

# Genetic and Non-Genetic Determinants of Circulating Levels of Adiponectin in a Cohort of Chilean Subjects

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## Abstract

**Background** Low adiponectin levels have been associated with obesity, diabetes, coronary disease and polymorphisms. The aim of this study was to assess the clinical and genetic factors related to adiponectin levels in a Chilean adult population.

**Methods** Four groups of 27 individuals, stratified by gender and abdominal obesity, were included in this cross-sectional study. Physical activity level, cardiovascular risk factors and therapy were assessed. Anthropometric measurements, adiponectin, glucose, insulin, hs-CRP, rs1501299, rs2241766, rs266729 and rs17300539 single nucleotide polymorphisms of the adiponectin gene were determined.

**Results** Adiponectin levels were inversely correlated with waist circumference, waist-to-hip ratio, weight, body mass index, insulin levels, homeostasis model insulin resistance index (HOMA-IR) and physical activity. Mean adiponectin levels were lower in men ( $10.1 \pm 3.6$  vs  $11.9 \pm 2.9$   $\mu\text{g}/\text{mL}$ ,  $p = 0.0051$ ), obese ( $9.9 \pm 3.2$  vs  $12.1 \pm 3.3$ ,  $p < 0.01$ ), physically inactive ( $8.9 \pm 3.2$  vs  $11.0 \pm 2.8$ ,  $p = 0.015$ ) and insulin-resistant individuals ( $10.2 \pm 3.1$  vs

$12.9 \pm 3.9$ ;  $p = 0.005$ ). Adiponectin levels were lower in subjects with GG genotype (rs1501299) compared with those with TT or GT variants ( $6.9 \pm 4.6$  vs  $12.2 \pm 2.9$  and  $11.5 \pm 3.4$   $\mu\text{g}/\text{mL}$ ,  $p = 0.006$ ) but were similar in the other polymorphisms studied.

**Conclusion** Lower adiponectin levels were related to gender, abdominal obesity, physical activity and insulin resistance and were less influenced by the polymorphisms studied.

## Key Points

Gender, obesity, physical activity and insulin resistance influence adiponectin levels.

Genotype GG (rs1501299) was associated with lower adiponectin levels.

Adiponectin levels were not related to rs2241766, rs266729 and rs17300539 variants.

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## 1 Introduction

Obesity has become one of the major health problems in the last few decades and is related to hypertension, dyslipidemia, sedentary life style and increased risk of diabetes mellitus and cardiovascular disease [1]. Adiponectin, an abundant circulating adipocytokine present in human plasma has been one of the fundamental mediators in obesity-related risk [2]. Data from both animal and human studies indicate that adiponectin has insulin-enhancing

effects, modulating insulin sensitivity and glucose homeostasis and anti-inflammatory actions. Clinical studies implicate low adiponectin levels in the occurrence of obesity, the pathogenesis of type 2 diabetes, coronary artery disease and hypertension [3–5].

In humans, plasma adiponectin concentrations are influenced by age, gender, physical activity and visceral adiposity [6, 7]. Adiponectin levels are also influenced by genetic variables; they are considered to be highly heritable, and several single nucleotide polymorphisms (SNPs) in adiponectin-related genes (*ADIPOQ*, *ADIPOR1*, *ADIPOR2*) have been examined in relation to circulating adiponectin levels and obesity phenotypes [8–19]. However, the results of these studies have been contradictory with regard to whether variability at this locus has an impact on metabolic phenotypes and which polymorphisms are responsible for such an effect.

The purpose of this study was to assess the influence of genetic and non-genetic factors, such as anthropometric measurements of obesity, physical activity, glucose levels, insulin levels, insulin resistance, inflammatory markers (high-sensitivity C-reactive protein) and four adiponectin polymorphisms on plasma adiponectin levels.

