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Adiponectin level and gene variability are obesity and metabolic syndrome markers in young population

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Abstract

Background and Aims

Human obesity is accepted as an important risk factor for development of MetS. Adiponectin is linked to central obesity and *ADIPOQ* variants are promising markers for understanding the genetic base of obesity-related disorders. We performed analyses of adiponectin concentrations and *ADIPOQ* variants and tested their associations with obesity and MetS in young subjects of Croatian origin.

Methods

Biochemical and anthropometric parameters of MetS were obtained for 149 unrelated subjects. Adiponectin levels were measured by ELISA assay. *ADIPOQ* -11391G>A and -11377C>G were genotyped by real-time PCR.

Results

BMI and WC, TG and GLUC showed inverse correlation, whereas HDL-C showed a positive correlation with adiponectin concentrations. For central obesity, we found association with -11377C>G and with -11391G>A polymorphisms. *ADIPOQ* -11377GG and -11391GA significantly increased the risk for the development of central obesity (OR 5.57 and OR 3.37, respectively). Significant association was found between -11391A, -11377G allele and haplotype and increased TG. -11377C>G and -11391G>A variant were significantly associated with the incidence of MetS. C>G mutation at position -11377 significantly increased the risk of MetS development (OR=2.93). Compared with the -11391G homozygotes, the carriers of the A allele had the significantly increased risk for the development of MetS (OR=3.15). The test of overall association showed statistically significant correlation of MetS with -11377C>G and -11391G>A haplotypes (p=0.008).

Conclusion

Analysis of adiponectin concentration and *ADIPOQ* -11391G>A and -11377C>G gene variants could be clinically meaningful for estimation of MetS risk in young population.

Key Words: obesity, metabolic syndrome, adiponectin, gene polymorphism, young population

Abbreviations: MetS, metabolic syndrome; *ADIPOQ*, adiponectin gene; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; BMI, body mass index; WC, waist circumference; TG, triglycerides, GLUC, glucose; HDL-C, high-density lipoprotein cholesterol; SNP, single nucleotide polymorphism; OR, odds ratio.

Introduction

Overweight and obesity are some of the most complex clinical syndromes whose prevalence in the world has reached epidemic proportions in recent decades; these syndromes affect both children and adults (1). Obesity has been widely accepted as an important risk factor for development of certain syndromes and many chronic diseases. Body mass index (BMI) was most widely used obesity indicator, but it seems that the best measure is waist circumference (WC) because it estimates the amount of visceral adipose tissue which contributes most to the metabolic changes that occur in the obese. Visceral fat accumulation is associated not only with quantitative and qualitative changes in serum lipids and lipoproteins (2) but also with

dysregulation in the secretion of adipocytokines which participate in the development of the metabolic syndrome (MetS) (3). The increasing prevalence of obesity in the European Union and the USA is a major health concern, and it is also becoming worrying in Croatia (4) In Croatia, the prevalence of overweight, obesity and central obesity is estimated to be 38.11%, 20.34% and 43.52%, respectively (5). The genetics of obesity is complex and involves interactions between genes, gene and environment, and gene and nutrition and behavioral factors (6). Genome-wide association studies (GWAS) have identified numerous obesity-related loci but precise identification of correlation between DNA sequence variation in specific gene and obesity phenotypes has been difficult (7). More than 400 chromosomal regions that encompass gene variants have been found to be involved in weight regulation and development of obesity, and considerable emphasis has been focused on gene polymorphisms related to MetS (8).

MetS is a combination of several factors and is recognized as an important risk factor for both cardiovascular disease (CVD) and type 2 diabetes (T2D). The core risk factors leading to MetS include i) independent risk factors (atherogenic dyslipidemia, elevated blood pressure and elevated plasma glucose), ii) emerging risk factors (e.g., prothrombotic and proinflammatory state) and iii) underlying risk factors (e.g., obesity and insulin resistance). Other factors that aggravate MetS include physical inactivity, advancing age, hormonal imbalance and genetic factors which can affect both the underlying causes and individual metabolic risk factors (9,10). Adiponectin is the most abundant adipose tissue-derived cytokine with anti-inflammatory and anti-atherogenic properties which was linked to central obesity and proposed as the major contributor to MetS in addition to insulin resistance (IR) and CVD (11-13). The role of adiponectin in energy metabolism has been confirmed by its reverse correlation with the adverse features of MetS (14). Moreover, serum adiponectin

concentrations are highly heritable and are linked to adiponectin gene (*ADIPOQ*) (15, 16), underlining the importance of studying *ADIPOQ* as one of the gene candidates of obesity and consequently MetS. The *ADIPOQ* gene is located on chromosome region 3q27 (17) with other susceptibility loci for risk factors of MetS (18), obesity, T2D and CVD (12). Also, pedigree-based analysis using a variance component linkage model demonstrated a quantitative trait locus on chromosome 3q27 that is strongly linked to MetS (19). Taking into consideration all the above, the association of adiponectin with MetS and its traits seems more likely.

