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Adiponectin gene polymorphisms and the risk of insulin resistance

Moushira Erfan zaki[1], Sanaa Kamal[1], Salah Alsayed Ibrahim Ali[2] and Ragaa Abd-elsalam Mohamed[3]

[1] Biological Anthropology Department, Medical Research Division, National Research Centre, **Egypt**.
[2] Clinical Pathology Department, Faculty of Medicine, Zagazig University **Egypt.**
[3] Department of Pediatrics, Faculty of Medicine (for Girls), Al-Azhar University, Cairo, **Egypt.**

***Correspondence: moushiraz@yahoo.com** **Accepted: 03** April, 2017 **Published online:** 29 Apr. 2017

Insulin sensitivity has been modulated by Adiponectin gene which regulates energy homeostasis. The aim of the present study was to evaluate the contribution of the Adiponectin gene polymorphisms in the genetic susceptibility of insulin resistance (IR) in female adolescents and investigate their relations to adiponectin levels and biochemical features. The study included 400 children, 200 unrelated with IR between and 200 age- sex and BMI- healthy matched children with no family history of IR. Their age ranged between 10-11 years. Two SNPs: -11,391G/A (rs17300539) and -11,377C/G (rs 266729) were studied by polymerase chain reaction–restriction fragment length polymorphism. Serum adiponectin was studied by ELISA method and its relation with polymorphisms was analyzed. Cases with IR showed higher triglyceride, HOMA-IR, SBP, DBP and lower serum adiponectin levels as compared to control group. Significant association was observed between IR and variant alleles -11,391A (OR: 4.33(2.14-8.79) and the -11,377G (OR: 4.82, 95% CI: 2.14-8.79). The risk of IR was increased in recessive genetic model (AA vs. GG+ GA) (OR: 4.84, 95% CI: 1.61- 14.58) and (GG vs. CC+ CG) (OR: 5.21, 95% CI: 1.12 - 14.09). Regarding -11,377C/G, cases with the GG genotype had significantly higher HOMA-IR, SBP, DBP, TG levels and lower adiponectin concentration compared with those with the combined genotypes group CC+CG (p<.03). Similar findings were found in the between AA carriers and GG+ GA genotypes. The two adiponectin gene polymorphisms -11,391G/A and -11,377C/G are associated with increased risk of IR, abnormal metabolic markers and low serum adiponectin levels. These polymorphisms can be used as good early biomarkers for IR risk and metabolic complications

Keywords: Children, Single-nucleotide polymorphisms, Adiponectin, Biomarkers

INTRODUCTION

Insulin sensitivity from the fasting glucose and insulin concentrations is a professional and noninvasive method to estimate the homeostasis model assessment of insulin resistance (HOMA-IR). It is accepted as the standard method to detect insulin resistance in epidemiologic studies. When the adipose tissue volume is increased, adiponectin is the exclusive adipokine of which the plasma concentration is reduced. Insulin sensitivity is improved by Increasing levels of plasma adiponectin (Yamauchi et al. 2001) , probably through metabolism that increase fatty acid oxidation(Tomas et al. 2002)(Yamauchi et al. 2002). Consistent with these findings, serum adiponectin levels are decreased with obesity (Pajvani and Scherer 2003), despite increased adipose mass. Adiponectin plays a significant role in atherosclerosis and inflammatory reactions. Adiponectin suppresses the adhesion of monocytes to endothelial cells leading to inhibition of inflammation and atherosclerotic progression

(Ouchi et al. 2000). The concentrations of plasma adiponectin is inversely related to body mass index(Huang et al. 2004), high density lipoprotein cholesterol (Berg et al. 2002) and directly related to insulin(Berg et al. 2002)(Hoffstedt et al. 2004)(Comuzzie et al. 2001) and triglyceride levels (Comuzzie et al. 2001) in obese subjects.

The promoter region of the adiponectin gene modulates insulin sensitivity. Circulating protein adiponectin is encoded by the adiponectin gene (ADIPOQ). It is expressed primarily in the adipose tissue of different organs and expressed in vascular tissue as fit(Hermann et al. 2006).