## 2 Material and Methods

### 2.1 Study Population

A random sample of the urban population of the Prospective Urban and Rural Epidemiology (PURE) study in Temuco city was selected stratified by gender and abdominal obesity. The PURE study is a cohort of 154,000 individuals from 628 rural and urban communities in 17 high-, middle- and low-income countries. The details of study design and sampling methods of the PURE study have been published previously [20]. Briefly, in Temuco a 4-stage stratified sampling method was used to select a representative sample of 2808 subjects from the general population. In the first stage, communities were randomly selected and stratified by socio-economic level. In the second stage, a number of blocks proportional to the community size were randomly selected. In the third stage, households within each block were selected by systematic random sampling. In the final stage of sampling, all members aged between 30–74 years in the selected households were included in the PURE study. However, only one individual per household could be selected for this sub-study, excluding individuals with diabetes mellitus. The Ethics Committee of the Servicio de Salud Araucanía Sur approved this study and all patients signed an informed consent.

### 2.2 Data Collection

Study data were collected during both home and clinical visits. During the home survey, information on demographic characteristics, including age, sex, education, occupation; personal history of cardiovascular disease and risk factors, including coronary artery disease, stroke, hypertension, diabetes and dyslipidemia; pharmacological treatment of hypertension, diabetes and dyslipidemia; and lifestyle risk factors, including cigarette smoking, alcohol consumption and physical activity was obtained. We collected data at individual levels with standardized questionnaires. Information about physical activity at work, in transport and during recreational and leisure-time activities was gathered using the International Physical Activity Questionnaire—Long Form (IPAQ) [21]. For each individual, the recorded activities were converted into their metabolic equivalent (MET).

During the clinical examination, blood pressure (BP) and anthropometric measurements were obtained by trained and certified observers using standard protocols and techniques. Two BP measurements were obtained with the participant in a seated position after 5 min of rest using an Omron automatic digital blood pressure monitor (Omron HEM-757), and the mean of the readings was used for analysis. Body weight, height and hip and waist circumferences were measured twice during the examination. Weight in kilograms was measured in light indoor clothing without shoes to one decimal place. Height was measured without shoes in centimeters to one decimal place with a stadiometer. Waist circumference was determined at the narrowest point between the costal margin and iliac crest, at minimal respiration, and hip circumference was determined at the level of the widest diameter around the buttocks in centimeters to one decimal place. Overnight fasting blood specimens were obtained for measurement of glucose, insulin, high-sensitivity C-reactive protein (hs-CRP) and adiponectin levels and SNP determination. Fasting glucose and insulin were used to calculate the homeostasis model insulin resistance index (HOMA-IR) [22].

### 2.3 Definitions

Abdominal obesity was defined as a waist circumference of >102 cm in men and >88 cm in women [23]. Obesity was defined as a body mass index (BMI)  $\geq 30$  kg/m<sup>2</sup>. Hypertension was defined as a mean systolic BP  $\geq 140$  mmHg and/or diastolic BP  $\geq 90$  mmHg and/or current use of antihypertensive medications. Diabetes was defined as a fasting glucose level of  $\geq 126$  mg/dL or self-reported history of diabetes. Low physical activity was defined as less than 600 MET-minutes/week of total physical activity.

Insulin resistance was defined with HOMA-IR values over 2.6 [24].

## 2.4 Laboratory Measurements

Adiponectin concentrations were measured using an enzyme-linked immunosorbent assay kit (ALPCO Diagnostics, Salem, NH, USA). Determination of insulin was performed by Microplate Enzymatic Immuno-Assay (Insulin Assay; AxSYM, Abbott, Abbott Park, IL, USA). Plasma hs-CRP concentration was measured using a Behring BN II nephelometer (Dade Behring Inc., Marburg, Germany).

