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Relation of a variant in adiponectin gene (rs266729) with metabolic syndrome and diabetes mellitus type 2 in adult obese subjects

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Abstract. – OBJECTIVE: Some studies in the literature indicate that ADIPOQ rs266729 polymorphism functionally regulates adiponectin promoter activity and secondarily adiponectin levels. The aim of the present investigation was to describe the association of rs266729 with diabetes mellitus (DM2), components of Metabolic syndrome (MS) and serum adiponectin levels.

PATIENTS AND METHODS: The study involved a population of 1004 adult obese subjects. Measurements of anthropometric parameters, blood pressure, fasting blood glucose, C-reactive protein (CRP), insulin concentration, insulin resistance (HOMA-IR), lipid profile, adipokines levels and prevalence of MS and DM2 were recorded. The genotype of ADIPOQ gene polymorphism (rs266729) was evaluated.

RESULTS: The distribution of the rs266729 polymorphism in this population was 56.7% (n=569) (CC), 33.1% (n=332) (CG) and 10.2% (n=103) (GG). Insulin and HOMA-IR levels were higher in G allele carriers than non G allele carriers. Adiponectin levels were lower in G allele carriers than non G allele carriers. In total group carriers of G allele, logistic regression analysis showed an increased risk of hyperglycaemia (OR=1.70, 95% CI=1.19-2.76, *p*=0.03) and prevalence of diabetes mellitus type 2 (OR=1.81, 95% CI=1.13-5.14, *p*=0.04), after adjusting by body mass index and age

CONCLUSIONS: G allele of SNP (rs266729) of the ADIPOQ gene showed high values of insulin and HOMA-IR, and low values of adiponectin levels than non G allele carriers. G allele carriers showed higher rate of diabetes mellitus and hyperglycemia.

Key Words:
Rs266729, Diabetes mellitus type 2, Adiponectin, Metabolic syndrome.

Introduction

Metabolic syndrome (MS) is defined by the clustering of several factors; abdominal obesity, glucose intolerance and/or insulin resistance, dyslipemia and increased blood pressure[1]. In the other hand, diabetes mellitus type 2 (DM2) is an important health problem, ranging from predominantly insulin resistance to predominantly an insulin secretory defect with insulin resistance. Its prevalence is increasing rapidly because of population and surge of obesity in many countries. Some studies pointed out that the total prevalence of DM2 was 13.8% in Spanish population[2], so it is important to investigate for the causes and risks of DM2, one factor of these causes is genetic factors and advances have enable the identification of a number of genes associated with DM2 risk. One of these target genes is the adiponectin gene and several studies investigated the SNPs of this gene.

Adiponectin is the most quantitatively abundant adipokine produced by adipocytes, which is known to regulate insulin sensitivity[3] and concentrations of adiponectin are reduced in obese subjects. Adiponectin is encoded by the adiponectin C1Q and collagen domain containing (*ADIPOQ*) gene, which located on chromosome 3q27. In humans, associations have been found between many polymorphisms in ADIPOQ and adiponectin levels, insulin resistance and obesity[4]. The most common single nucleotide polymorphism (SNP) of this gene is rs266729 (-11,377C>G), it is located in the proximal promoter region of the *ADIPOQ* gene. Some scholars[5] in the literature indicate that *ADIPOQ* rs266729 polymorphism functionally regulates adiponectin promoter activity and secondarily adiponectin levels. This *ADIPOQ* variant has been identified to be associated with adiponectin levels, high body mass index, insulin resistance and diabetic nephropathy[6-8]. This gene variant rs266729 has been reported to be associated with DM2[9,10] in some populations. Thus, this present study is important because it is the first

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one that investigated the relationship of this SNP with diabetes mellitus type 2 and metabolic syndrome in the same Caucasian population.

The aim of the present investigation was to describe the association of rs266729 with diabetes mellitus type 2, components of MS and plasma adiponectin levels.