Two promoter single nucleotide polymorphisms (SNPs) at the *ADIPOQ* locus, the -11391G>A and -11377C>G, have been shown to alter the plasma adiponectin concentration and abdominal obesity and consequently affect the risk of MetS, T2D and CVD (20,12). Although the importance of *ADIPOQ* gene variants for the development of obesity and MetS has been widely recognized, their clear contribution has not yet been fully understood. The results of association studies are conflicting due to differences in age and genetic or ethnic background of study populations (21). In recent years, attention has shifted from reliance on clinical events and focused on screening for preclinical symptoms of disease.

This study aimed to perform analysis of the possible associations of the *ADIPOQ* gene variants with abdominal obesity and increased susceptibility to the development of MetS in young subjects of Croatian origin.

Materials and Methods

Study subjects

A total of 149 unrelated young subjects (65 male, 84 female) aged 20-33 (mean 26±3 years) were chosen during routine medical check-up. Exclusion criteria included known cardiovascular diseases, confirmed diabetes, endocrine disease, significant renal or hepatic disease and other chronic disease, use of medications that could alter blood pressure, glucose or lipid metabolism. The participants had their blood tests completed and anthropometric measurements taken. All participants signed informed consent forms, and the study protocol was approved by Ethics Committee of the University Hospital Centre Zagreb.

Clinical and Biochemical Measurements

Clinical and biochemical parameters were measured by standard laboratory procedures. The measurings of weight, height and WC were done in a standardized manner. The weights and heights of participants were measured while wearing light clothing and without shoes. Weight was measured to the nearest 0.1 kg on a medical balance scale. Height was measured to the nearest 0.1 cm with stadiometer. BMI was calculated as weight (kg)/height(m^2). WC was measured twice to the nearest 0.5 and the mean was used for subsequent analyses. Overweight and obesity were defined as a BMI between 25.0 kg/m^2 and 29.9 kg/m^2, and ≥30 kg/m^2, respectively, as proposed by WHO (22). Central obesity was defined as a WC≥94 cm for men and the WC≥80 cm for women according to the criteria of the International Diabetes Federation (IDF) (23). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from two readings with a minimal interval of 5 minutes. Elevated blood pressure was defined according to IDF criteria by a SBP that was ≥ 135 mm Hg and a DBP that was ≥ 85 mm Hg for men and women, respectively (23). Blood samples for serum biochemical analyses (total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and glucose (GLUC)) were collected after overnight fasting. Plasma concentrations of GLUC, TC and TG were measured by standard enzymatic methods (24)

with Olympus System Reagent 800 kit on an Olympus AU2700 autoanalyzer (Olympus, Tokyo, Japan). HDL-C was also determined enzymatically after precipitation of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) by polyethylene glycol solutions (Quantolip Immuno AG, Vienna, Austria) and also measured on an Olympus AU2700. LDL-C was calculated using Friedewald Equation (25). If TG concentration was higher than 3.0 mmol/L, HDL-C was measured by direct immunoinhibition method (Olympus Diagnostica GmbH, Lismeehan, Ireland), and if TG concentrations were higher than 4.5 mmol/L, LDL-C was measured by homogenous assay (Randox Laboratories, Crumlin, United Kingdom). Fasting plasma adiponectin concentration was determined using a validated sandwich enzyme-linked immunosorbent assay (ELISA kit, adiponectin: E09, Mediadiagnost, Reutlingen, Germany). The assay uses two specific and high affinity antibodies and determines total adiponectine in human serum and plasma samples. Blueher et al. (26) demonstrated that total adiponectine, as measured by Mediagnost test, is significantly correlated with insulin sensitivity and metabolic variables. Additionally, their study showed that total adiponectin, as measured by Mediagnost test, was clearly superior to HMW at identifying the presence of insulin resistance and predicting the levels of metabolic variables (26). The values of adiponectin were interpreted in relation to BMI and age of subjects. MetS was defined using the diagnostic criteria of the IDF (23). We used this definition, and not ATPIII definition, because central obesity is the key point in IDF definition. This point is further elaborated in a way that various reference intervals for waist circumference have been stated as valid for different ethnic groups. This approach enabled the application of this definition in different populations and at the global level. An individual with a combination of central obesity (defined as WC $\geq$ 94cm for European men and $\geq$ 80cm for European women) and any two or more of the following risk factors was classified as having MetS: elevated triglyceride levels (TG$\geq$1.695 mmol/L), reduced HDL-C (HDL-C<1.036mmol/L for men and