## 2.5 Genotyping of Single Nucleotide Polymorphisms (SNPs)

Four SNPs were chosen for genotyping because they have been linked to adiponectin levels. Polymorphisms rs1501299 (276 G>T) and rs2241766 (45 T>G) were previously associated with BMI and obesity risk [25], while rs266729 (-11377C>G) and rs17300539 (-11391G>A) promoter variants have been associated with hypoadiponectinemia in humans and decreased adiponectin promoter activity in adipocytes [26]. DNA was isolated from whole blood samples using a previously described method [27]. DNA purity and quantification were assessed by UV spectrophotometry using a microplate reader (Infinite<sup>®</sup> 200PRO Nanoquant, Tecan). The genotypes for rs1051299 and rs2241766 polymorphisms were obtained by PCR restriction fragment length polymorphism (PCR-RFLP). For rs1051299, DNA was amplified using a primer pair (forward 5' GGC CTC TTT CAT CAC AGA CC 3' and reverse 5' AGA TGC AGC AAA GCC AAA GT 3') designed to obtain a fragment of 196 bp, which was subjected to enzymatic digestion using the endonuclease *Mva*I269I. Thus, in presence of the G allele, the initial fragment of 196 bp was sectioned (150 and 46 bp), whereas in presence of the T allele the initial length remains unaltered (196 bp). To identify the genotypes for the rs2241766 polymorphism, a DNA fragment of 372 bp was amplified using a primer pair specific for flanking the sequence that contains the polymorphic site (forward 5' GAA GTA GAC TCT GCT GAG ATG G 3' and reverse 5' TAT CAG TGT AGG AGG TCT GTG ATG 3'). Then, the PCR product was digested using the endonuclease *Sma*I to obtain two fragments of 219 and 153 bp when the G allele was present, and the initial fragment remained unaltered in presence of the T allele (372 bp). The PCR and digestion products were visualized after electrophoresis separation using a 2 % agarose gel and ethidium bromide staining. Finally, the genotypes for the rs266729 and rs17300539 polymorphisms were obtained by real time PCR in a

StepOne<sup>™</sup> Real-Time PCR system (Applied Biosystems<sup>®</sup>) using allele-specific TaqMan<sup>®</sup> probes following the manufacturer's recommendations (TaqMan<sup>®</sup> SNP Genotyping Assay, Life Technologies). Data were collected at the extension step and then analysed in the StepOne Software v2.2 using the genotyping mode to obtain the allelic discrimination plots. Positive controls of each genotype and no template controls were used for both PCR-RFLP and TaqMan<sup>®</sup> genotyping assays.

## 2.6 Statistical Methods

The summary results are presented as the numbers (and corresponding percentages) for categorical variables and the mean and standard deviation (SD) for continuous variables. The differences in baseline characteristics were tested by analyses of variance for continuous variables. The Spearman test was used to assess correlations between continuous variables. Associations between individual SNPs and adiponectin levels were examined using mean values of adiponectin and stratified multiple linear regression models. Linear regression models were used to assess for differences in unadjusted adiponectin-factor associations. After that, logistic regression modeling was performed, including statistically significant variables in the linear regression analysis and also interactions for polymorphisms. A two-sided *p* value of <0.05 was considered to be statistically significant. All statistical analyses were performed with STATA (Version 11; Stata, College Station, TX, USA) statistical software packages.

## 2.7 Sample Size

The estimated sample size for the two-sample comparison of means was performed, considering a mean difference of 2.0 μ/mL in plasma adiponectin and a standard deviation of 3.0 μ/mL, with a power of 0.90 and an  $\alpha$  value of 0.05. The estimated required sample size was 48 individuals in each group if the proportion of exposed is 50 %. This sample size has a power of 0.8 if the proportion of exposed is 20 % and the power drops to 0.55 if the proportion of exposed is 10 %. Ten percent was added for possible missing data. Men and women were selected in the same proportion.

## 3 Results

Fifty-three men were included; of those men, 27 had abdominal obesity, but one male without abdominal obesity was excluded for missing laboratory information.

Fifty-four women were included, of whom 27 had abdominal obesity, and the mean age was similar between obese and non-obese men and women. The mean values for waist, waist-to-hip ratio (WHR), weight and BMI were significantly higher in the obese group (Table 1).