Patients and Methods

Subjects and Clinical Investigation

The population studied was selected from obese patients sent by the primary care physicians of our Health Area, obesity is defined by a body mass index (BMI) ≥30 kg/m^2. One thousand and four obese Caucasian subjects were enrolled in a non-probabilistic consecutive method of sampling from 24 Primary Care Centers of our Health Area. In the recruitment, we only took into account the temporary order of referral of patients from their primary care physician to our Nutrition Unit, as long as they met the inclusion criteria. The recruited subjects fulfilled the following inclusion criteria; body mass index ≥30 kg/m^2, had no a history of cardiovascular disease, thyroid disease, renal or hepatic disorders, had no history of alcoholism, malignant tumor, bariatric surgery, and within the 6 months before the study were not receiving medications known to influence lipid levels (statins, hormonal therapy, glucocorticoids and anti-inflammatory drugs). The exclusion criteria were ages under 18 years or older than 65 years, BMI over 45 kg/m^2 years old, and a dietary treatment 6 months prior to the current study.

The Ethics Committee (HCUVA Committee) approved the study and was in accordance with the guidelines laid down in the Declaration of Helsinki. All participants provided written informed consent. After signed consent was obtained, all participants underwent a medical evaluation including physical examination and complete medical history. Data on blood pressure, anthropometric parameters [weight, height, body mass index (BMI), fat mass by impedance and waist circumference] have been collected. Venous blood samples were collected in EDTA-treated and plain tubes after a 10 hour overnight fasting state for analysis of plasma C reactive protein (CRP), adipokine levels (leptin, total adiponectin and resistin), insulin, total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. To estimate the prevalence of diabetes mellitus type 2 was considered American Diabetes Association criteria (elevated fasting glucose above 126 mg/dl or HBA1c >6.5% or glucose oral overload with a glucose levels at 120 minutes above 200 mg/dl)[11] and the prevalence of Metabolic Syndrome (MS), the definitions of the ATPIII was considered[1]. Subjects need to fulfill at least 3 of the following 5 criteria in order to be diagnosed of MS; hyperglycaemia elevated fasting glucose or treatment for diabetes as hyperglycemia, elevated triglycerides (>150 mg/dl) or treatment for dyslipidemia, low HDL cholesterol < 40 mg/dl (males) or <50 mg/dl (females), elevated systolic or diastolic blood pressure (>130/85 mmHg or antihypertensive treatment) and increased waist circumference [>94 cm (males) or >80 cm (females)].

Anthropometric Parameters and Blood Pressure

Body weight was measured in the morning while the subjects were unclothed and not wearing shoes. They were measured using scales (Omrom, Los Angeles, CA, USA) and recorded to the nearest 50 g. Height was measured with a tape measure (Omrom, Los Angeles, CA, USA) while patients were standing with shoulders in normal alignment and no wearing shoes. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Waist circumferences (WC) were measured at the umbilical level with the use of an upstretched tape measure while the subjects were standing after normal expiration. Bio impedance was used to determine body composition with an accuracy of 5 g[12] (EFG, Akern, Pisa, IT). Mean systolic and diastolic blood pressures were calculated by averaging three measurements (Omrom, Los Angeles, CA, USA).

Biochemical Procedures

Total cholesterol and triglyceride levels were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, NY, USA), while HDL cholesterol was measured in the supernatant after precipitation of other lipoproteins by enzymatic methods. LDL cholesterol was calculated using Friedewald formula (LDL cholesterol=total cholesterol-HDL cholesterol-triglycerides/5)[13]. Glucose levels were measured by an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, CA, USA). Insulin was determined by radioimmunoassay (RIA) (RIA Diagnostic Corporation, Los Angeles, CA, USA) with a sensitivity of 0.5

