	4	2.9	3	2.2	0	0.0	1	2.0
		$\chi^2 = 0.078$, $\chi_{ст} = 3.84$, $p < 0.962$				$\chi^2 = 0.310$, $\chi_{ст} = 3.84$, $p < 0.856$				$\chi^2 = 1.180$, $\chi_{ст} = 3.84$, $p < 0.554$			

Notes: SNP — single-nucleotide polymorphism, n — number of patients, p — value of significance.

were significantly higher in the NAFLD group than in the non-NAFLD ($\chi^2 = 10.61$, $p < 0.005$). The genotype frequencies also were significantly different between NAFLD group and control subjects ($p < 0.01$). We evaluated also the frequencies of *ADIPOQ* –11391G/A alleles in Kharkiv population and the possibility of their further use as a prognostic marker for the risk of T2D complicated by NAFLD. Frequencies of *ADIPOQ* –11391G/A alleles for healthy individuals were: $p_G = 0.911$, $p_A = 0.088$. The frequencies of *ADIPOQ* –11391G/A alleles for total T2Ds were $p_G = 0.758$, $p_A = 0.134$. No significant differences in allele frequencies between studied groups were found ($p > 0.05$). The distribution of genotypes in the case and control groups corresponds to the HWE. Frequencies of *ADIPOQ* –11391G/A alleles for T2Ds with NAFLD were $p_G = 0.863$, $p_A = 0,137$. No statistically significant differences of the allele frequencies between the control group and T2Ds with NAFLD were found ($p > 0.05$). Also, there were any significant differences of allele frequencies between T2Ds with or without NAFLD ($p_G = 0.873$, $p_A = 0.127$). Thus, the initial data shown that homo- or heterozygous carriers of allele A (AA + GA) have increased risk of T2D (OR = 1.45, 95% CI 0.60–4.57), and the absence of this allele (GG-genotype) reduces the risk (OR = 0.69, 95% CI 0.29–1.22) as compared to the average for the whole population.

We did also haplotype analysis in order to get more accurate assessment about the influence of the two *ADIPOQ* gene polymorphisms.

As a result of a haplotypic analysis of the 2 polymorphic variants of the *ADIPOQ* gene in patients with T2D and NAFLD (table 2), the tendency to increase the frequency of the haplotype GT/GG was shown in comparison with the sample of patients without the liver disease, but the difference was not statistically significant ($p > 0.05$). It was found that the haplotype of GT/GG was more common in patients with T2D complicated by NAFLD and was twice less often detected in patients without hepatic disease (33 and 16.49 %, respectively, $p < 0.05$).

According to the results of our study, there was no significant difference in adiponecin levels in blood and frequency distribution of the genotypes of the polymorphic loci G276T and G11391A of the adiponectin gene in patients with T2D with and without NAFLD ($p > 0.05$). But in the analysis of haplotypes at these polymorphic loci, it was found that the levels of adiponectin in the group of patients with T2D and NAFLD are numerically or, in some cases statistically, lower than in the group without NAFLD (table 3).

Patients with NAFLD had significantly lower total adiponectin levels than controls (5.32 ± 0.70 *vs* 15.23 ± 0.63 mg/ml, $p < 0.001$). In addition, patients

Table 2. Frequencies of genotypes for polymorphic variants +276G/T and –11391G/A adiponectin gene in type 2 diabetes patients in the presence and absence NAFLD

Genotype	Frequencies of genotypes	Patients with NAFLD		Genotype	Frequencies of genotypes	Patients without NAFLD	
		actual	theoretically expected			actual	theoretically expected
GG/GG	0.311	22	21	GG/GG	0.266	12	10
GG/AG	0.084	4	6	GG/AG	0.114	2	4
GG/AA	0.025	2	2	GG/AA	0.000	0	0
GT/GG	0.303	22	20	GT/GG	0.245	6	9
GT/AG	0.082	5	5	GT/AG	0.105	7	4
GT/AA	0.025	0	2	GT/AA	0.000	0	0
TT/GG	0.126	5	8	TT/GG	0.189	8	7
TT/AG	0.034	4	2	TT/AG	0.081	2	3
TT/AA	0.010	2	0	TT/AA	0.000	0	0
		$\chi^2 = 7.04$, $\chi_{ст} = 15.51$, $p > 0.05$				$\chi^2 = 5.12$, $\chi_{ст} = 15.51$, $p > 0.744$	

Note: p — value of significance.