ADIPOQ polymorphisms have been investigated in relation to circulating adiponectin concentrations in some studies but the functional variants responsible for these observed associations have not yet studied (Menzaghi et al. 2007). Several studies reported the association of single-nucleotide polymorphisms (SNPs) and haplotypes of ADIPOQ with circulating adiponectin concentrations, but these studies have had varied results and have concentrated on a small number of SNPs, usually within one ethnic group(Heid et al. 2006)(Filippi et al. 2005)(Qi et al. 2005) . A review and meta-analysis the frequency of the rare genotype (Menzaghi et al. 2007) reported an association of some SNPs of ADIPOQ with circulating adiponectin but many variations in ADIPOQ has not been examined.

Variants in the ADIPOQ gene have been associated with type 2 diabetes and/or insulin resistance in five different populations, including a Japanese population and German, French, American, and Swedish Caucasians. However, the susceptibility SNPs of this gene for type 2 diabetes and/or insulin resistance are different in different populations. This may reveal that the genetic defects of SNPs in the APM1 gene are influenced by genetic backgrounds and environmental factors in different ethnic populations. Interactions of ADIPOQ SNPs with BMI, waist circumference, as well as age and sex were reported(Schäffler et al. 2004).

There are many association studies from different populations analyzing Adiponectin gene effect on IR but there is not any report whether Adiponectin gene is associated with IR in Egypt, particularly in female adolescents.

We therefore investigated the role of Adiponectin gene polymorphisms in IR in Egyptian non obese female during adolescence.

MATERIALS AND METHODS

Genotyping for adiponectin polymorphisms:

This cross sectional study included 200 female adolescents with IR and 200 healthy control matches in age and BMI with patients. The mean age of the study subjects was 12.5 ± 2.5 years, ranged from 13-15 years.

Cases have been collected from the outpatient obesity clinic of National Research Centre, from April 2015 to December 2016 in Giza governorate as a metropolitan governorate. Inclusion criteria for patients were female adolescents with IR aged 13-15 years. Controls were randomly collected from the general population from the same region of residence and free of any medical complications. The study was approved by the Ethical Committee form of National Research Centre, Egypt, in accordance with the World Medical Association's Declaration of Helsinki.

An informed written consent was obtained from all participants.

Genotyping for adiponectin alleles 11391G/A and 11377C/G polymorphisms

Genomic DNA was isolated from the peripheral blood leucocytes with phenol-chloroform followed by ethanol precipitation. The polymerase chain reaction (PCR)- restriction fragment length polymorphism genotyping. Genomic DNA was extracted from peripheral blood leucocytes using the salting out method. Two adiponectin (ADIPOQ) single nucleotide polymorphisms (SNPs): 11391G/A and 11377C/G were genotyped in Egyptian adolescents.

The polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) genotyping procedure was carried out to the extracted genomic DNA. The following primers were used for (C-11377G): 5'-GCTCTGTGTGGACTGTGGAG-3' as forward and 5'AGAAGCAGCCTGGAGAACTG-3' as a reverse primer; and (G-11391A): 5'-CATC AGAA TGTG TGGC TTGC-3' as forward and 5'-AGAAGCAG CCTG GAGA ACTG-3' as a reverse primer. Each PCR reaction contained 25ul final volumes consisting of the following 250 ng genomic DNA, 200 uM dNTPS, 0.5 unit of DNA polymerase (DyNAZyme II, FINZYMES) and 20 pmol of each primer. The thermocycling conditions consisted of initial denaturation at 94°C for 10 minutes, followed by 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and the final extension at 72°C for 10 minutes. The PCR products were digested with MspI restriction endonuclease (Fermentas, Germany) in case of 11391G>A polymorphism, at 37°C for 15 minutes, giving DNA fragments of 137 and 26 bps when

the GG was present and 163 bps of undigested PCR product when the AA was present. 163, 137 and 26 bps when the GA was present. The products of the digest were then visualized on a 2.5% agarose gel stained with ethidium bromide.
We have sequenced 100 cases (50 IR and 50 controls) and compared the genotypes obtained from sequencing and RFLP. No discrepancies in the results between the two methods were observed. To certify genotyping quality, we also regenotyped approximately 10% of the random samples, and found no genotyping errors.

