Association between *+ 276 G>T* polymorphism of the adiponectin gene (*ADIPOQ*) and insulin resistance in Ukrainian patients with type 2 diabetes mellitus (T2DM)

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Introduction

Adipose tissue is a metabolic active tissue that secretes multiple metabolically important proteins known as 'adipokines'. Some adipokines could be relate to insulin resistance which has a causal role in Type 2 diabetes mellitus (T2DM) and its cardiovascular complications. Adiponectin, an adipocyte-secreted protein, is known to have anti-atherogenic, anti-inflammatory and anti-diabetic properties and its serum levels are decreased in obesity, T2DM, and coronary artery disease. Different polymorphisms have been identified in humans and examined for their possible association with insulin resistance indexes and circulating adiponectin concentrations. Variants of *ADIPOQ* have been inconsistently associated with adiponectin levels or diabetes in diverse populations.

Aim

We explored association of single nucleotide polymorphism (*SNP*) in the *ADIPOQ* (*+276 G>T*, rs1501299) with circulating total adiponectin and insulin resistance (IR) in Ukrainian T2DM cohort.

Materials and methods

•544 unrelated T2DM patients (M/F: 241/303) and 215 healthy control subjects (C) (M/F: 145/70) were genotyped (*Table 1*).

Table 1. Baseline parameters of the study groups

	Type 2 diabetes	Control group	p-value
Age (years)	56.30±0.42	53.80±0.48	p>0.05
Diabetes duration (years)	7.88±0.33	-	
BMI (kg/m^2)	31.50±0.24	26.80±0.76	p<0.001
WHR	0.99±0.02	0.78±0.01	p<0.001

BMI – body mass index, WHR – waist to hip ratio.

- The following parameters have been measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturers instructions: insulin (DRG insulin ELISA kit, Germany); total adiponectin (ALPCO Diagnostics, USA). Triglycerides have been determined with an autoanalyzer (LX20-Pro from Beckman-Coulter, Woerden, the Netherlands) with kits from Beckman-Coulter; non-etherified fatty acids (NEFA) have been evaluated with a kit of Wako Diagnostics (Richmond, VA, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI) were calculated. Fasting blood glucose (FBG), HbA1c were determined.
- The *SNP +276 G>T* in the *ADIPOQ* was detected using polymerase chain reaction restriction fragment length polymorphism – (PCR-RFLP): DNA was amplified with PCR (APM276F GGCCTCTTTCATCACAGACC, APM276R AGATGCAGCAAAGCCAAAGT) followed by restriction enzyme digestion (using *Mva1269I*). The products were size-separated on 2% agarose gel [DNA marker (M) *pUC19*], stained with ethidium bromide, and pictured on an ultraviolet light box (Fig. 1).