Table 3. Level of serum total adiponectin in according to haplotypes for polymorphic variants +276G/T and –11391G/A adiponectin gene in type 2 diabetes patients with or without NAFLD

Genotype	Adiponectin, mg/ml		p
	Patients with NAFLD	Patients without NAFLD	
GG/GG	3.90 ± 0.59	7.40 ± 1.66	**< 0.05**
GG/AG	1.38 ± 0.03	5.96 ± 0.85	**< 0.001**
GG/AA	–	2.25 ± 1.25	–
GT/GG	4.57 ± 0.94	6.69 ± 0.74	> 0.05
GT/AG	6.04 ± 1.01	7.27 ± 0.82	> 0.05
GT/AA	–	–	–
TT/GG	4.91 ± 1.55	6.51 ± 1.64	> 0.05
TT/AG	3.49 ± 0.32	6.88 ± 3.72	> 0.05
TT/AA	2.73 ± 0.84	–	–

with T2D and NAFLD who carried the GG/GG and GG/AG haplotypes of polymorphic variants +276G/T and -11391G/A adiponectin gene had significantly lower serum total adiponectin levels than patients without NAFLD who carried the GG/GG- or GG/AG-genotype. Moreover, in patients with T2D without NAFLD, there was no significant difference in plasma adiponectin levels between patients with different genotypes ($p > 0.05$).

It was found also, that SNP +276G/T, but not SNP −11391G/A, had the effect on adipose resistance to insulin in T2D patients with or without NAFLD. Regarding SNP +276G/T the TT-genotype exhibited numerically higher levels of Adipo-IR-index, which was statistically increased in TT-carriers with BMI ≥ 30 kg/m^2 (239.62 ± 36.89 *vs* 110.54 ± 25.97 mmol/L·pmol/L in T2Ds with BMI < 26 kg/m^2, $p < 0.05$). After the adiponectine gene haplotype analysis we revealed, that this trend (T-allele effect) had been demonstrated only in TT/GG- and GT/GG-carriers with NAFLD (314.56 ± 79,59 *vs* 144.22 ± 47.12 mmol/L·pmol/L in non-NAFLD T2D TT/GG-carriers, $p < 0.02$; 135.48 ± 21,88 *vs* 76.59 ± 15.60 mmol/L·pmol/L in non-NAFLD T2D GT/GG-carriers, $p < 0.05$) against the background of similarly enhanced (but less pronounced) Adipo-IR in all another genotypes and groups (data not shown).

Discussion

At present time, T2D is considered as a multifactorial, polygenic disease. The level of family risk incompatible with any of the hypotheses of monogenic inheritance, without the additional assumption of incomplete penetrance of the putative gene, which from a formally genetic point of view coincides with the polygenic hypothesis, indicates the polygenic nature. A multifactorial model of inheritance suggests that the manifestation of a disease is determined by the interaction of multiple environmental and genetic factors. By genetic factors, it means certain alleles of a number of polymorphic genes involved in the development of T2D, which in clinical practice are called alleles predisposing to the development of T2D. The study of hereditary predisposition to multifactorial diseases is extremely important for their diagnosis and selection of optimal therapy. Thus, of great practical value is the study of polymorphic markers in candidate genes, whose products are involved in the pathogenesis of a multifactorial disease. In this study, we investigated the possible association between *ADIPOQ* candidate gene polymorphisms of and NAFLD in the group of patients with T2D in Kharkiv population. It was recently shown that the level of fat in the liver correlated with such indicators of total fat as BMI and body fat percent. Furthermore, there is clear correlation between the liver fat accumulation of and WHR [31]. In addition, large population studies have proved an association of nonalcoholic steatohepatitis with increased BMI. It was shown that 91 % of individuals with a BMI over 30 kg/m^2 had signs of steatosis by ultrasound data [32].

