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Role of rs1501299 variant in the adiponectin gene on total adiponectin levels, insulin resistance and weight loss after a Mediterranean hypocaloric diet

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ABSTRACT

Background/Aim: Several adiponectin gene (*ADIPOQ*) single nucleotide polymorphisms (SNPS) have been related with adiponectin levels and risk for obesity. Our aim was to analyze the effects of *rs1501299 ADIPOQ gene* polymorphism on total adiponectin levels, insulin resistance and weight loss after a Mediterranean hypocaloric diet in obese subjects.

Methods: A Caucasian population of 82 obese patients was analyzed, before and after 3 months on a Mediterranean hypocaloric diet. Before and after 3 months on a hypocaloric diet, an anthropometric evaluation, an assessment of nutritional intake and a biochemical analysis were performed.

Results: After dietary treatment and in wild type group, weight, BMI, fat mass, leptin levels, systolic blood pressure and waist circumference decreases were similar to the mutant type group. In wild type group, the decrease in total cholesterol was −28.1 ± 15.3 mg/dl (mutant group: −12.6 ± 16.7 mg/dl:p = 0.009), LDL- cholesterol was −31.8 ± 20.5 mg/dl (−12.2 ± 11.5 mg/dl:p = 0.006), fasting glucose plasma −4.8 ± 2.5 mg/dL (−0.5 ± 0.1 mg/dL:p = 0.02), insulin −3.6 ± 1.5 mUI/L (+0.6 ± 1.1 mUI/L:p = 0.02) and HOMA-IR −1.2 ± 0.9 (−0.1 ± 1.1: p = 0.03).

Conclusion: The present study suggests that T allele of *ADIPO* (rs1501299) could be a predictor of a lack of response of HOMA-IR, insulin, fasting glucose and LDL cholesterol secondary to a Mediterranean hypocaloric diet in obese subjects.

1. Introduction

Adiponectin expressed in adipose tissue is an insulin sensitizer that regulates glucose tolerance in different tissues such as the liver and muscle and modulate energy homeostasis, too [1]. The evidence from human studies supports a main role for this adipokine in pathophysiology of metabolic syndrome and most of its components [2]. Concentrations of adi-

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ponectin are decreased in persons who are obese [3] and some studies have reported a decrease risk for type 2 diabetes mellitus with increasing levels of adiponectin [4].

Adiponectin levels have a very important genetic component, with heritability estimated 50% [5]. Several adiponectin gene (*ADIPOQ*) single nucleotide polymorphisms (SNPs) have been shown to influence adiponectin levels. One of the most commonly studied SNPs at the *ADIPOQ* locus are a G to T substitution in intron 2 (+276G > T, rs1501299). The G allele has been associated either with increased or decreased concentrations of plasma adiponectin in Caucasians populations [6,7]. Moreover, this allele has been positively [8] and negatively (9) associated with obesity in some populations. The relationship of this SNP with insulin resistance is contradictory, too [10–12].

These previous inconsistencies may be due to unknown interactions between the environmental influences and the gene, such as dietary factors. In the literature, results from interventional and observational studies have showed that diets high in unsaturated fat [13] and low in carbohydrates [14] might increase adiponectin levels. The effect of genetic variant rs1501299 of adiponectin gene on changes secondaries to weight loss has been scare investigated. Previous studies with this variant are cross sectional studies [15,16] and only one study [17] was an interventional study with fish oil without a hypocaloric diet as the main intervention.

Our aim was to analyze the effects of *rs1501299 ADIPOQ gene* polymorphism on total adiponectin levels, insulin resistance and weight loss after a Mediterranean hypocaloric diet in obese subjects.

2. Materials and methods

2.1. Subjects and procedure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Hospital Clinico Universitario Valladolid (HCUVA) ethics committee. A population of 82 Caucasians subjects with obesity (body mass index $\geq$ 30) was analyzed in a non randomized interventional study. The recruitment of subjects was a consecutive method of sampling among patients send from Primary Care Physicians with obesity to a Nutrition Clinic Unit (Valladolid, Spain) and all participants provided informed consent to a protocol approved by the local ethical review boards.