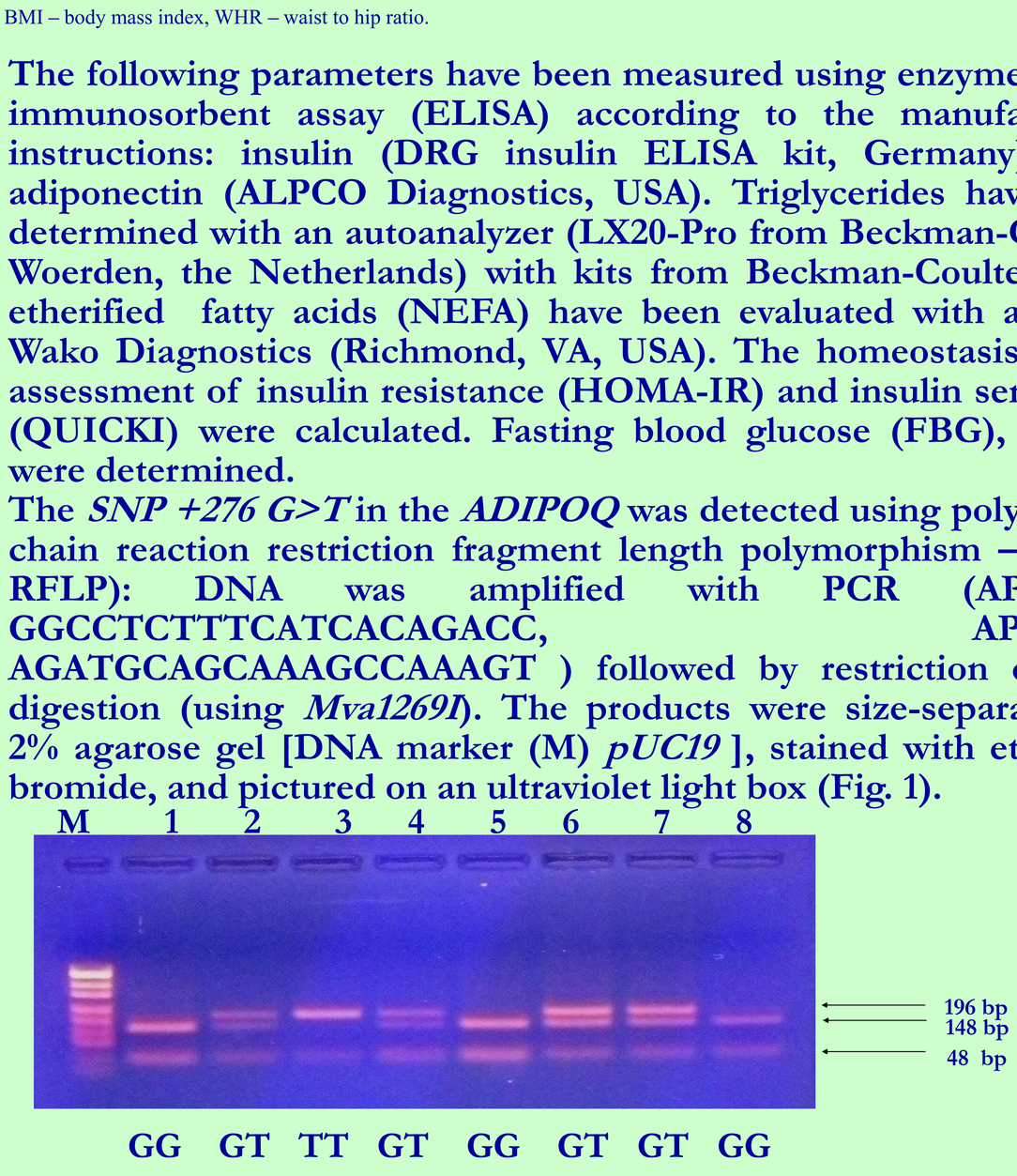

Fig. 1 Electrophoretic analyses of the *+276 G>T SNP*

• The statistical analysis was performed with Student's *t* test, genotype and allele frequencies were tested by χ^2. Data were presented as mean ± SEM.

Results

Comparing with C significant (p<0.001) increase in triglyceridemia, plasma NEFA levels, plasma insulin levels and HOMA-IR as well as hypoadiponectinemia were observed in T2DM patients (*Table 2*).

Table 2. Baseline fasting parameters of the study groups

	Type 2 diabetes	Control group	p-value
FBG (mmol/l)	9.62±0.22	5.52±0.49	p<0.001
HbA_{1c} (%)	7.57±0.17	5.4±0.10	p<0.001
Insulin (pmol/l)	132.47±6.11	85.21±8.00	p < 0.001
HOMA-IR (arbitrary units)	8.35±0.40	1.77±0.41	p < 0.001
QUICKI (arbitrary units)	0.47±0.01	0.50±0.01	p < 0.05
Triglycerides (mmol/l)	2.89±0.12	1.23±0.26	p < 0.001
NEFA (mmol/l)	1.35±0.03	0.34±0.07	p < 0.001
Total adiponectin (mg/l)	4.99±0.16	11.80±1.45	p < 0.001

The genotype frequencies were consistent with Hardy-Weinberg equilibrium in both groups. In T2DM patients allele frequencies for the *+ 276G>T SNP* were 0.399 for the *T* allele and 0.601 for the *G* allele, and they differ significantly from C (*T* 0.307; *G* 0.693). Major allele in the studied groups are G: its frequency in the control group was 0.693, and in the diabetic patients - 0.601. No gender differences were observed in allele frequencies in both studied groups. In comparison with C, T2DMs had more homozygotes *TT*: 16.9 vs 9.3%, p<0.05, but less homozygotes *GG*: 37.1% vs 47.4%, p<0.05, and heterozygotes *GT*: 46.0 vs 43.3 %, p>0.05.