Adipose tissue IR is present in the majority of patients with NAFLD, whether they are obese or not, and adipose tissue lipolysis provides approximately 60 % of the fatty acids used for hepatic triglyceride synthesis [33, 34]. Even though hepatic IR has been documented in T2D patients using homeostasis model assessment of IR (HOMA-IR) as a measure, there is insufficient data on adipose IR (Adipo-IR) and its relationship with the dysregulation of adipokines in T2D and NAFLD. We first found, that Adipo-IR index was significantly increased in T2D patients with NAFLD as compared to obesity-matched non-NAFLD diabetic subject with pronounced negative correlation between Adipo-IR and adiponectin level in T2D patients with NAFLD only. We found also, that the minor allele of SNP +276G/T, but not SNP −11391G/A, had the substantial effect on adipose resistance to insulin in T2D patients with NAFLD. Therefore, we can recommend Adipo-IR index as a predictive marker for the NAFLD development and the indicator for monitoring therapy success in T2D patients.

Hipoadiponectinemia considered as unfavorable prognostic sign because adiponectin has angioprotective properties [35]. In NAFLD patients, adiponectin levels are generally decreased [36]. Decreased plasma adiponectin levels were observed in obese and T2D patients, as well as in the ob/ob mouse line (mice with congenital obesity and hyperglycaemia) [37, 38]. In our previous study hipoadiponectinemia was found in all patients with T2D without modulating influence of the NAFLD [39]. This trend is confirmed in the current work, in both diabetic groups, with or without NAFLD, adiponectin levels are significantly lower ($p < 0.001$) than in control subjects. Our results shown, in contrast to previous studies in the other countries [40, 41], that levels of total adiponectin in circulation were characterized by no gender difference in T2D patients. Moreover, there was no effect of NAFLD on adiponectin levels in T2D patients likely due to the fact that adiponectin is synthesized exclusively by immature adipocytes and depends largely on insulin, glycemia and NEFA levels, which were not statistically different in patients with T2D with or without NAFLD ($p > 0,05$). On the other hand, we found that total adiponectin levels in patients with T2D and NAFLD who carried the GG/GG- and GG/AG-haplotypes of *ADIPOQ* SNPs +276G/T and −11391G/A were significantly lower than in T2D patients without NAFLD who carried the GG/GG- or GG/AG-genotype.

Low levels of adiponectin can promote the development of IR by blocking the phosphorylation of insulin receptor, increasing the flow of fatty acids in the liver and slowing their oxidation, thus, improving liver glucose, TG, and LDL production. So far, there are no comprehensive information about genetic determination of fat accumulation in the whole body and in the liver in humans. It is believed that gene polymorphism that exists in patients with NAFLD, associated with a large number of substances involved in the metabolism of lipids and carbohydrates in the liver.

The distribution of genotypes in the control group for *ADIPOQ* SNP +276G/T, which at some assumptions can be considered as a sample of the population, the ratio deviates from HWE toward high heterozygosity. The share of heterozygotes +276G/T at 1.53 times higher ($p < 0.001$) than the theoretically expected value at equilibrium [42]. Our data correspond in part to the

other study which revealed that adiponectin +276 G/T polymorphism was not significantly different between NAFLD and controls, but among females, the GG genotype was reported to be significantly more prevalent in patients with NAFLD [43].

Genetic variation in *ADIPOQ* –11391G/A was associated with plasma levels of adiponectin, obesity and resistance to insulin, also tested the association between insulin resistance and –11391G and BMI, suggest that the impact of the variant adiponectin gene polymorphism on glucose homeostasis may depend on the body fat [44]. Due to reduced activity and low circulating levels of adiponectin associated with an increased risk of serious diseases such as T2D and coronary heart disease, experimental data suggest that minor haplotype (by promoter polymorphisms) affects the expression of adiponectin in vivo to such measures that might be unfavorable [45]. SNPs in the promoter and intron 2 regions of *ADIPOQ* gene play a functional role in adiponectin regulation [46].

G. Dolley et al. (2008) did not found the influence of *ADIPOQ* gene polymorphisms –11391G/A and –11377C/G on anthropometric indicator associations separately, but haplotype was associated with WHR. If there was a genotype –11391A and 11377C, WHR was significantly higher compared to other haplotypes. Also AC/AC-haplotype carriers had higher levels of adiponectin than the carriers of GG/GG-haplotype ($p < 0.0001$) [47].