Inclusion criteria were body mass index $\geq$ 30, absence of a diet during the 6 months prior to the study and an adult age ranged from 20 to 65 years. Exclusion criteria included history of cardiovascular disease or stroke during the previous 24 months, total cholesterol > 200 mg/dl, triglycerides > 200 mg/dl, blood pressure > 140/90 mmHg, as well as the use of metformin, sulfonylurea, dypeptidil type IV inhibitors drugs, thiazolidinedions, insulin, glucocorticoids, antineoplasic agents, angiotensin receptor blockers, angiotensin converting enzyme inhibitors, psychoactive medications, statins and other antidyslipidemic drugs.

2.2. Procedure

Venous blood specimens (5 ml) were collected in EDTA-treated and plain tubes after a minimun 8-h overnight fast. Basal and after 3 months of intervention; fasting glucose, c-reactive protein (CRP), insulin, insulin resistance as homeostasis model assessment (HOMA-IR), total cholesterol, LDL-cholesterol, HDL-cholesterol, plasma triglycerides concentration and adipokines levels (leptin, adiponectin and resistin) were measured. Weight, height, and blood pressure measures were measured within the start of the trial and repeated 3 months of intervention. A bioimpedance was performed in order to measure fat mass. These measures were performed at same time of the day (morning). Genotype of *ADIPOQ* gene (*rs1501299*) was studied.

2.3. Dietary intervention

The study duration was 12 months, from the inclusion to the first patient until the end of the last patient. The lifestyle modification program was a Mediterranean hypocaloric diet (1502 calories per day) during three months, the distribution of calories from macronutrient was; 52% of carbohydrates, 25% of lipids and 23% of proteins. Percentage of fats was: 50.8% of monounsaturated fats, 37.4% of saturated fats and 11.8% of polyunsaturated fats. All participants had two individual sessions (60 minutes with diet sheets and example menu plans) with the dietitian at the start of the trail to explain the diet. A dietitian assessed the adherence of this diet each 14 days with a phone call, in order to improve compliment of the calorie restriction and macronutrient distribution. All enrolled subjects received instruction to record their daily dietary intake for three days including a weekend day. Records were reviewed by a dietitian and analysed with a computer-based data evaluation system (Dietosource®, Ge, Swi). National composition food tables were used as Ref. [18].

2.4. Assays

Plasma glucose levels were determined by using an automated glucose oxidase method (Glucose analyser 2, Beckman Instruments, Fullerton, California). Insulin was measured by RIA (RIA Diagnostic Corporation, Los Angeles, CA) with a sensitivity of 0.5mUI/L (normal range 0.5–30 mUI/L) [19] and the homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using these values [20]. CRP was measured by immunoturbimetry (Roche Diagnostics GmbH, Mannheim, Germany), with a normal range of (0–7 mg/dl) and analytical sensivity 0.5 mg/dl. Serum total cholesterol and triglyceride concentrations were determined by enzymatic colorimetric assay (Technicon Instruments, Ltd., New York, N.Y., USA), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using Friedewald formula.

Adiponectin was measured by ELISA (R&D systems, Inc., Mineapolis, USA) (DRP300) with a sensitivity of 0.246 ng/ml, a normal range of 8.65–21.43 ng/ml and a CV% 3.8% [21].

Resistin was measured by ELISA (Biovendor Laboratory, Inc., Brno, Czech Republic) (RD191016100) with a sensitivity of 0.2 ng/ml, a normal range of 4–12 ng/ml [22] and a CV% 3.2%. Leptin was measured by ELISA (Diagnostic Systems Laboratories, Inc., Texas, USA) (DSL1023100) with a sensitivity of 0.05 ng/ml, a normal range of 10–100 ng/ml and a CV% 3.5% [23].

2.5. Anthropometric measurements and blood pressure

Body weight and height were measured in the morning while the subjects were unclothed. They were measured to an accuracy of 0.1 Kg with a calibrated weight (Omrom, LA, CA) and a stadiometer 0.1 mm (Omrom, LA, CA), respectively. Body mass index (BMI) was calculated as body weight (in kg) divided by height (in m^2). Waist (narrowest diameter between xiphoid process and iliac crest) and hip (widest diameter over greater trochanters) circumferences to derive waist-to hip ratio (WHR) were measured, too. A bioimpedance was used to determine body composition with an accuracy of 50 g [24]. The same investigator evaluated all patients. Blood pressure was measured twice after a 10 minutes rest with a random zero mercury sphygmomanometer, and averaged (Omrom, LA, CA).