<1.295 mmol/L for women), elevated blood pressure (SBP ≥ 130 mm Hg or DBP ≥ 85 mm Hg), and increased fasting plasma glucose (GLUC≥5.6 mmol/L.). Hypercholesterolemia and increased LDL-C levels were identified when TC≥5 mmol/L and LDL-C>3.0 mmol/L were determined, respectively.

ADIPOQ (-11391G>A and -11377C>G) genotyping

Human DNA was extracted from leukocytes in whole blood samples, following the standard salting out method (27). DNA was amplified in a 20 μL reaction volume. The *ADIPOQ* gene promoter SNPs -11391G>A (rs17300539) and -11377C>G (rs266729) were genotyped by applying DNA assay that uses real-time PCR and fluorescence resonance energy transfer (FRET) with the LightCycler (Roche Diagnostics, Germany). Analyses for SNPs -11391G>A and -11377C>G were performed using primers and hybridization probes as previously described by Schwarz et al. (28). The reaction mixture for each PCR consisted of 100 ng of genomic DNA, 0.5 μl of each primer (0.125 μmol/L), 0.4 μl of each DNA hybridization probe (0.04 μmol/L), 2.0 μl DNA "Fast start'' mix, and 3.0 μl of MgCl2 (3.75 mmol/L). After initial denaturation at 94°C for 3 sec, 40 PCR cycles were performed with 1 s of denaturation at 95°C; 14 s of annealing at 58°C and extending at 72°C for 10 s. Genotyping was carried out via melting curve analysis. Replicate quality control samples were included and genotyped with 100% concordance.

Statistical analysis

SPSS 17.0 (SSPS Inc., Chicago, IL) and MedCalc 10.2.0.0. (Frank Schoonjans, Mariakerke, Belgium) were used for descriptive statistical analysis. The comparisons between the study groups were analyzed using Student's t-test or Mann-Whitney test, depending on distribution normality or χ^2 and Fischer exact test for categorical variables. Relationships between adiponectine and other variables were presented as Spearman's rank correlation coefficients. The association of *ADIPOQ* -11377C>G and -11391G>A gene variants and haplotypes with the development of central obesity, MetS and their components was tested using program UNPHASED ver. 3.0.10. (29). A test for Hardy-Weinberg equilibrium using Markov chain method (30), as implemented in Arlequin ver. 3.01 (31) was applied. A linkage disequilibrium likehood-ratio test between loci was performed by Arlequin ver. 3.01 (31). The level of significance was set at $p<0.05$.

Results

Population sample description

The clinical and metabolic characteristics of participants are shown in Table 1. 43.6% of the study population included men and 56.4% of the population was female. Eighty-eight (59.1%) young adults had normal weight, 37 (24.8%) were overweight and 24 (16.1%) were obese. Central obesity was found in 46,6% (39/84) of women and in 44,6% (29/65) of men. Hypertriglyceridemia and hypercholesterolemia were detected in 21.5% and 30.9% of subjects, respectively. Increased LDL-C was found in 32.2% of subjects. Decreased HDL-C was found in 4.8% (4/84) of women and in 12.3% (8/65) of men. Elevated SBP and DBP were detected in 15.4% and 19.5% of subjects, respectively. Increased fasting plasma glucose was detected in 4.0% of subjects.

With the exception of BMI and adiponectin, there were no differences between sexes for the measured variables (data not shown). No differences in genotype distribution according to

gender were detected (data not shown). Therefore, all analyses were performed by including male and female subjects in the same group. Of 149 subjects, MetS was defined in 34 subjects (22.82%). All MetS variables (SBP, DBP, WC, HDL-C and GLUC) were significantly different in the MetS group as compared to the group without MetS. Moreover, the MetS group had significantly higher BMI, LDL-C and TC concentrations, and lower adiponectin level compared to the group without MetS (Table 1) The Mets variables (DBP, WC, HDL-C and GLUC) and LDL-C and TC were significantly different between the two groups even after adjustment for BMI (data not shown).