Our data shown that homo- or heterozygous allele A (AA + GA) increases the risk of T2D (OR = 1.45, 95% CI 0.60–4.57), and the absence in the genotype the A-allele reduced the risk (OR = 0.69, 95% CI 0.29–1.22) compared to the average for the whole population. However, the odds ratio was not statistically significant, perhaps due to the limited amount of samples, which did not allow to achieve the minimum capacity criteria which justifies further studies with increased samples.

Thus, verification of SNPs of adiponectin gene and characteristics of its association with the level of the hormone in the circulation for patients with T2D complicated by NAFLD allow improved preventive therapy algorithm.

NAFLD is a complex disease, which may be the results of multiple loci commonly affected mutations [48, 49]. Previous studies have shown that polymorphisms of adiponectin gene were associated with NAFLD [50]. At the same time, in our study the combination of GG/GG- and GT/GG-haplotypes (33 % respectively) in patients with NAFLD was more frequent. Some combinations of alleles of different loci (haplotypes) can give a highly adapted phenotype, a combination of other alleles give phenotypes poorly adapted. Such deviations from equilibrium may mean that carriers of some genotypes are likely to have a selective advantage, possibly GT/AA have reduced adaptivity, and their absence may be due to the action of selection or the small number of samples.

Conclusions

Our results demonstrate for the first time, that Adipo-IR index was significantly increased in Ukrainian T2D patients with NAFLD as compared to obesity-matched non-NAFLD diabetic subject with pronounced negative correlation between Adipo-IR and adiponectin level in T2D patients with NAFLD only. We grounded the prospects of using the Adipo-IR index for monitoring of T2D therapy and associated liver disease development. Against the background of the research the adiponectin gene polymorphic variant haplotype association with NAFLD in T2Ds in the Kharkiv region population it have been established new markers (rs1501299 G-allele, rs17300539 and rs1501299 GG-/GG- and GT/GG-haplotypes, respectively) of an increased risk of NAFLD development in T2D patients. Thus, the contribution of various factors to the development of the NAFLD is significantly different depending on the population. Further identification of genetic markers for NAFLD risk in patients with T2D will allow to understand the main pathological mechanisms of the diseases, and to select the respectively treatment.

Conflicts of interests. Authors declare the absence of any conflicts of interests that might be construed to influence the results or interpretation of their manuscript.

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Yu. Karachentsev — coordination of the project, within the framework of which the research was conducted, the concept of work;

M. Gorshunska — key participation in data collection, diagnosis of type 2 diabetes mellitus, discussion of the data;

T. Tyzhnenko — creating a control group, analysis of genetic data and their discussion, participation in creating the database, writing the text of the article;

N. Krasova — conducting biochemical studies (carbohydrate/lipid metabolism, assessment of insulin resistance), analysis and discussion, writing the text of the article;

I. Dunaeva — participation in data collection, diagnosis of non-alcoholic fatty liver disease, performing and analyzing anthropometric studies;

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T. Mishchenko — search for information and literature sources, participation in the systematization of the material;

O. Plohotnichenko — conducting statistical analysis of the data;

L. Atramentova — coordination of molecular genetic studies, the choice of statistical methods of the research;

N. Kravchun — coordination of the project, within the framework of which the research was conducted, the design of the study, determination of the cohort of patients with non-alcoholic fatty liver disease;

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Однонуклеотидні поліморфізми гена адипонектину у хворих на цукровий діабет 2-го типу та неалкогольну жирову хворобу печінки