mUI/L (normal range 0.5-30 mUI/L)[14] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these value[15]. CRP was measured by immunoturbidimetry (Roche Diagnostics GmbH, Mannheim, Baden-Wurtemberg, Germany), with a normal range of (0-7 mg/dl) and analytical sensitivity 0.5 mg/dl. All adipokines were determined by enzyme linked immunosorbent assay (ELISA); resistin (Biovendor Laboratory, Inc., Brno, Moravia, Czech Republic) with a sensitivity of 0.2 ng/ml and a normal range of 4-12 ng/ml[16], adiponectin (R&D systems, Inc., Minneapolis, MN, USA) with a sensitivity of 0.24 ng/ml and a normal range of 8.63-21.42 ng/ml[17] and leptin (Diagnostic Systems Laboratories, Inc., Houston, TX, USA) with a sensitivity of 0.05 ng/ml and a normal range of 11-100 ng/ml[18].

Genotyping ADIPOQ Gene

Hardy Weinberg equilibrium was assessed with a statistical test (Chi-square) to compare our expected and observed counts. The variant was in Hardy Weinberg equilibrium (p=0.31). DNA was isolated from blood samples using QIAamp® DNA blood kit following the manufacturer's instructions. To extract DNA we use 200 uL of blood. Oligonucleotide primers and probes were designed with the Beacon Designer 5.0 (Premier Biosoft International®, Los Angeles, CA, USA). The Polymerase Chain Reaction (PCR) was carried out with 50 ng of genomic DNA, 0.5 uL of each oligonucleotide primer (primer forward: 5'-ACGTTGGATGATGTGTGGCTTGCAAGAACC-3' and reverse 5'-ACGTTGGATGCAACATTCAACACCTTGGAC-3' in a 2 uL final volume (Termociclador Life Tecnologies, Los Angeles, CA, USA). DNA was denatured at 90ºC for 2 min; this was followed by 50 cycles of denaturation at 90ºC for 20 s and annealing at 56.1°C for 50 s). The PCR were run in a 25 uL final volume containing 10.5 uL of IQTM Supermix (Bio-Rad®, Hercules, CA, USA) with hot start Taq DNA polymerase. We used as internal standard for RT-PCR (GAPDH) with a forward sequence: GTCTCCTCTGACTTCAA and reverse sequence: ACCACCCTGTTGCTGTA.

Statistical Analysis

All the data were analyzed using SPSS for Windows, version 19.0 software package (SPSS Inc. Chicago, IL, USA). Sample size was calculated to detect differences over 5 ng/dl of adiponectin levels between genotype groups with 90% power and 5% significance. All analysis was performed under a dominant genetic model with rs266729 G- allele as the risk allele (GG+GC *vs.* CC). The results were expressed as average ± standard deviation. Variables were analyzed with ANOVA test (for normally distributed variable) or Kruskal-Wallis test (for non-normally-distributed variable). Logistic regression analyses adjusted by age, gender and BMI were used to calculated odds ratio (OR) and 95% confidence interval (CI) to estimate the association of the rs266729 SNP with the risk of Metabolic syndrome, components of MS and diabetes mellitus type 2. A p-value under 0.05 was considered statistically significant.

Results

The sample comprised of 1004 Caucasian obese subjects. The mean age was 45.9±6.1 years (range: 26-64) and the mean body mass index (BMI) 36.4±5.1 kg/m^2 (range: 31.4-41.1). Gender distribution was 735 females (73.2%) and 269 males (26.8%). The distribution of the rs266729 polymorphism in this adult population was 56.7% (n=569) (CC), 33.1% (n=332) (CG) and 10.2% (n=103) (GG). The allele frequency was C (0.73) and G (0.27). The subjects were grouped into two genotype groups (CC *vs.* CG+GG). Age was similar in both genotype groups (CC; 46.1±6.1 years *vs.* CG+GG; 45.6±7.2 years: ns). Gender distribution was similar in both genotype groups (CC 26.9% males *vs.* 73.1% females *vs.* CG+GG; 26.6% males *vs.* 73.4% females).