2.6. Genotyping of ADIPOQ gene polymorphism

Genomic DNA was extracted from 5 ml whole blood by using commercial kit extraction (Biorad, LA, CA) according to the manufacturer's protocol. Primers were designed with the Sequenom Assay Design v4 (SEQUENOM, Inc.San Diego, California CA). Genotyping for the rs1015299 polymorphism was performed by chain reaction real time analysis. This polymerase chain reaction (PCR) was carried out with 20–25 ng of genomic DNA, 0.1–0.15 μl each of oligonucleotide primer for rs1501299 (primer forward: 5′- ACGTTGGATGAAAGCTTTGCTTTCTCCCTG-3′ and reverse 5′- ACGTTGGATGAAAGCTTTGCTTTCTCCCTG-3′ in a 2-μl final volume (Termociclador Lifetecnologies, LA, CA). DNA was denatured at 85 °C for 5 min; this was followed by 45 cycles of denaturation at 95 °C for 15 s, and annealing at 58.1 °C for 45 s). The PCRs were run in a 2-μl final volume containing 0.1 μl of iPLEx Termination mix (Bio-Rad®, San Diego, CA) with hot start Taq DNA polymerase. Hardy Weinberg equilibrium was calculated with a statistical test (Chi-square). The variant of *ADIPOQ* gene was in Hardy Weinberg equilibrium ($p = 0.49$).

2.7. Statistical analysis

Sample size was calculated to detect differences over 2 kg in body weight loss with 90% power and 5% significance ($n = 80$). The Kolmogorov–Smirnov test was used to determine variable distribution. The results were expressed as average ± standard deviation. Numerical variables with normal distribution were analyzed with a two-tailed Student's *t*-test. Non-parametric variables were analyzed with the Mann-Whitney's U test. Categorical variables were analyzed with the chi-square test, with Yates correction as necessary, and Fisher's test. The statistical analysis to evaluate the gene –diet interaction was an univariate ANCOVA. A Chi square test was used to evaluate the Hardy–Weinberg equilibrium. The statistical analysis was performed for the combined *TT* and *GT* as a group and GG genotype as second group (wild type genotype), with a dominant model. A p-value < 0.05 was considered significant. SPSS version 19.0 has been used to realize statistical analysis.

3. Results

Eighty two obese subjects were enrolled in the study. The mean age was 47.1 ± 10.3 years (range: 24–65) and the mean BMI 35.7 ± 4.3 kg/m^2 (range:30.2–40.1). Sex distribution was 62 women (75.6%) and 20 men (24.4%). Forty two patients (51.2%) had the genotype GG, 31 patients GT (37.8%) and 6 patients *TT* (11.0%). Age was similar in the three genotype groups (GG; 47.2 ± 8.2 years vs GT; 46.9 ± 13.8 years vs TT; 46.6 ± 8.2 years: ns). Sex distribution was similar in all genotype groups (GG; 23.8% males vs 76.2% females vs GT; 25.8% males vs 74.2% females vs TT; 33.3% males vs 76.7% females: ns). All patients completed the 3-month follow-up period without drop-outs.

After 3 months of intervention all patients achieved dietary recommendations in both genotype groups without statistical differences between calorie intake (wild type: 1518.1 ± 210.9 kcal/day vs mutant type: 1514.9 ± 488.1 kcal/day) or macronutrient intakes (wild type: 50.6% from carbohydrates vs 50.8% mutant type), (wild type: 25.1% from fats (50.0% of monounsaturated fats, 38.2% of saturated fats and 12.8% of polyunsaturated fats) vs 24.9% mutant type (50.2% of monounsaturated fats, 39.0% of saturated fats and 11.8% of polyunsaturated fats) and (wild type: 24.1% from proteins vs 24.3% mutant type). Fiber intake was similar in both genotypes (15.8 ± 2.9 g/day vs 16.1 ± 3.9), too.

Anthropometric characteristics and blood pressure according to the rs1015299 genotype are shown in Table 1. Basal and post-treatment BMI, weight, fat mass, waist circumference, systolic pressures and diastolic pressures were similar in genotype groups. After dietary treatment weight, BMI, fat mass, systolic blood pressure and waist circumference decreases in both genotypes without statistical differences between groups. In mutant type group, the deltas between both times were; weight −3.0 ± 3.2 kg (decrease in wild type group −2.6 ± 3.0 kg:$p > 0.05$), BMI −0.8 ± 0.3 kg (decrease in wild type group −1.1 ± 0.4 kg:$p > 0.05$), fat mass −1.4 ± 1.0 kg (decrease in wild type group −1.3 ± 1.1 kg: $p > 0.05$) and waist circumference the decrease was −4.6 ± 3.8 cm (decrease in wild type group −5.4 ± 3.6 cm:$p > 0.05$). After dietary treatment and in wild type group, systolic blood pressure decreases were similar to the mutant type group. In mutant type group, the decrease in systolic blood pressure were −9.0 ± 7.9 mmHg (decrease in wild type group −8.6 ± 8.9 mmHg: $p > 0.05$). After dietary intervention, no differences were detected in other variables (waist to hip ratio and diastolic blood pressure).