Correlation of adiponectin concentrations with anthropometric and metabolic variables

The levels of total adiponectin in serum ranged from 0.87 mg/L to 38.5 mg/L. The serum concentrations of adiponectin in women (13.9±8.2 mg/L) were significantly higher ($p<0.001$) that those in men (6.14±4.32 mg/L). As shown in Table 2, anthropometric parameters (BMI and WC) and the concentration of TG showed the strongest significant inverse correlation with adiponectin concentrations ($p<0.001$), whereas HDL-C showed a strong positive correlation with adiponectin concentrations ($p<0.001$). The concentration of GLUC also showed an inverse significant correlation with adiponectin ($p=0.012$), as well as SBP and DBP ($p<0.001$) (Table 2). The correlation between serum adiponectin level and TG, HDL-C, GLUC, SBP and DBP were significant even after adjustment for sex and anthropometric parameters (BMI and WC) (data not shown).

Genetic analysis

The allele frequencies of the two SNPs in the *ADIPOQ* gene were as follows: -11377C>G (C:G=0.68:0.32) and -11391G>A (G:A=0.79:0.21). The frequencies of the *ADIPOQ* -

11377C>G variants CC, CG and GG were 68 (45.6%), 67 (45.0%) and 14 (9.4%), respectively. The frequencies of the *ADIPOQ* -11391G>A variants GG, GA and AA were 89 (59.7%), 57 (38.3%) and 3 (2.0%), respectively. There was no deviation from the expected proportions of genotypes in the population predicted by the Hardy–Weinberg equilibrium (*ADIPOQ* -11377C>G p=0.573, *ADIPOQ* -11391G>A p=0.133). Test results for linkage disequilibrium were found to be significant (r^2 =0.1278, χ^2=38.098, p<0.001) between the two loci.

Association of ADIPOQ -11377C>G and -11391G>A polymorphisms with central obesity

We compared the genotype distribution of SNPs of -11377C>G and -11391G>A of *ADIPOQ* in the group of subjects with central obesity (WC ≥94 cm for men; WC ≥ 80 cm for women) and in the group of subjects who did not meet the criteria for central obesity (WC <94 cm for men; WC<80 cm for women) to examine if the above SNPs were significantly related to central obesity (Table 3). The genotype distribution of *ADIPOQ* -11377C>G and -11391G>A was different between subjects with and those without central obesity (p=0.025 and p=0.002, respectively). In relation to central obesity, a significant association was found for the -11377GG variant (16.1% vs. 3.7%,). The frequency of the -11377C and -11377G alleles was significantly different between the two groups (p=0.031), and the carriers of the -11377C allele were more frequent among the subjects who did not meet the criteria for central obesity (73.4% vs. 61.8%). The G allele and the GG genotype of the -11377 SNP significantly increased the risk for the development of central obesity [OR 1.71 (95%Cl 1.03-2.85), p=0.032 and OR 5.57 (95%Cl 1.42-21.82), p=0.014, respectively]. The -11377CC genotype decreased the odds ratio for the development of central obesity compared with G homozygotes [OR 0.17 (95%Cl 0.05-0.70), p=0.014]. The frequencies of the -11377CG

genotype were not different between the groups and the odds ratio was not significant. The frequency of -11391GG SNP was significantly lower among the subjects with central obesity (44.1% vs. 72.8%). The frequency of the –11391G and -11391A alleles was significantly different between the two groups (p=0.001) and the carriers of the -11391A allele were more frequent among the subjects with central obesity (29.4% vs. 14.2%). The A allele and the GA genotype of the -11391G>A SNP significantly increased the risk of central obesity [OR 2.52 (95%Cl 1.34-4.72), p=0.002 and OR 3.37 (95%Cl 1.68-6.75), p<0.001, respectively]. No significant OR was established for the AA homozygotes at -11391 position since only two subjects in the group with central obesity and one in the group without central obesity were found. Each mutation of G>A allele at position -11391 significantly increased the odds ratio for central obesity [OR 3.40 (95%Cl 1.71-6.74), p<0.001].

Associations of ADIPOQ -11377C>G and -11391G>A polymorphisms with metabolic parameters

Analysis of association of *ADIPOQ* -11377C>G and -11391G>A with hypertriglyceridemia is presented in Table 4. The genotype distributions at position -11391 were different between the groups of subjects with (TG≥1.695 mmol/L) and without (TG<1.695) (p=0.048). hypertriglyceridemia. Among subjects with hypertriglyceridemia, the frequency of -11391GG was lower compared to those without hypertriglyceridemia (43.8% vs. 64.2%), and mutation of G>A allele at position -11391 significantly increased the incidence of hypertriglyceridemia [OR 2.30 (95%Cl 1.04-5.08), p=0.04]. The frequency of the -11391G and 11391A alleles was significantly different between the two groups (p=0.025) and the carriers of the -11391A allele were more frequent among subjects with increased TG levels

(31.2% vs. 18.4%). We found no association of *ADIPOQ* -11377C>G variants with hypertriglyceridemia.