By applying a dominant genetic model, we did not detect a significant association between rs266729 G-allele and fat mass, weight, waist circumference, blood pressure and BMI in the total group. We did not find statistically significant differences between the genotypes in the analysis carried out taking into account females and males of isolated groups (Table I). Males had higher weight, fat mass and waist circumference than females in both genotype groups.

Biochemical characteristics according to genotype are shown in Table II. In males, insulin levels (CC *vs.* CG+GG: delta: 2.7±1.2 UI/L; p=0.03) and HOMA-IR (delta: 1.1±0.8 units; p=0.01) were higher in G allele carriers than non G allele carriers. In females, insulin levels (CC vs CG+GG: delta: 2.5±1.0 UI/L; p=0.02) and HOMA-IR (delta: 0.9±0.5 units; p=0.01) were higher in G allele carriers than non G allele carriers (Table II). In both genotypes, HOMA-IR and insulin levels

Table I. Anthropometric variables and blood pressure.

Parameters	Total group (n = 1004)		Male (n = 269)		Female (n = 735)	
	CC n = 569	CG + GG n = 435	CC n = 152	CG + GG n = 117	CC n = 417	CG + GG n = 318
BMI	36.2 ± 5.0	36.5 ± 5.1	36.3 ± 4.8	36.6 ± 4.9	36.0 ± 4.2	36.3 ± 3.1
Weight (kg)	94.1 ± 11.0	95.0 ± 9.7	106.3 ± 12.1*	107.8 ± 9.3*	89.6 ± 9.2	90.1 ± 7.7
Fat mass (kg)	39.2 ± 5.1	39.6 ± 4.0	34.0 ± 3.2*	33.8 ± 3.0*	40.9 ± 4.0	41.0 ± 6.3
WC (cm)	111.3 ± 8.0	111.9 ± 7.0	118.1 ± 6.2	137.9 ± 5.1*	108.0 ± 4.1$	109.1 ± 6.3
SBP (mmHg)	127.1 ± 9.1	128.2 ± 8.0	129.2 ± 6.8	130.3 ± 6.1	126.9 ± 6.8	127.1 ± 5.1
DBP (mmHg)	82.0 ± 5.1	82.1 ± 3.9	82.4 ± 5.2	82.3 ± 4.3	81.3 ± 5.1	82.0 ± 4.0

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; *p<0.05, between genders. NO statistical differences between genotype groups.

were higher in males than females. All previous statistical analysis was realized after exclusion of patients using lipid lowering drugs (n=36), antidiabetic drugs (n=39) and antihypertensive drugs (n=99).

Plasma adipokine levels and C reactive protein are shown in Table III. In both genotypes, leptin levels were higher in females than males. In males, adiponectin levels (CC *vs.* CG+GG: delta: 9.0±1.9 ng/ml; p=0.03) and in females (delta: 12.5±1.8 ng/ml; p=0.01) were lower in G allele carriers than non G allele carriers.

The frequency of patients with metabolic syndrome, different components of MS (central obesity, hypertriglyceridemia, hypertension or hyperglycemia) and diabetes mellitus type 2 have been reported in Table IV. According to the results of metabolic characteristics, the percentage of individuals who had metabolic syndrome (MS) was 53.6% (n=540) and 46.4% patients without MS (n=464). In both genotypes, metabolic syndrome rate, percentage of central obesity, percentage of hyperglycemia and diabetes mellitus type 2 prevalence were higher in males than females. G allele carriers had a higher percentage of hyperglycemia and diabetes mellitus type 2. This statistical association of G allele with DM2 and rate of hyperglycemia was reported in both gender groups (male and female) (Table IV).