Adiponectin plasma levels were lower in obese individuals, categorized by their WHR ( $10.2 \pm 3.1$  vs  $11.8 \pm 3.4$   $\mu\text{g/mL}$ ,  $p = 0.012$ ) or a BMI of  $>30$   $\text{kg/m}^2$  ( $10.3 \pm 2.5$  vs  $11.9 \pm 3.8$   $\mu\text{g/mL}$ ,  $p = 0.013$ ) compared with non-obese individuals and in men compared with women ( $10.0 \pm 3.6$  vs  $12.0 \pm 2.8$   $\mu\text{g/mL}$ ,  $p = 0.003$ ) (Table 1). Lower adiponectin levels were observed in low physical activity persons ( $8.9 \pm 3.2$  vs  $11.2 \pm 3.1$   $\mu\text{g/mL}$ ,  $p = 0.014$ ), those with hypertension ( $10.4 \pm 3.4$  vs  $12.2 \pm 2.7$   $\mu\text{g/mL}$ ,  $p = 0.01$ ) and those with insulin resistance ( $10.2 \pm 3.1$  vs  $12.2 \pm 3.1$   $\mu\text{g/mL}$ ,  $p = 0.005$ ). Mean values of adiponectin were not significantly different between the younger and older individuals.

Adiponectin circulating levels showed statistically significant correlations with several variables: a strong negative correlation ( $>0.5$ ) with weight, insulin levels and HOMA-IR, a moderate (0.3–0.5) negative correlation with waist circumference, WHR and BMI and a modest (0.1–0.3) inverse correlation with physical activity, glucose and hs-CRP (Table 2). In a linear regression model that includes gender, anthropometric measurements and physical activity, only gender (regression coefficient  $-2.35$ ,  $p < 0.001$ ) and BMI ( $-0.28$ ,  $p < 0.001$ ) remained statistically significant. However, when gender was excluded from the model, WHR and BMI were significantly associated with adiponectin plasma levels, demonstrating an interaction between gender and WHR.

Regarding the molecular analysis, the genotype distributions were consistent with the Hardy-Weinberg equilibrium as shown in Table 3. Subjects with the GG genotype of the SNP rs1501299 had lower adiponectin plasma levels at  $6.9 \pm 4.6$   $\mu\text{g/mL}$  compared with those with TT or TG variants, whose adiponectin plasma levels were  $12.2 \pm 2.9$  and  $11.5 \pm 3.4$   $\mu\text{g/mL}$ , respectively ( $p = 0.006$ ). The HOMA index was higher in this group, although the difference did not reach statistical significance; in addition, age, gender, anthropometric measurements, plasma insulin levels and hs-CRP were similar between those with the GG genotype compared with the TT and GT genotypes. After adjusting the effects of SNP rs1501299 on adiponectin levels in a logistic regression model with gender and BMI, no interaction between SNP polymorphisms with gender and BMI was observed, and its effect remained significant (coefficient  $-3.99$ ,  $p = 0.001$ ). For the rs2241766, rs266729 and rs17300539 SNPs, the mean value of plasma adiponectin was similar in the different genotypes and alleles (Table 3).

**Table 1** Clinical and demographic characteristics of the study group

Variable	Men		Women		Total	Gender comparison		Abdominal obesity	
	WC < 102	WC > 102	WC < 88	WC > 88		p value	No	Yes	
Age (years)	$50.6 \pm 10.2$	$51.8 \pm 10.0$	$49.5 \pm 10.6$	$52.1 \pm 10.1$	$50.8 \pm 10.3$	0.85	$50.1 \pm 10.3$	$51.9 \pm 10.3$	
WHR	$0.89 \pm 0.06$	$1.06 \pm 0.02$	$0.79 \pm 0.03$	$0.98 \pm 0.04$	$0.89 \pm 0.1$	$<0.0001$	$0.84 \pm 0.07$	$1.02 \pm 0.05^*$	
Waist (cm)	$87.1 \pm 9.7$	$106.2 \pm 9.4$	$80.1 \pm 8.8$	$104.5 \pm 13.3$	$91.3 \pm 15.9$	0.11	$83.6 \pm 9.8$	$105.4 \pm 11.4^*$	
BMI ( $\text{kg/m}^2$ )	$26.0 \pm 3.6$	$30.4 \pm 3.7$	$26.7 \pm 3.6$	$32.9 \pm 6.0$	$29.8 \pm 5.8$	0.12	$26.4 \pm 3.2$	$31.7 \pm 5.1^*$	
Weight (kg)	$70.9 \pm 10.6$	$86.6 \pm 12.5$	$64.5 \pm 10.9$	$78.7 \pm 17.4$	$71.6 \pm 16.1$	0.013	$67.6 \pm 11.1$	$82.7 \pm 15.6^*$	
Physical activity (METs)	$2861 \pm 1115$	$1292 \pm 2459$	$1996 \pm 1321$	$1029 \pm 2426$	$1512 \pm 1914$	0.16	$2428 \pm 1220$	$1161 \pm 2225^*$	
Adiponectin ( $\mu\text{g/mL}$ )	$10.4 \pm 3.9$	$9.6 \pm 3.3$	$13.1 \pm 2.2$	$10.8 \pm 2.9$	$12.0 \pm 2.8$	0.00014	$11.8 \pm 3.4$	$10.2 \pm 3.1^{\#}$	
Insulin ( $\mu\text{U/mL}$ )	$6.8 \pm 9.6$	$10.4 \pm 5.3$	$6.1 \pm 3.4$	$11.0 \pm 6.4$	$8.6 \pm 5.7$	0.97	$6.5 \pm 7.0$	$10.7 \pm 5.8^*$	
HOMA-IR	$1.36 \pm 1.9$	$2.53 \pm 1.4$	$2.15 \pm 4.3$	$3.2 \pm 3.0$	$2.65 \pm 3.7$	0.28	$1.40 \pm 1.4$	$3.0 \pm 2.1^*$	
hs-CRP (mg/L)	$2.7 \pm 4.0$	$2.9 \pm 3.8$	$1.8 \pm 2.4$	$4.4 \pm 5.5$	$3.2 \pm 4.4$	0.67	$2.26 \pm 3.3$	$3.7 \pm 4.7$	