Association between other analyzed parameters and -11377C>G and -11391G>A SNPs did not reach statistical significance.

Associations of ADIPOQ -11377C>G and -11391G>A polymorphisms with MetS

We compared the genotype distribution of *ADIPOQ* -11377C>G and -11391G>A in the group of subjects who met (MetS group) and the group who did not meet the criteria for MetS (control group) to examine if they were significantly related to MetS subjects. Distribution frequency of -11377C>G and -11391G>A *ADIPOQ* alleles and genotypes of the studied subjects are shown in Table 5. The *ADIPOQ* genotype distributions at positions -11377 and -11391 were different between MetS and control subjects (p=0.031 and p=0.008, respectively). Among subjects with MetS, the frequency of -11377CC genotype was significantly lower compared to the subjects without MetS (26.5% vs. 51.3%). The subjects with -11377CG genotype had higher risk for development of MetS [OR 2.79, (95%Cl 1.16-6.69), p=0.013] than -11377CC genotype carriers (CC genotype vs. others, χ^2=6.87, p=0.009). C>G mutation at position -11377 significantly increased the risk of MetS development [OR=2.93 (95%Cl 1.26-6.81), p=0.013]. The frequency of the -11377C and -11377G alleles was significantly different between the two groups (p=0.015) and the carriers of the -11377G allele were more frequent in the MetS group (44.1% vs. 28.2%). The G allele at position -11377 significantly increased the risk of MetS [OR 2.00 (95%Cl 1.15-3.50), p=0.015].

The frequency of -11391GG genotype was lower among the subjects with MetS compared to the control subjects (38.2% vs.66.1%). The subjects with -11391GA genotype had higher risk

for the development of MetS [OR=2.92, (95%Cl 1.31-6.54), p=0.009] than the -11391GG genotype carriers (GG genotype vs. others, χ^2=7.72, p=0.005). Compared with the -11391G homozygotes, the carriers of the A allele significantly increased the risk of MetS development [OR=3.15 (95%Cl 1.43-6.95), p=0.005]. The allele frequency at position -11391 was significantly different between the two groups (p=0.005) and the carriers of the -11391A allele were more frequent in the MetS group (33.8% vs. 17.4%). The A allele at position -11391 significantly increased the risk for MetS [OR 2.43 (95%Cl 1.32-4.46), p=0.004].

Haplotype analysis

Test of overall association showed statistically significant correlation of MetS with *ADIPOQ* -11377C>G and -11391G>A haplotypes (χ^2= 11.72; dF=3 p=0.008), with specific haplotype -11377G-11391A having the strongest association (OR=4.09, 95%CI 1.61-10.41, p=0.001). Also, statistically different concentrations of triglycerides were observed according to different haplotypes (χ^2= 10.34 dF=3 p=0.016), with -11377G-11391A haplotype contributing to the highest triglyceride levels (χ^2 = 7.79, p=0.005).

Discussion

The goal of this study was to test the role of adiponectin level and gene variability as markers of abdominal obesity and MetS in a cohort of young adults of Croatian (Caucasian) origin. In this investigation, waist circumference was used as a valid marker of central obesity. Central obesity was detected in 46,6% of women and 44,6 % of men, which is similar to the data

described for the entire population of Croatian origin (5). The research of scale and dynamics of overweight and obesity epidemic in Croatia showed that in the recent years the age-stratified prevalence of obesity showed the highest increase in a young group of adults (18-34 years) (32), which is why we chose them as subjects in our study. The youngest adults should be treated as a priority population group that requires substantial public health intervention aimed to reduce the epidemic of obesity and obesity comorbidities. As a marker of central obesity, WC is one of the diagnostic criteria of MetS because subjects with central obesity have been suggested to be more prone to develop MetS than lean subjects (33). According to IDF diagnostic criteria, 50.75% of subjects with central obesity have MetS in our study. This means that, although progression to MetS occurs more frequently in abdominal obese humans compared with lean subjects, this association is highly dependent on genetic background (34). MetS was defined in 22.82% of participants. The prevalence of MetS in the adult population differs depending on the diagnosis criteria and ethnicity, and usually varies between 22 and 39% (35). Unfortunately, the lack of published data on the MetS prevalence in general Croatian population makes the comparison between the investigated young adult populations and general population difficult. Adiponectin is the most abundant adipokine in human plasma and low levels of adiponectin seem to be associated with poor prognosis (36). The data presented in this study support this association. In this study, adiponectin was significantly lower in subjects with increased WC and in subjects with developed MetS, which is consistent with the claim that hypoadiponectinemia may represent the dysfunction of adipose tissue in obesity and is closely associated with the clinical phenotype of MetS (37, 38). We demonstrated significant correlations between adiponectin plasma concentration and clinical (BMI, WC, SBP and DBP) and metabolic variables (HDL-C, TG, GLUC). Other authors have also found serum adiponectin levels to be negatively correlated with BMI and WC (6) and with adverse features of MetS (11). Because both adiponectin and metabolic