In total group carriers of G allele, logistic regression analysis showed an increased risk of hyperglycemia (OR=1.70, 95% CI=1.19-2.76, p=0.03) and prevalence of diabetes mellitus type 2 (OR=1.81, 95% CI=1.13-5.14, p=0.04), after adjusting by BMI and age. This association remained in males; risk of hyperglycemia (OR=1.94, 95% CI=1.13-3.21, p=0.03) and prevalence of diabetes mellitus type 2 (OR=2.03, 95% CI=1.10-6.24, p=0.04). The analysis in female group showed the same results; risk of hyperglycemia (OR=1.61, 95% CI=1.12-2.79, p=0.02) and prevalence of diabetes mellitus type 2 (OR=1.70, 95% CI=1.07-6.02, p=0.03), after adjusting by BMI and age, too.

Table II. Biochemical parameters (mean ± SD).

Parameters	Total group (n = 1004)		Male (n = 269)		Female (n = 735)	
	CC n = 569	CG + GG n = 435	CC n = 152	CG + GG n = 117	CC n = 417	CG + GG n = 318
Fasting glucose (mg/dl)	102.1 ± 8.9	101.9 ± 7.1	103.1 ± 7.1	102.8 ± 6.1	100.1 ± 8.1	99.9 ± 7.1
Total cholesterol (mg/dl)	205.3 ± 21.8	204.6 ± 18.7	202.5 ± 28.6	201.7 ± 21.1	207.5 ± 24.6	207.7 ± 22.1
LDL-cholesterol (mg/dl)	128.0 ± 10.9	126.6 ± 12.1	127.2 ± 12.3	125.3 ± 12.1	128.5 ± 12.1	128.3 ± 16.1
HDL-cholesterol (mg/dl)	53.6 ± 7.1	53.7 ± 8.2	52.9 ± 8.4	52.8 ± 7.9	56.9 ± 8.0	55.3 ± 7.1
Triglycerides (mg/dl)	126.7 ± 28.1	126.6 ± 29.7	140.1 ± 36.9	143.9 ± 37.9	124.1 ± 23.1	123.9 ± 17.1
Insulin (mUI/l)	13.0 ± 5.0	15.8 ± 3.9*	13.3 ± 4.0$	16.0 ± 4.2*$	12.3 ± 4.1	14.8 ± 5.2*
HOMA-IR	3.0 ± 1.0	3.9 ± 0-9*	4.0 ± 1.1$	5.1 ± 1.2*$	2.9 ± 1.1	3.8 ± 1.0*

HOMA-IR (homeostasis model assessment of insulin resistance).; *p<0.05, in CC *vs*. CG+GG genotypes. $$p$<0.05, between genders.

Table III. Serum adipokine levels and C reactive protein (mean±SD).

Parameters	Total group (n = 1004)		Male (n = 269)		Female (n = 735)	
	CC n = 569	CG + GG n = 435	CC n = 152	CG + GG n = 117	CC n = 417	CG + GG n = 318
Resistin (ng/dl)	5.1 ± 1.3	5.2 ± 1.2	5.3 ± 1.2	5.5 ± 1.5	5.0 ± 1.2	5.1 + 1.3
Adiponectin (ng/dl)	21.7 ± 8.0	10.9 ± 5.3*	19.1 ± 8.1	10.2 ± 5.1*	23.9 ± 4.9	11.4 ± 3.0*
Leptin (ng/dl)	66.1 ± 16.9	65.9 ± 12.3	31.2 ± 10.2	35.8 ± 10.4	77.2 ± 8.1$	79.8 ± 10.4$
CRP (ng/dl)	5.3 ± 1.4	5.4 ± 1.5	5.6 ± 1.9	5.7 ± 1.8	5.2 ± 1.1	5.1±.3

HOMA-IR (homeostasis model assessment of insulin resistance).; *p<0.05, in CC *vs*. CG+GG genotypes. $$p$<0.05, between genders.

Discussion

The main finding of this cross-sectional study was the fact that the G allele of SNP (rs266729) of the *ADIPOQ* gene showed high values of insulin and HOMA-IR, and low values of adiponectin levels than non-G allele carriers. G allele carriers showed higher rate of diabetes mellitus and hyperglycemia than non-G allele carriers, too.