BMI body mass index, HOMA-IR homeostasis model assessment insulin resistance, hs-CRP high sensitivity C-reactive protein, METs metabolic equivalents, WC waist circumference, WHR waist to hip ratio

\*  $p < 0.001$ ,  $^{\#} p = 0.012$

**Table 2** Correlations of clinical variables with plasma adiponectin levels

Variable	Spearman rho	<i>p</i> values
Age (years)	0.15	0.1239
Waist (cm)	-0.45	<0.0001
Hip (cm)	-0.27	0.0048
WHR	-0.36	0.0001
Weight (kg)	-0.53	<0.0001
Height (cm)	-0.34	0.0003
BMI (kg/m <sup>2</sup> )	-0.40	<0.0001
Physical activity (METs)	0.26	<0.001
hs-CRP (mg/L)	-0.24	0.0149
Glucose (mg/dL)	-0.21	0.0285
Insulin (μU/mL)	-0.51	0.0001
HOMA-IR	-0.52	0.0001
Systolic BP (mmHg)	-0.11	0.2522
Diastolic BP (mmHg)	-0.16	0.0937

*BMI* body mass index, *BP* blood pressure, *hs-CRP* high sensitivity C-reactive protein, *HOMA-IR* homeostasis model assessment insulin resistance, *METs* metabolic equivalents, *WHR* waist to hip ratio

## 4 Discussion

Our findings confirm the relationship of decreased adiponectin levels with male gender, obesity, abdominal obesity, physical activity, insulin resistance and the G allele of SNP rs1501299 in the adiponectin gene. However, no relationship was found between other adiponectin SNPs examined.

In our results, the strongest negative correlation of adiponectin was with insulin levels and HOMA-IR. Adiponectin exerts a potent insulin-sensitizing effect through binding to its receptors AdipoR1 and AdipoR2, leading to

activation of adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor  $\alpha$  (PPAR- $\alpha$ )[28]. The adiponectin receptor 2 shows a high level of expression in the liver, pancreatic  $\beta$  cells and muscle, which are all important sites for glucose metabolism [29]. The effect of adiponectin in insulin sensitivity involves the suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in the muscle and the stimulation of insulin secretion [30].

We observed a significant 16.7 % lower value of adiponectin in men ( $p = 0.00014$ ), similar to the reported difference in the literature; this difference does not have a clear explanation [31, 32], but the difference in our data was observed primarily in women with lower waist circumference. Although cardiovascular risk has been associated more with abdominal obesity than with BMI [33], our results showed that adiponectin levels have a similar correlation with WHR and BMI, which remain significant after adjusting for both in a linear regression model. This can be explained by the association of adiponectin levels with subcutaneous and visceral adipose tissue as demonstrated with imaging techniques [34].