The T2DM genotype groups were well matched for age, diabetes duration, fasting glycaemia and HbA1c levels. It was determined lower severity of dyslipidemia, i.e. total cholesterol (p<0.01), triglycerides (p<0.02) and LDL-cholesterol (p<0.05) against the background of more pronounced hypoadiponectinemia (on 15.4%) in *GG* homozygotes (p<0.1) compared to *TT*-carriers (Fig. 2). Obesity significantly (p<0.05) decreased insulin sensitivity and increased IR in carries of all studied genotypes at 276 position *vs* similar genotype carries with normal BMI. In TT homozygotes were revealed the higher HOMA-IR and the lower QUICKI in comparison with other genotypes against the background of normal, overweight and obesity. Higher HOMA-IR was found in *TT* compared to other genotypes with maximal difference in patients with obesity (HOMA-IR indexes in carriers of *TT*, *GT* and *GG* genotypes were 10.96±1.52, 8.40±0.65 and 8.96±0.74, respectively, p<0.02 for *TT vs GT* and *GG*). The more pronounced increase in triglycerides was revealed for T2DM homozygotes *TT* vs T2DM heterozygotes and homozygotes *GG* (p<0.01) (Fig. 2).

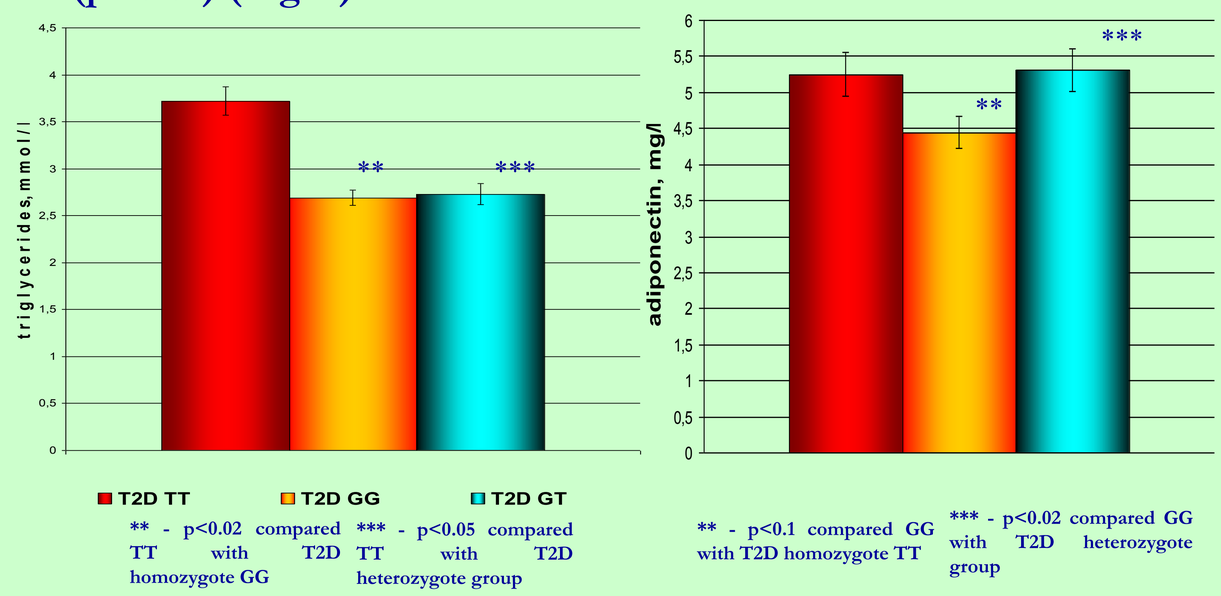

Fig. 2 Plasma triglycerides and adiponectin levels in T2DM patients with different genotypes

Conclusions

The study demonstrates for the first time that *ADIPOQ* variants are associated with IR phenotype in Ukrainian T2DM patients. We suggest the predominant impact of metabolic disturbances and hyperinsulinemia on diabetic hypoadiponectinemia genesis.

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Diabetes (to include obesity, pathophysiology & epidemiology)

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