According to literature, more than 600 SNPs map within the adiponectin locus. One of these common genetic variants is rs266729. The potential physiological mechanisms to explain the relationship of the insulin levels and HOMA-IR and G-allele of this genetic variant remain unknown. *ADIPOQ* encodes adiponectin expressed exclusively in both brown and white adipose tissues[19]. Recent findings have indicated that G allele alters the sequence for one transcriptional stimulatory protein binding sites (Sp) and secondarily reduces adiponectin promoter activity[20]. Sp1 is associated with adiponectin promotor and Sp1 over-expression enhanced gene promotor activity. Perhaps a molecular pathway relates this genetic variant of the *ADIPOQ* gene with adiponectin secretion and secondarily with insulin resistance and insulin levels. In fact, one study demonstrated that the minor allele G alter the DNA sequence of Sp1 binding site of the transcriptional elements, resulting in a reduction inx the transcriptional activity of adiponectin gene promoter[21]. Thus, this negative regulation leads to low plasma concentration of adiponectin and so high susceptibility of diabetes mellitus. In addition, some studies have demonstrated that there was an association between adiponectin and adipose tissue mass and suggested that adiponectin secretion and circulating levels were inversely proportional to body fat[22]. Another potential hypothesis to explain these associations is a gene-nutrient interaction. Fergusson et al[23] have demonstrate that G allele for this SNPs was identified as having degrees of insulin resistance, and was highly responsive to differences in plasma saturated fatty acids. Unfortunately, in our work we have not measured either the circulating levels of fatty acids or the intake of fats.

Table IV. Metabolic syndrome, components of MetS and diabetes mellitus.

Parameters	Total group (n = 1004)		Male (n = 269)		Female (n = 735)	
	CC n = 569	CG + GG n = 435	CC n = 152	CG + GG n = 117	CC n = 417	CG + GG n = 318
Percentage of MetS	53.6%	53.9%	62.0%	55.5%	40.1%$	43.5%$
Percentage of central obesity	79.5%	82.6%	96.6%	95.6%	73.3%$	77.8%$
Percentage of Hypertriglyceridemia	11.0%	12.0%	14.2%	12.2%	9.8%	10.3%
Low HDL cholesterol	10.6%	12.1%	14.9%	15.3%	11.5%	11.8%
Percentage of Hypertension	43.3%	44.8%	53.8%	50.0%	39.7%	43.1%
Percentage of hyperglycemia	19.8%	30.1%*	23.6%	37.6% *	18.4%$	27.3%*$
Diabetes Mellitus	6.3%	10.3%*	7.2%	13.6%*	5.9%$	9.1%*$

The cut-off points for the criteria of; central obesity (waist circumference >88 cm in female and >102 in male), hypertension (systolic BP>130 mmHg or diastolic BP>85 mmHg or specific treatment), hypertriglyceridemia (triglycerides >150 mg/dl or specific treatment) or hyperglycemia (fasting plasma glucose >110 mg/dl or drug treatment for elevated blood glucose). Diabetes mellitus by American Diabetes Association (ref 10). *p<0.05, in CC vs. CG+GG genotypes. $$p$<0.05 between genders.

The results of our study demonstrated that G allele carriers have increased risk of hyperglycemia and diabetes mellitus type 2, after adjustment for sex, age and BMI. Such data suggested a role of adiponectin gene polymorphism rs266729 in the pathogenesis of DM2 in this Caucasian population. The current findings are consistent with results in Asian Population[24], French Caucasians[25], Swedish Caucasians[26], and Chinese Populations[27]. However, studies in Indians[28], Hispanic and African-American[29] and Chinese populations[30] failed to detect significant relationship with this significant SNP. A possible explanation for these contradictory results in the literature is due to the influence of other genes, which could be in linkage disequilibrium. Other potential hypothesis to explain this fact may be due to differences in study design, sample size, genetic background, and level of BMI, gender or dietary environment. Finally, diabetes mellitus type 2 has a multifactorial polygenic origin, so that an abnormality in the promotor region of ADIPOQ such as rs266729 may act or interact with other environmental or genetic factors and induce to development diabetes