Hypertension was associated with lower adiponectin plasma levels in our results. A systematic review and meta-analysis that includes 48 studies with 17,598 individuals reported that hypertensive adults had adiponectin levels of 1.64  $\mu\text{g/mL}$  lower than normotensive persons [35]. However, when we adjust the effect of hypertension by WHR and HOMA-IR, the association between adiponectin levels and hypertension was no longer significant, reflecting the fact that the basis of the low adiponectin levels in hypertension is primarily a consequence of increased adipose tissue and insulin resistance. Adiponectin plasma levels

**Table 3** Effect of *ADIPOQ* polymorphisms on plasma adiponectin levels and insulin resistance

SNP	Genotype	Subjects, <i>n</i> (%)	HWE, $\chi^2$ ( <i>p</i> value)	Adiponectin ( $\mu\text{g/mL}$ )	<i>p</i> value	HOMA-IR	<i>p</i> value
276 G>T (rs1501299)	GG	6 (5.6)	0.620 (0.431)	6.9 $\pm$ 4.6	0.006	3.6 $\pm$ 3.5	0.130
	TG	45 (42.1)		11.5 $\pm$ 3.4		2.4 $\pm$ 2.7	
	TT	56 (52.3)		11.2 $\pm$ 2.9		1.9 $\pm$ 1.4	
45 T>G (rs2241766)	TT	72 (67.3)	0.083 (0.772)	11.0 $\pm$ 3.5	0.970	2.3 $\pm$ 2.4	0.610
	TG	31 (29.0)		11.1 $\pm$ 3.4		2.0 $\pm$ 1.5	
	GG	4 (3.7)		10.8 $\pm$ 1.3		1.5 $\pm$ 0.7	
-11377 CG (rs266729)	CC	35 (32.7)	1.385 (0.239)	11.2 $\pm$ 3.3	0.510	2.4 $\pm$ 2.1	0.510
	GC	47 (43.9)		10.6 $\pm$ 3.5		2.3 $\pm$ 2.5	
	GG	25 (23.4)		11.5 $\pm$ 3.2		1.8 $\pm$ 1.3	
-11391G>A (rs17300539)	GG	92 (86.0)	0.608 (0.436)	10.9 $\pm$ 3.3	0.400	2.2 $\pm$ 2.2	0.560
	GA	15 (14.0)		11.7 $\pm$ 3.2		1.9 $\pm$ 1.6	
	AA	0 (0.0)		–		–	

*HOMA-IR* homeostasis model assessment insulin resistance, *HWE* Hardy-Weinberg equilibrium, *SNP* single nucleotide polymorphism

were also inversely associated with physical activity as assessed by IPAQ. Evidence exists that physical training increases circulating adiponectin levels and AdipoR1/R2 mRNA expression in muscle [36]. However, in a systematic review that included 33 studies with different designs and 20,131 subjects, an increased serum adiponectin was observed in only 38 % of the randomized clinical trials [37]; some data suggest that moderate- or high-intensity resistance or aerobic training of adequate duration is needed to produce substantive changes in body composition as a means to augment circulating adiponectin [37], suggesting that, like in hypertension, in physical activity, obesity is the leading factor in decreased adiponectin plasma level. Levels of hs-CRP were increased in obese individuals, showing an inverse correlation with adiponectin plasma levels. Recent studies suggest that adiponectin modulates inflammatory vascular responses by inhibiting the expression of adhesion molecules on endothelial cells, inhibiting endothelial cell nuclear factor- $\kappa$ B signaling and suppressing macrophage function. In addition, adiponectin reduces the inflammatory response induced by tumor necrosis factor (TNF $\alpha$ ) and indirectly decreases the levels of C-reactive protein [38].

The gene coding for adiponectin, named *ADIPOQ*, is placed on chromosome 3q27. We analyzed the four SNPs that have been more extensively studied and that were among the first to be discovered by targeted resequencing efforts. Two of these, rs17300539 and rs17300539, are placed in the first LD block, in the immediate five flanking regions of the gene, and the other two, rs2241766 and rs1501299, are placed in the second LD block, in exon 2 and intron 2, respectively.