Table 2 shows the classic cardiovascular risk factors. No differences were detected among basal and post-treatment values of variables between both genotypes. In wild type group, fasting plasma glucose, insulin, HOMA-IR, total choles-

Table 1 – Anthropometric variables and blood pressure.

Parameters	GG Basal	3 months	GT-TT Basal	3 months
BMI	35.8 ± 4.1	34.8 ± 5.2*	35.7 ± 4.1	34.9 ± 5.1*
Weight (kg)	93.6 ± 19.7	91.1 ± 18.7*	94.8 ± 15.3	91.8 ± 15.7*
Fat mass (kg)	41.4 ± 15.1	40.0 ± 13.2*	39.9 ± 9.8	38.5 ± 10.8*
WC (cm)	110.6 ± 15.7	105.2 ± 15.6*	111.1 ± 8.2	106.5 ± 9.1*
Waist to hip ratio	0.94 ± 0.7	0.93 ± 0.6	0.95 ± 0.7	0.94 ± 0.6
SBP (mmHg)	135.6 ± 15.8	126.5 ± 11.0*	133.9 ± 10.6	124.9 ± 9.6*
DBP (mmHg)	80.4 ± 5.3	83.1 ± 4.2	83.3 ± 5.1	84.3 ± 3.1

BMI: body mass index DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference; *$P < 0.05$, basal vs 3 months. No statistical differences among genotypes.

Table 2 – Biochemical parameters (mean ± SD).

Parameters	GG Basal	3 months	GT-TT Basal	3 months
Glucose (mg/dl)	104.9 ± 9.1	100.1 ± 10.1*	95.6 ± 9.1	95.1 ± 8.1
Total cholesterol (mg/dl)	211.1 ± 42.8	183.3 ± 30.1*	220.8 ± 31.7	207.1 ± 29.7
LDL-cholesterol (mg/dl)	134.6 ± 32.1	102.8 ± 27.1*	138.8 ± 19.1	126.6 ± 31.7
HDL-cholesterol (mg/dl)	50.3 ± 10.5	52.9 ± 8.1	54.1 ± 9.1	56.1 ± 9.0
Triglycerides (mg/dl)	125.6 ± 61.1	128.8 ± 64.4	124.5 ± 51.6	127.5 ± 39.6
Insulin (mUI/l)	15.8 ± 9.3	12.2 ± 7.6*	11.6 ± 4.2	12.4 ± 4.1
HOMA-IR	4.3 ± 1.5	3.1 ± 1.1*	3.8 ± 1.4	3.7 ± 1.5

HOMA-IR (homeostasis model assessment); *$P < 0.05$, basal vs 3 months. No statistical differences among genotypes.

terol and LDL cholesterol levels decreased significantly. In mutant type group and after dietary treatment, all parameters remain unchanged. In wild type group, the decrease in total cholesterol was −28.1 ± 15.3 mg/dl (decrease in mutant type group −12.6 ± 16.7 mg/dl:p = 0.009), LDL- cholesterol was −31.8 ± 20.5 mg/dl (decrease in mutant type group −12.2 ± 11.5 mg/dl:p = 0.006), fasting glucose plasma −4.8 ± 2.5 mg/dL (decrease in mutant type group −0.5 ± 0.1 mg/dL:p = 0.02), insulin −3.6 ± 1.5 mUI/L (decrease in mutant type group +0.6 ± 1.1 mUI/L:p = 0.02) and HOMA-IR −1.2 ± 0.9 (decrease in mutant type group −0.1 ± 1.1:p = 0.03).

Table 3 shows levels of adipokines and inflammatory status. No differences were detected among basal and post-treatment values of adipokines and inflammatory parameters between both genotypes. Leptin levels decrease in both genotypes after dietary treatment (−14.1 ± 9.2 ng/dL in wild type group vs −22.1 ± 0.2 ng/dL in mutant type group:p > 0.05). Other adipokines and inflammatory parameters remained unchanged after dietary intervention in both groups.