Limitations of our study are firstly that the study has been realized in obese subjects, so the data are not generalizable to the entire population. The second, the design as a cross-sectional design does not allow to extract causality. The third, the lack of assessing the combined effect of other genetic factors on insulin resistance. Finally, we have evaluated only Caucasian subjects, ethnic differences in genetic background and the environment they live in would play a crucial role in genetic effects[31].

Conclusions

The G allele of SNP (rs266729) of the *ADIPOQ* gene showed high values of insulin and HOMA-IR, and low values of adiponectin levels than non-G allele carriers. G allele carriers showed higher rate of diabetes mellitus and hyperglycemia. More studies are necessary to evaluate the role of these associations with possible therapeutic measures in obese patients at risk of diabetes mellitus[32,33].

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the Ethical standards of the Institutional and/or National Research Committee (HVUVA committee 3/2017) and with the 1964 Helsinki Declaration and its later amendments or comparable Ethical standards.

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Informed Consent

Informed consent was obtained from all individual participants included in the study.

Authors' Contribution

D.A. de Luis designed the study and wrote the article; O. Izaola realized nutritional evaluation; R. Aller realized laboratory analysis; D. Primo realized nutritional evaluation.

References

1) Expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Executive summary of the Third Report of the National Cholesterol Education Program (NCEP). JAMA 2001; 285: 2486-2497.
2) Valdés S, García-Torres F, Maldonado-Araque C, Goday A, Calle-Pascual A, Soriguer F. Prevalence of obesity, diabetes and other cardiovascular risk factors in Andalusia (southern Spain). Comparison with national prevalence data. The Diabet.es study. Rev Esp Cardiol (Engl Ed) 2014; 67: 442-448.
3) Yang WS, Chuang LM. Human genetics of adiponectin in the metabolic syndrome. J Mol Med 2006; 84: 112-121.
4) Takahashi M, Arita Y, Yamagata K, Matsukawa Y, Okutomi K, Horie M, Shimomura I, K Hotta, H Kuriyama, S Kihara, T Nakamura, S Yamashita, T Funahashi, Y Matsuzawa. Genomic structure and mutations in adipose-specific gene, adiponectin. Int J Obes Relat Metab Disord 2000; 24: 861-868.
5) Gu HF. Biomarkers of adiponectin: plasma protein variation and genomic DNA polymorphisms. Biomark Insights 2009; 4: 123-133.
6) Bouatia-Naji N, Meyre D, Lobbens S, Séron K, Fumeron F, Balkau B, Heude B, Jouret B, Scherer PE, Dina C, Weill J, Froguel P. ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. Diabetes 2006; 55: 545-550.
7) Yang M, Qiu CC, Chen W, Xu LL, Yu M, Xiang HD. Identification of a regulatory single nucleotide polymorphism in the adiponectin (APM1) gene associated with type 2 diabetes in Han nationality. Biomed Environ Sci 2008; 21: 454-459.

8) Rasmussen-Torvik LJ, Pankow JS, Jacobs DR Jr, Steinberger J, Moran A, Sinaiko AR. The association of SNPs in ADIPOQ, ADIPOR1, and ADIPOR2 with insulin sensitivity in a cohort of adolescents and their parents. Hum Genet 2009; 125: 21-28.

9) Chu H, Wang M, Zhong D, Shi D, Ma L, Tong N, Zhang Z. AdipoQ polymorphisms are associated with type 2 diabetes mellitus: a meta-analysis study. Diabetes Metab Res Rev 2013; 29: 532-545.

10) Han LY. Associations between single-nucleotide polymorphisms (+45TNG, +276GNT, –11377CNG, 11391GNA) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetologia 2011; 54: 2303-2314.