variables, as well as blood pressure, were correlated with BMI and WC, and because the concentration of adiponectin is sex-dependent, we made the necessary adjustment in analyses for BMI, WC and sex, but the correlations between adiponectin, HDL-C, TG, GLUC, SBP and DBP were still significant. The results indicate that adiponectin and those variables were independent of sex and anthropometric variables (BMI and WC). The present study shows the protective/positive influence of adiponectin level on the components of MetS. Some other reports have also identified the significant role of adiponectin in the development of MetS and of metabolic comorbidities (12), and have suggested that measuring the plasma concentration of adiponectin may be a useful biomarker (39). However, regarding the relationship between adiponectin and lipid profile indicators, there are still contradicting findings and no general consensus has been achieved. We did not find significant correlation between adiponectin and LDL-C and TC, which indicates that the effects of adiponection on LDL-C and TC are indirect. While some authors found significant relationship between adiponectin and lipid parameters (40-42), others could not confirm these findings (43). Studies in humans have shown that adiponectin decreases body weight by increasing lipid oxidation in muscles and in other organs such as the pancreas and liver (44). In this study, a statistically significant correlation between SBP, DBP and plasma adiponectin concentration was found, which is in agreement with published data on association between decreased plasma adiponectin levels and elevated blood pressure (45). Adamczak et al. reported on significantly lower levels of adiponectin in patients with essential hypertension compared to normotensive controls (46). While the presence of MetS has been linked to decreased adiponectin values, the association of *ADIPOQ* variants with MetS and its components remains vague (15). The effect of the *ADIPOQ* gene on the risk of obesity and MetS may vary according to ethnicity, age and the degree of obesity across populations (47). Some studies indicate that *ADIPOQ* variants may contribute to MetS and its components (12,20,36,48) while other reports point to the limited

impact of *ADIPOQ* gene on MetS parameters (15,49). In this study, we found *ADIPOQ* promoter variants -11377C>G and -11391G>A, independently and as haplotypes that are associated with elevated blood lipids, central obesity and MetS. Minor -11377G and -11391A alleles were associated with central obesity and MetS, with the -11391A variant having shown more significant impact. The associations in this study also extend to another feature of the MetS, i.e. increased serum concentrations of triglyceride in -11391A carriers. Also, we found a correlation between -11377G-11391A haplotype and a risk of development of MetS, where this haplotype also contributes to the highest triglyceride levels. Similar results were obtained in other studies in other ethnic groups with regard to associations of rare -11377G (9,18,36, 47,50), or -11391G>A (51) allele/genotype/haplotype carriers with obesity and obesity-related phenotypes. In contrast, some other studies demonstrated that the common -11377C allele was strongly associated with obesity and MetS parameters (17,52). Our findings are also consistent with previous reports in different populations and different age groups (53,54,55) stating that *ADIPOQ* gene may play an important role in MetS. Results of these reports pointed that regional differences in body fat affected the risk of metabolic abnormalities, and also *ADIPOQ* gene variants were suggested as modulators of visceral fat accumulation. Identification of genes controlling adiponectin levels may aid our understanding of how genes influence MetS and possibly obesity (56). Many study results were published on associations of *ADIPOQ* SNPs (27,36,57) as well as haplotypes with plasma adiponectin concentrations (22, 54), but the data are controversial. Association between serum adiponectin level and *ADIPOQ* variants represents one of the several mechanisms that can be considered as a link between *ADIPOQ* and MetS. Other mechanisms that could explain the *ADIPOQ*-MetS association are: insulin resistance, WC, body fat distribution, lipid profile. Although the molecular mechanisms underlying the pathogenesis of MetS are still far from being fully understood, insulin resistance is in general considered to be

the core defect of MetS (58). The association of plasma adiponectin and insulin sensivity was also determined in humans, and lower plasma adiponectin levels were found in more insulin resistant subjects (21).