In our results, patients carrying the genotype GG (rs1501299) had significant lower adiponectin values. Similarly, Woo et al. found that non-Hispanic white adolescents in the USA exhibited significantly lower values in individuals with the GG genotype:  $10.1 \pm 0.2$  vs  $10.8 \pm 0.3$  in GT and  $10.6 \pm 0.6$  in TT individuals, and similar results were found in black adolescents [19]. However, only 5.6 % of our participants had the GG genotype, compared with 55 % in the Woo study. In a review article that includes 12 publications and 6570 subjects, Caucasian and Asiatic individuals with the GG genotype also had lower adiponectin plasma levels [11]. Alternatively, discordant results demonstrated no association in the Arab [8], Brazil [9] or Finland [13] population. Even in South India, those with the TT genotype had the lowest values, with heterozygous individuals having intermediate values and those with the GG genotype having the highest [39].

We found that adiponectin levels were not related to the rs2241766, rs266729 and rs17300539 polymorphisms. Our results for SNP rs2241766 were similar to a study

performed in Thailand in individuals with metabolic syndrome and controls [12], in young Finnish men [13] and in type 2 diabetes mellitus and control subjects in Arabs [8]. However, another study in Finland, including participants in the Finnish Diabetes Prevention Study, reported that those with the TT genotype had lower adiponectin levels [14], which was similar to the results reported in Sao Paulo in subjects referred to coronary angiogram [9]. However, in Qatar, patients with acute coronary syndrome with the GG genotype exhibited lower adiponectin levels [18]. Regarding our findings of no association between the SNP rs266729 polymorphism and adiponectin levels, similar results were reported by the study mentioned in Finland [14], in the RISC (Relationship between Insulin Sensitivity and Cardiovascular disease) study that includes healthy participants from European countries [15] and in individuals attending the seventh examination (1998–2001) of the Framingham Offspring Study [16]. However, in India, obese and non-obese, diabetics or those with normal glucose tolerance with the genotype GG had lower adiponectin values [39], consistent with similar results in the GOLDN (Genetics of Lipid Lowering Drugs and Diet Network) study in the USA [17]. Finally, our finding of no difference in adiponectin levels in SNP rs17300539 was also observed in Brazil [9] but, in Southern India, those with the GG allele had lower adiponectin values [37]. Similar results were reported for the GOLDN study in the White population from the USA [17] and in non-Hispanic Whites and African Americans also in the USA [19]. In our results, insulin resistance, as defined by the surrogate marker HOMA-IR, was a strong negative predictor of adiponectin serum levels, with a correlation index of  $-0.52$ . However, no difference was observed in HOMA-IR values between the polymorphisms studied, suggesting a predominance of environmental factors in insulin resistance. In a meta-analysis that included 2926 subjects in seven publications with rs2241766 polymorphism studies, no differences were found in HOMA-IR levels and 45 TG genotype; in 2,688 subjects in six publications with rs1501299 polymorphism studied, a statistical significant difference in HOMA-IR levels was observed in three of the six studies comparing G/T with G/G genotype and in four between T/T and G/G genotype [11]. The heterogeneity of the results of studies of genetic determinants of adiponectin levels can be explained by ethnic differences in populations, the inclusion of special groups, environmental exposure and the limited sample size.

Our study has limitations given the small sample size that restricts the conclusion for weak associations and low frequency variables, like low frequency genotypes, and by the cross-sectional design that limits the conclusions about causality. The major strength is the representation of individuals obtained through general population sampling

and the detailed assessments of variables. In summary, our results demonstrated the importance of lifestyle factors compared with genetic polymorphisms in the plasma adiponectin levels, a major determinant of insulin resistance and cardiovascular events.

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**Author contributions** FL, PS and LAS conceived and designed the experiment; NS, JR and LAS performed the experiments; FL, PS, NS and LAS analysed the data; LAS and FL contributed reagents/materials/analysis tools; FL and LAS wrote the paper

### Compliance with Ethical Standards

**Disclosure statement** None of the authors has a relationship with a for-profit company or institution. No competing financial interests exist.

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