11) Standards of Medical Care in Diabetes-2017: summary of revisions. Diabetes Care 2017; 40 (Suppl 1): S4-S5.

12) Lukaski H, Johson PE. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985; 41: 810-817.

13) Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.

14) Duart MJ, Arroyo CO, Moreno JL. Validation of an insulin model for the reactions in RIA. Clin Chem Lab Med 2002; 40: 1161-1167.

15) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-414.

16) Meier U, Gressner M. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004; 50: 1511-1525.

17) Pfutzner A, Langefeld M, Kunt T. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin Lab 2003; 49: 571-576.

18) Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem 2004; 50: 219-221.

19) Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006; 116: 1784-1792.

20) Gu HF. Biomarkers of adiponectin: plasma protein variation and genomic DNA polymorphisms. Biomark Insights 2009; 4: 123-133.

21) Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. Adiponectin - a key adipokine in the metabolic syndrome. Diabetes Obes Metab 2006; 8: 264-280.

22) Suriyaprom K, Phonrat B, Tungtrongchitr R. Association of adiponectin gene -11377CNG polymorphism with adiponectin levels and the metabolic syndrome in Thais. Asia Pac J Clin Nutr 2014; 23: 167-173.

23) Ferguson JF, Phillips CM, Tierney AC, Pérez-Martínez P, Defoort C, Helal O. Gene-nutrient interactions in the metabolic syndrome: single nucleotide polymorphisms in ADIPOQ and ADIPOR1 interact with plasma saturated fatty acids to modulate insulin resistance. Am J Clin Nutr 2010; 91: 794-801.

24) Alkhateeb A. Genetic association of adiponectin with type 2 diabetes in Jordanian Arab population. Gene 2013; 512: 61-63.

25) Vasseur F, Helbecque N, Dina C. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002; 11: 2607-2614.

26) Harvest FG, Abulaiti A, Ostenson CG. Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type two diabetes in Swedish Caucasians. Diabetes 2004; 53 (Suppl 1): S31-S35.

27) Min Y, Chen JN, Zhu LL, Li XL, Wei JZ. Association of 211377 C/G polymorphism in proximal promoter region of adiponectin gene with type diabetes and diabetes nephropathy. Shandong Med J 2011; 51: 65-66.

28) Gupta V, Khadgawat R, Hon Keung Tony, NG, Kumar S, Aggarwal A, Raghavendra R, Vadlamudi S. A validation study of type 2 diabetes-related variants of the TCF7L2, HHEX, KCNJ11, and ADIPOQ genes in one endogamous ethnic group of north India. Ann Hum Genet 2010; 74: 361-368.

29) An S, Sandy H, Anthony J.G., Ziegler JT, Brown, WM, Haffner SM. Association between ADIPOQ SNPs with plasma adiponectin and glucose homeostasis and adiposity phenotypes in the IRAS family study. Mol Genet Metab 2012; 107: 721-728.

30) Han L.Y. Associations between single-nucleotide polymorphisms (+45TNG, +276GNT, –11377CNG, 11391GNA) of adiponectin gene and type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetologia 2011; 54: 2303-2314.

31) Chu H, Wang M, Zhong D, Shi D, Ma L, Tong N. AdipoQ polymorphisms are associated with type 2 diabetes mellitus: a meta-analysis study. Diabetes Metab Res Rev 2013; 29: 532-545.

32) de Luis DA, Calvo SG, Pacheco D, Ovalle HF, Aller R. Adiponectin gene variant RS rs266729: Relation to lipid profile changes and circulating adiponectin after bariatric surgery. Surg Obes Relat Dis 2018; 14: 1402-1408.

33) de Luis DA, Primo D, Izaola O, Gomez E, Ortola A, Lopez JJ, Aller R. Role of the variant in adiponectin gene rs266729 on weight loss and cardiovascular risk factors after a hypocaloric diet with the Mediterranean pattern. Nutr 2018; 60: 1-5.