No significant associations were found between plasma adiponectin values and selected *ADIPOQ* SNPs in this study. The reasons for this may be the following : i) limited number of subjects, ii) we measured total adiponectin and did not separate the trimere and hexamere structure of adiponectin, limiting the clinical interpretation because recent evidence has suggested that the high-molecular-weight adiponectin may be more strongly related to several characteristics of the metabolic syndrome complex (59) iii) we analyzed only two promoter SNPs while for some other *ADIPOQ* SNPs have also been reported to be independently associated with serum adiponectin. Interactions with other genes related to susceptibility to obesity and MetS could have interfered. Various studies reported a multitude of loci identified in different chromosomes, varying across populations and reflecting substantial complexity of the metabolic syndrome etiology (60-63).

Potential limitations of our study should be mentioned:

A rather low number of subjects was included. The effects of environmental factors, controlling the risk of obesity and MetS, were not evaluated in this study. The effects of the candidate gene markers could be validly evaluated after controlling for environmental factors (e.g. physical activity, diet, smoking) and ethnicity (6, 55). Finally, the answer to the question of whether or not the findings from this study can be generalized to Croatian population remains uncertain because analysis was performed in a rather small group.

In spite of these limitations, the data obtained suggest that analysis of adiponectin concentrations and -11391G>A and -11377C>G *ADIPOQ* gene promoter variants could

provide information that could be clinically meaningful for estimation of the risk of metabolic syndrome and its traits in young adult population. This points to the need of personalized behavioral recommendations for prevention of chronic disorders.

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Conflict of Interest Statement: None.

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Table 1. Clinical features of study participants

	All subjects	Without MetS	With MetS	p-value
n	149	115	34	
***BMI (kg/m^2)**	24.17±5.03	23.04±3.72	30.29 ±4.69	<0.001
WC (cm)	88.14±15.6	82.4±11.5	107.3±11.9	<0.001
***TG (mmol/L)**	1.05±1.01	0.95±0.54	1.88±1.32	<0.001
TC (mmol/L)	4.67±1.08	4.53±0.96	5.13±1.32	0.008
HDL-C (mmol/L)	1.42±0.34	1.50±0.32	1.13±0.20	<0.001
***LDL-C (mmol/L)**	2.69±0.93	2.40±0.85	3.00±1.06	0.004
***GLUC (mmol/L)**	5.06±0.59	5.00±0.43	5.30±0.85	<0.001
***DBP (mmHg)**	80.0±10.9	80.0±8.3	87.5±14.3	<0.001
***SBP (mmHg)**	120.0±17.79	120.0±10.7	138.5±17.5	<0.001
***Adiponectin (mg/L)**	9.0±7.74	12.36±7.84	4.77±2.09	<0.001

BMI, body mass index; WC, waist circumference; TG, triglycerides, TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLUC, glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Data are expressed as mean or *median ± standard deviation (SD) depending on parametric or nonparametric distribution.

Differences between groups were evaluated by Student's t-test or Mann-Whitney test, depending on distribution normality.

Table 2. Spearman Rank Correlations Coefficients of the adiponectin with different clinical parameters in the whole subject group (n = 149)

Parameter	Correlation coefficient (r)	95 % CI for r	p-value
BMI (kg/m^2)	-0.434	-0.561 to -0.288	<0.001
WC (cm)	-0.467	-0.616 to -0.361	<0.001
TG (mmol/L)	-0.499	-0.517 to -0.231	<0.001
TC (mmol/L)	-0.031	-0.197 to 0.137	0.718
HDL-C (mmol/L)	0.540	0.410 to 0.649	<0.001
LDL-C (mmol/L)	-0.131	-0.292 to -0.037	0.124
GLUC (mmol/L)	-0.214	-0.368 to -0.485	0.012
DBP (mmHg)	-0.387	-0.523 to -0.233	<0.001
SBP (mmHg)	-0.346	-0.487 to -0.187	<0.001

BMI, body mass index; WC, waist circumference; TG, triglycerides, TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLUC, glucose; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 3. Distribution of the -11377C>G and -11391G>A *ADIPOQ* genotypes and alleles in subjects without (WC< 94 cm (M); WC< 80 cm (F); control) and with (WC≥ 94 cm (M); WC≥ 80 cm (F)) central obesity.