4. Discussion

Our results confirmed the association between the (rs1501299) of the *ADIPOQ* gene and metabolic response after body weight loss in a short-term nutritional intervention study. There are some cross-sectional studies evaluating the above-mentioned association between this SNP on *ADIPOQ* gene and insulin resistance or adipokines concentrations [6–12], but there is a lack of information about the influence of weight loss on this association in interventional designs.

Although it was not the objective of our work, in our data we didn't find differences between anthropometric and biochemical variables as a function of genotype of obese patients. This SNP rs1015299 has been studied in a lot of populations including Americans, Asians and Europeans [1] and there is a lack of consistency among studies. While in a Mediterranean population T allele was associated with higher insulin levels and HOMA-IR [9], in other studies have been reported opposite results [11,25–27]. For example in a two Asiatic populations (Korean and Japanese), T allele was found to

Table 3 – Serum adipokine levels and C reactive protein (mean ± SD).

Parameters	GG Basal	3 months	GT-TT Basal	3 months
Resistin (ng/dl)	4.7 ± 1.2	4.3 ± 1.1	3.7 ± 1.4	3.8 ± 2.4
Adiponectin (ng/dl)	25.7 ± 11.4	23.6 ± 9.4	28.7 ± 13.4	25.2 ± 10.4
Leptin (ng/dl)	83.5 ± 21.4	69.9 ± 19.4	82.1 ± 18.4	80.2 ± 13.1
CRP (ng/dl)	6.9 ± 1.8	7.1 ± 1.9	5.0 ± 1.2	5.1 ± 1.7

CRP: C reactive protein. *$P < 0.05$, basal vs 3 months. No statistical differences among genotypes.

be protective for insulin resistance [11,27]. Finally, the frequency of rare T allele of rs1501299 was consistent with data reported from HapMap project for European populations [28].

To the best of our knowledge, this is the first study that analyzes the relationship of a Mediterranean hypocaloric diet and the rs1501299 of the *ADIPOQ* gene on body weight loss and subsequent changes of metabolic parameters. Previously, in a non-interventional study on childhood obesity [16] the rs1501299 and fiber intake was significantly associated with adiponectin levels; GG homozygotes showed higher adiponectin levels compared to T carriers under conditions of lower fiber intake. Other investigators [29] have studied whether adiponectin gene variants interact with dietary fat intakes and insulin resistance. In this study [29] realized in Caucasian men found no association for rs1501299 with HOMA-IR in response to dietary fat consumption in healthy volunteers. However, they showed that homozygous subjects of rs16861194 had lower HOMA-IR following a diet rich in carbohydrates and MUFA than a diet rich in saturated fatty acids. In our study, the lack of a relation between rs1501299 variant and diet affecting adiponectin levels could be due to the age of subjects, the health status (presence of obesity without diabetes mellitus) and other unknown factors. In other study [15] with a replacement of dietary saturated fatty acids (SFAs) with monounsaturated fatty acids (MFAs) or carbohydrates showed that subjects with rs10066 GG had a 3.8% increase and A allele carriers a 2.6% decrease adiponectin concentrations. The analysis of the effect of (rs1501299) x diet interaction didn't found statistical differences [15]. The authors hypothesized that the switch from SFAs to MFAs could have led in the increase expression of ADIPOQ gene and serum adiponectin concentrations through the increased availability of PPARgamma-activating MUFA ligands. Finally, the Modulation of Atherosclerosis Risk by increasing Doses of n3 Fatty acids study (MARINA) [17] is a double blind controlled trial with different doses of omega 3 fatty acids vs placebo for 12 months. In this trial [17] rs1501299 variant did not show significant effects on metabolic response after fish oil supplementation. However, individuals homozygous for the +45 T-allele has a 22% increase in serum adiponectin concentration compared with baseline after the highest dose of fish oil (2.8 g per day).