Резюме. ***Актуальність.*** Зазвичай вважають, що фактори навколишнього середовища та генетичні фактори взаємодіють з утворенням фенотипу неалкогольної жирової хвороби печінки (НАЖХП) та визначають її прогресування. Як НАЖХП, так і цукровий діабет 2-го типу (ЦД2) становлять собою гетерогенні захворювання зі спільними патогенетичними шляхами. Адипонектин — це адипокін, що підвищує чутливість гепатоцитів та м'язів до інсуліну, модулює енергетичний гомеостаз, глюкозний/ліпідний метаболізм та запальну відповідь. Ряд значущих поліморфізмів гена адипонектину відомі в цієї галузі. ***Метою*** дослідження була оцінка можливої асоціації між двома варіантами гена адипонектину (*ADIPOQ*) — +276 G/T (rs1501299) і −11391 G/A (rs17300539) та чутливістю до НАЖХП у хворих на ЦД2 української популяції. ***Матеріали та методи.*** Дослідження за типом «випадок — контроль» включало 155 хворих на ЦД2 (ч./ж. — 77/78, вік — 54,55 ± 0,73 року, тривалість діабету — 6,66 ± 0,49 року, індекс маси тіла — 32,20 ± 0,43 кг/м², обсяг талії/стегон — 0,98 ± 0,01 м, HbA_{1c} — 7,26 ± 0,11 %) для біохімічної

характеристики (ліпідний профіль, неестерифіковані жирні кислоти (НЕЖК), інсулін, загальний адипонектин та ін.), у тому числі 90 хворих на ЦД2 із НАЖХП, 245 хворих на ЦД2 для генотипування rs1501299, 155 хворих на ЦД2 для генотипування rs17300539 та 51 контрольну особу відповідного віку (К). Варіанти +276 G/T та –11391 G/A визначали за допомогою полімеразної цепної реакції — довжини рестрикційних фрагментів з ендонуклеазами *Mva1269I* (*BsmI*) та *MspI (HpaII*). Інсулінорезистентність (ІР) оцінювали за допомогою алгоритму HOMA та як жирову ІР (адипо-ІР, НЕЖК × інсулін). Для статистичного аналізу використовували непарний t-критерій Стьюдента, χ^2 та ранговий критерій Спірмана. Для оцінки генетичного ризику НАЖХП розраховували відношення шансів та 95% довірчий інтервал (ДІ). ***Результати.*** Хворі на ЦД2 характеризувалися надлишковою масою тіла або ожирінням, що було більш вираженим за наявності НАЖХП ($p < 0,01$). Це супроводжувалося підвищенням рівня HOMA-ІР ($p < 0,05$) та тригліцеридів ($p < 0,001$). Відмічено, що адипо-ІР була вищою у хворих на ЦД2 порівняно з К ($p < 0,001$) та цей індекс суттєво підвищений у хворих на ЦД2 із НАЖХП порівняно з хворими на ЦД2 без НАЖХП такого ж ступеня ожиріння (190,18 ± 22,15 проти 133,32 ± 13,58 ммоль/л · пмоль/л, $p < 0,02$) з оберненою кореляцією між адипо-ІР та рівнем адипонектину тільки у хворих на ЦД2 із НАЖХП ($r_s = –0,350$, $p = 0,021$). Стратифікація хворих без НАЖХП за генотипом +276 G/T свідчить про поширеність GT- та TT-генотипів. Таким чином, G-алель rs1501299 підвищує ризик розвитку НАЖХП порівняно з Т-алелем (OR = 4,44, 95% ДІ = 2,89–6,81, $p < 0,05$). Ми також виявили значущу різницю в частотах –11391G/A між хворими на ЦД2 та К, але не між хворими з НАЖХП та без неї. Ми спостерігали, що гаплотип GT/GG частіше зустрічався серед хворих на ЦД2 із НАЖХП та удвічі менше в пацієнтів без хвороби печінки (33 та 16,49 % відповідно, $p < 0,05$). ***Висновки.*** Обґрунтовано перспективність використання індексу адипо-ІР як предиктивний маркер розвитку НАЖХП та індикатор успішності терапії хворих на ЦД2. Виявлено новітні генетичні маркери (G-алель rs1501299, GG/GG- та GT/GG-гаплотипи за rs17300539 та rs1501299 відповідно) для ризику розвитку НАЖХП у хворих на ЦД2.