Genotype /allele	Control (n=81)	Central obesity (n=68)	OR (95% CI), p	[a]Specific p	p
-11377C>G	n (%)	n (%)			
CC	41 (50.6)	27 (39.7)	1 (1-1)	χ^2=1.84, p=0.175	χ^2=7.36;
CG	37 (45.7)	30 (44.1)	1.23 (0.62-2.44), 0.551	χ^2=0.04, p=0.846	df=2
GG	3 (3.7)	11 (16.2)	5.57 (1.42-21.82), 0.014	χ^2=8.98, p=0.003	p=0.025
C	119 (73.5)	84 (61.8)	1 (1-1)		χ^2=4.65; df=1,
G	43 (26.5)	52 (38.2)	1.71 (1.03-2.85), 0.032		p=0.031
-11391G>A	n (%)	n (%)			
GG	59 (72.8)	30 (44.1)	1 (1-1)	χ^2=12.59, p<0.001	χ^2= 12.82;
GA	21 (25.9)	36 (52.9)	3.37 (1.68-6.75), <0.001	χ^2=11.42, p<0.001	df=2
AA	1 (1.2)	2 (2.9)	3.93 (0.34-45.15), 0.271	χ^2=0.61, p=0.436	p=0.002
G	139 (85.8)	96 (70.6)	1 (1-1)		χ^2=10.29;df=1,
A	23 (14.2)	40 (29.4)	2.52 (1.34-4.72), 0.002		p=0.001

WC, waist circumference; M, male and F, female; OR, odds ratio

[a]Specific p = individual *vs*. other genotypes

Comparisons of subject groups were performed using χ^2 test or Fisher's exact test.

Table 4. Distribution of the -11377C>G and -11391G>A *ADIPOQ* genotypes and alleles in subjects without (TG<1.695 mmol/L) and with (TG≥1.695mmol/L) hypertriglyceridemia

Genotype /allele	TG < 1.695 mmol/L (n=117)	TG > 1.695 mmol/L (n=32)	p
-11377C>G	n (%)	n (%)	
CC	58 (49.6)	10 (31.3)	χ^2=4.084;df=2
CG	50 (42.7)	17 (53.1)	p=0.130
GG	9 (7.7)	5 (15.6)	
C	166 (70.9)	37 (57.8)	χ^2=3.988; df=1
G	68 (29.1)	27 (42.2)	p=0.046
-11391G>A	n (%)	n (%)	
GG	75 (64.1)	14 (43.8)	χ^2=6.069; df=2
GA	41 (35.0)	16 (50.0)	p=0.048
AA	1 (0.9)	2 (6.3)	
G	191 (81.6)	44 (68.8)	χ^2=4.996; df=1
A	43 (18.4)	20 (31.2)	p= 0.025

TG, triglycerides;

Comparisons of subject groups were performed using χ^2 test or Fisher's exact test.

Table 5. Distributions of the -11377C>G and -11391G>A *ADIPOQ* genotypes in the group of subjects with and without MetS

Genotype/ allele	Control (n=115)	MetS (n=34)	OR (95% CI), p	[a]Spec.p	p
-11377C>G	n (%)	n (%)			
CC	59 (51.3)	9 (26.5)	1 (1-1)	χ^2=6.87, p=0.009	χ^2 = 6.96;df=2
CG	47 (40.9)	20 (58.8)	2.79 (1.16-6.69), 0.013	χ^2=3.35, p=0.067	p=0.031
GG	9 (7.8)	5 (14.7)	3.64 (0.99-13.35), 0.052	χ^2=1.14, p=0.285	
C	165 (71.7)	38 (55.9)	1 (1-1)		χ^2 = 5.86;df=1
G	65 (28.2)	30 (44.1)	2.00 (1.093.67), 0.015		p=0.015
-11391G>A	n (%)	n (%)			
GG	76 (66.1)	13 (38.2)	1 (1-1)	χ^2=7.72, p=0.005	χ^2=9.65; df=2
GA	38 (33.0)	19 (55.9)	2.92 (1.31-6.54), 0.009	χ^2=5.29, p=0.021	p=0.008
AA	1 (0.9)	2 (5.9)	11.69 (0.99-138.4), 0.051	χ^2=2.62, p=0.106	
G	190 (82.6)	45 (66.2)	1 (1-1)		χ^2=7.87; df=2
A	40 (17.4)	23 (33.8)	2.43 (1.22-4.83), 0.004		p=0.005

MetS, metabolic syndrome; OR, odds ratio

[a]Specific. p = individual *vs.* other genotypes

Comparisons of subject groups were performed using χ^2 test or Fisher's exact test.