These contradictories results in various populations suggest a complex relationship between *ADIPOQ* rs1501299 and dietary intakes or weight loss after dietary modifications. The exact molecular mechanism responsible for the interaction between diet and this genetic variant remained unknown. Perhaps this SNP could directly influence metabolic response, it has been reported that intronic SNPs might modulate gene expression levels [30]. Other authors have found that the impact of *ADIPOQ* +276 variation on insulin resistance is dependent on degree of obesity as indicated by fat mass upper 40% [31] or BMI upper 26 kg/m^2 [32]. Therefore, weight loss induced in our study might be related with metabolic variations depending on the genotype of the obese patient. Previous studies [32] have shown an association of this SNP and LDL cholesterol levels. Leu et al [33] reported a protective effect of T allele of rs1501299 on blood pressure and Gupta et al [34] showed a reverse effect of T allele in hypertension. To date, there were no studies that assessed the effect of this SNP in the lipid profile and blood pressure modification after weight loss. The mechanisms by which the presence of the T allele make disappear the beneficial effects of weight loss on lipid profile and blood pressure remain unexplained. The unclear relation of this SNP with diabetes mellitus and insulin resistance could explain these unknown results [35,36].

Our study has some limitations. First, many uncontrolled non-genetic factors could influence the relationships of our design (exercise, hormones, so on). Secondly, a relative small sample size, which reduce the power to detect some responses to weight loss. And finally, our population are an obese adult sample without comorbidities, these factors could modulate the response to dietary intervention.

In conclusion, the present study suggests that T allele of *ADIPO* (rs1501299) could be a predictor of a lack of response of HOMA-IR, insulin, fasting glucose and LDL cholesterol secondary to a Mediterranean hypocaloric diet in obese subjects. Further studies are needed to analyze this unclear topic area with clinical and therapeutic implications.

Conflict of interest

The authors declare no conflicts of interest.

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Author statement

Daniel Antonio de Luis designed the study and wrote the article.

Olatz Izaola realized clinical evaluations.

David Primo realized biochemical measurements.

Rocio Aller wrote the article.

REFERENCES

[1] Gable DR, Hurel SJ, Humphries SE. Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. Atherosclerosis 2006;188:231–44.

[2] Sheng T, Yang K. Adiponectin and its association with insulin resistance and type 2 diabetes. J Genet Genom 2008;35:321–6.

[3] Oh DK, Ciaraldi T, Henry RR. Adiponectin in health and disease. Diabetes Obes Metab 2007;9:282–9.

[4] Heidemann C, Sun Q, van Dam RM, Meigs JB, Zhang C, Tworoger SS, et al. Total and high-molecular-weight adiponectin and resistin in relation to the risk for type 2 diabetes in women. Ann Intern Med 2008;149:307–16.

[5] Comuzzie AG, Funahashi T, Sonnenberg G, et al. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab 2001;86:4321–5.

[6] Filippi E, Sentinelli F, Romeo S, Arca M, Berni A, Tiberti C, et al. The adiponectin gene SNP+276G>T associates with early-onset coronary artery disease and with lower levels of adiponectin in younger coronary artery disease patients (age <or = 50 years). J Mol Med 2005;83:711–9.