Ключові слова: ген адипонектину; однонуклеотидний поліморфізм; частоти генотипів; цукровий діабет 2-го типу; неалкогольна жирова хвороба печінки; інсулінорезистентність

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Однонуклеотидные полиморфизмы гена адипонектина у больных сахарным диабетом 2-го типа и неалкогольной жировой болезнью печени

Резюме. ***Актуальность.*** Обычно полагают, что факторы окружающей среды и генетические факторы взаимодействуют с формированием фенотипа неалкогольной жировой болезни печени (НАЖБП) и определяют ее прогрессирование. Как НАЖБП, так и сахарный диабет 2-го типа (СД2) представляют собой гетерогенные заболевания с общими патогенетическими путями. Адипонектин — это адипокин, повышающий чувствительность гепатоцитов и мышц к инсулину, модулирующий энергетический гомеостаз, глюкозный/липидный метаболизм и воспалительный ответ. В данной области известен ряд значимых полиморфизмов гена адипонектина. ***Целью*** исследования была оценка возможной ассоциации между двумя вариантами гена адипонектина (*ADIPOQ)* — +276 G/T (rs1501299) и –11391 G/A (rs17300539) и чувствительностью к НАЖБП у больных СД2 украинской популяции. ***Материалы и методы.*** Исследование по типу «случай — контроль» включало 155 больных СД2 (м./ж. — 77/78, возраст — 54,55 ± 0,73 года, длительность диабета — 6,66 ± 0,49 года, индекс массы тела — 32,20 ± 0,43 кг/м², объем талии/бедер — 0,98 ± 0,01 м, HbA_{1c} 7,26 ± 0,11 %) для биохимической характеристики (липидный профиль, неэстерифицированные жирные кислоты (НЭЖК), инсулин, общий адипонектин и др.), в том числе 90 больных СД2 с НАЖБП, 245 больных СД2 для генотипирования rs1501299, 155 больных СД2 для генотипирования rs17300539 и 51 контрольное лицо соответствующего возраста (К). Варианты +276 G/T и –11391 G/A определяли с помощью полимеразной цепной реакции — длины рестрикционных фрагментов с эндонуклеазами *Mva1269I* (*BsmI*) и *MspI (HpaII*). Инсулинорезистентность (ИР) оценивали с использованием алгоритма HOMA и как жировую ИР (адипо-ИР, НЭЖК × инсулин). Для статистического анализа применяли непарный t-критерий Стьюдента, χ^2 и ранговый критерий Спирмана. Для оценки генетического риска НАЖБП рассчитывали отношение шансов и 95% доверительный интервал. ***Результаты.*** Больные СД2 характеризовались избыточной массой тела или ожирением, более выраженным при НАЖБП ($p < 0,01$). Это сопровождалось повышением уровня HOMA-ИР ($p < 0,05$) и триглицеридов ($p < 0,001$). Выявлено, что адипо-ИР была выше у больных СД2 по сравнению с К ($p < 0,001$) и этот индекс существенно больше у больных СД2 с НАЖБП по сравнению с больными СД2 без НАЖБП с сопоставимой степенью ожирения (190,18 ± 22,15 против 133,32 ± 13,58 ммоль/л · пмоль/л, $p < 0,02$) с отрицательной корреляцией между адипо-ИР и уровнем адипонектина только у больных СД2 с НАЖБП ($r_s = –0,350$, $p = 0,021$). Стратификация больных без НАЖБП по генотипу +276 G/T свидетельствует о повышенной частоте встречаемости GT- и TT-генотипов. Таким образом, G-аллель rs1501299 повышает риск развития НАЖБП по сравнению с Т-аллелем (OR = 4,44, 95% доверительный интервал = 2,89–6,81, $p < 0,05$). Мы также выявили значимое различие в частотах –11391G/A между больными СД2 и К, но не между больными с НАЖБП и без нее. Мы обнаружили, что гаплотип GT/GG встречался чаще у больных СД2 с НАЖБП и вдвое реже у пациентов без болезни печени (33 и 16,49 % соответственно, $p < 0,05$). ***Выводы.*** Обоснована перспективность использования индекса адипо-ИР в качестве предикторного маркера развития НАЖБП и индикатора успешности терапии больных СД2. Выявлены новые генетические маркеры (G-аллель rs1501299, GG/GG- и GT/GG-гаплотипы по rs17300539 и rs1501299 соответственно) для риска развития НАЖБП у больных СД2.

Ключевые слова: ген адипонектина; однонуклеотидный полиморфизм; частоты генотипов; сахарный диабет 2-го типа; неалкогольная жировая болезнь печени; инсулинорезистентность