[7] Kyriakou T, Collins LJ, Spencer-Jones NJ, Malcolm C, Wang X, Snieder H, et al. Adiponectin gene ADIPOQ SNP associations with serum adiponectin in two female populations and effects of SNPs on promoter activity. J Hum Genet 2008;53:718–27.
[8] Vasseur F, Helbecque N, Dina C, Lobbens S, Delannoy V, Gaget S, et al. 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;11:2607–14.
[9] Salmenniemi U, Zacharova J, Ruotsalainen E, Vauhkonen I, Pihlajamaki J, Kainulainen S, et al. Association of adiponectin level and variants in the adiponectin gene with glucose metabolism, energy expenditure, and cytokines in offspring of type 2 diabetic patients. J Clin Endocrinol Metab 2005;90:4216–23.
[10] Filippi E, Sentinelli F, Trischitta V, Romeo S, Arca M, Leonetti F, et al. Association of the human adiponectin gene and insulin resistance. Eur J Hum Genet 2004;12:199–205.
[11] Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, et al. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 2002;51:536–40.
[12] Szopa M, Malczewska-Malec M, Wilk B, Skupien J, Wolkow P, Malecki MT, et al. Variants of the adiponectin gene and type 2 diabetes in a Polish population. Acta Diabetol 2009;46 (4):317–22.
[13] Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. JAMA 2003;289:1799–804.
[14] Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am J Clin Nutr 2005;81:780–6.
[15] AlSaleh A, O'Dell SD, Frost GS, Griffin BA, Lovegrove JA, Jebb SA, et al. Single nucleotide polymorphisms at the ADIPOQ gene locus interact with age and dietary intake of fat to determine serum adiponectin in subjects at risk of the metabolic syndrome. Am J Clin Nutr 2011;94:262–9.
[16] Ntalla I, Dedoussis G, Yannakoulia M, Smart MC, Louizou E, Sakka SD, et al. ADIPOQ gene polymorphism rs1501299 interacts with fibre intake to affect adiponectin concentration in children: the GENe-Diet Attica Investigation on childhood obesity. Eur J Nutr 2009;48:493–7.
[17] Alsaleh A, Crepostnaia D, Maniou Z, Lewis FJ, Hall WL, Sanders TA, et al. MARINA study team. Adiponectin gene variant interacts with fish oil supplementation to influence serum adiponectin in older individuals. J Nutr 2013;143 (7):1021–7.
[18] Mataix J, Mañas M. Tablas de composición de alimentos españoles. Ed: University of Granada; 2003.
[19] Duart MJ, Arroyo CO, Moreno JL. Validation of a insulin model for the reactions in RIA. Clin Chem Lab Med 2002;40:1161–7.
[20] Mathews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–4.
[21] 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;50:1511–25.
[22] Pfutzner A, Langefeld M, Kunt T, et al. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin lab 2003;49:571–6.
[23] Suominen P. Evaluation of an enzyme immunometric assay to measure serum adiponectin concentrations. Clin Chem 2004;50:219–21.
[24] Lukaski H, Johnson PE. Assessment of fat-free mass using bioelectrical impedance measurements of the human body. Am J Clin Nutr 1985;41:810–7.
[25] Stumvoll M, Tschritter O, Fritsche A, Staiger H, Renn W, Weisser M, et al. Association of the T-G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. Diabetes 2002;51:37–41.
[26] Xita N, Georgiou I, Chatzikyriakidou A, Vounatsou M, Papassotiriou GP, Papassotiriou I, et al. Effect of adiponectin gene polymorphisms on circulating adiponectin and insulin resistance indexes in women with polycystic ovary syndrome. Clin Chem 2005;51:416–23.
[27] Jang Y, Lee JH, Kim OY, Koh SJ, Chae JS, Woo JH, et al. The SNP276G>T polymorphism in the adiponectin (ACDC) gene is more strongly associated with insulin resistance and cardiovascular disease risk than SNP45T>G in nonobese/ nondiabetic Korean men independent of abdominal adiposity and circulating plasma adiponectin. Metabolism 2006;55:59–66.
[28] <http://www.genecards.org> [accessed 18 ocobrer2015].
[29] Perez Martinez P, Lopez Miranda J, Crus Teno C, Delgado Lista J, et al. Adiponectin gene variants are associated with insulin sensitivity in response to dietary fat consumption in Caucasian men. J Nutr 2008;45:309–15.
[30] Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, et al. Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 2000;26:163–75.
[31] Melistas L, Mantzoros CS, Kontogianni M, Antonopoulou S, Ordovas JM, Yiannakouris N. Association of the +45T>G and +276G>T polymorphisms in the adiponectin gene with insulin resistance in nondiabetic Greek women. Eur J Endocrinol 2009;161(6):845–52.
[32] Jang Y, Lee JH, Chae JS, Kim OY, Koh SJ, Kim JY, et al. Association of the 276G→T polymorphism of the adiponectin gene with cardiovascular disease risk factors in nondiabetic Koreans. Am J Clin Nutr 2005;82:760–7.
[33] Leu HB, Chung CM, Lin SJ, Jong YS, Pan WH, Chen JW. Adiponectin gene polymorphism is selectively associated with the concomitant presence of metabolic syndrome and essential hypertension. PLoS ONE 2011;6(5):e19999. https://doi.org/10.1371/journal.pone.0019999.
[34] Gupta V, Khadgawat R, Ng HK, Walia GK, Kalla L, Rao VR, et al. Association of TCF7L2 and ADIPOQ with body mass index, waist-hip ratio, and systolic blood pressure in an endogamous ethnic group of India. Genet Test Mol Biomarkers 2012;16:948–51.
[35] Wang WL, Zhu H, Xie Y, Li J. Relation between ADIPOQ gene polymorphisms and type 2 diabetes in a Chinese population. Int J Clin Exp Med 2015;15:6124–8.
[36] Li ZP, Zhang M, Gao J, Zhou GY, Li SQ, An ZM. Relation between ADIPOQ gene polymorphisms and Type 2 diabetes. Genes (Basel) 2015;6:512–9.