Biochemical analysis
Venous blood samples were centrifuged at 3000 g at 4 °C for 15 min. Serums were frozen at -20 °C until enzyme-linked immune sorbent assay (ELISA) analysis. Adiponectin serum concentrations were measured by using human ELISA kit. Serum insulin concentration was analyzed by chemiluminescent immunoassay (Immulite 2000, Siemens, Germany). Insulin resistance was then determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) calculated as the product of the fasting plasma insulin level (μU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 (Matthews et al. 1985).
Fasting plasma glucose and serum lipids (total cholesterol, HDL-C, LDL-C, triglycerides) were measured by enzymatic colorimetric methods using a Hitachi auto analyzer 704 (Roche Diagnostics Switzerland). Serum adiponectin was measured by ELISA with intra-assay and inter assay coefficients of variation of <5.4% and <8.5%, respectively. The study used the criteria of the National Cholesterol Education Program's Adult Treatment Panel (5) to diagnose the metabolic syndrome among Egyptian adolescents.
Insulin resistance was then determined by the Homeostasis Model Assessment of
Insulin Resistance (HOMA-IR) calculated as the product of the fasting plasma insulin level (μU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5. Insulin resistance was defined if HOMA-IR >3.5 (Keskin et al. 2005)
Anthropometric and body composition measurements were performed with the subject wearing light clothing and without shoes. For all subjects, body weight and height were measured using a scale and a wall-mounted stadiometer to the nearest 0.5 kg and 0.5 cm respectively. Body mass index (BMI) was computed as weight (in kilograms) divided by height (in meters) squared. Waist circumference (cm) was measured in the middle between the 12th rib and the iliac crest, and hip circumference (cm) was measured around the buttocks, at the level of the maximum extension. Blood pressure was measured three times and was averaged for analysis

Statistical analysis
Statistical analysis was performed by SPSS Software 16.0. Data were shown as mean ± standard deviation and odds ratio, 95% confidence interval. The Chi square test test or Fisher's exact test was used to assess whether the genotypes were in Hardy–Weinberg equilibrium and to compare the genotype and allele frequencies between case and control subjects. Differences in mean values between groups were analyzed with student-t test or with Mann-Whitney U-test, when sample size is smaller than 30. All p values are two tailed and group differences or correlations with $p < 0.05$ were accepted as significant

RESULTS AND DISCUSSION
Anthropometric and clinical data of total of 400 children were included. 200 formed the IR group and 200 healthy children accounted for the control group. There were no statistical differences between IR and control groups with regard to age, body weight and BMI.
Cases with IR showed higher triglyceride, HOMA-IR, SBP, DBP and lower serum adiponectin levels as compared to control group ($p < 0.05$) as shown in Table 1

Table: 1. Clinical and biochemical characteristics of IR and control group.

Characteristics	IR N=200	Controls N=200	p
Age (years)	10.2 ± 2.2	9.6 ± 2.3	NS
BMI (kg/m^2)	20.09± 5.49	19.2± 3.763	NS
Waist circumferences	81.9 ± 8.5	82.4 ± 4.6	**NS**
Waist to hip ratio	0.73 ± 0.03	.74± 0.01	**NS**
Cholesterol (mg/dl)	113.0±18. 6	112.7±15.4	**NS**
Triglycerides(mg/dl)	129.3± 12.5	88.9± 16.7	<.01
HDL-C (mg/dl)	40.7 ±19.9	41.3 ±16.4	**NS**
LDL-C (mg/dl)	89.5 ± 19.8	88.5± 16.4	**NS**
SBP (mmHg)	124.3±14. 1	102 ± 10.8	<.01
DBP (mmHg)	99.0 ± 10.6	69.6 ± 11.7	<.01
Glucose (mg/dl)	89.3 ± 9.8	85.9 ± 8.8	**NS**
HOMA-IR	4.5± .95	2.41± .82	<.01

NS: statistical non-significant

The genotype distributions of -11,391G/A (rs17300539) and -11,377C/G (rs 266729) in ADIPOQ are presented in Table 2.

Table: 2. ADIPOQ gene polymorphisms in IR cases and healthy controls

ADIPOQ polymorphisms		IR n = 200, (%)	Controls n = 200, (%)	P	OR
-11377C/G	CC	170 (85)	193(96.5)	.03	
	CG	20 (10)	5 (2.5)		
	GG	10 (.5)	2 (1.0)		
Recessive model	GG CC+CG	10 (.5) 190 (90)	2 (1.0) 198 (97.5)	<.001	5.21 (1.12- 14.09)
Alleles	C G	360 (90) 40 (10)	391 (97.75) 9 (2.25)	<.001	4.82 (2.31 - 10.08)
−11391G/A	GG	171(85.5)	195(97.5)	.02	
	GA	18(9)	4(2)		
	AA	11(5.5)	1(.5)		
Recessive model	AA GG +GA	18 (9) 182 (91)	4 (2) 196 (98)	<.001	4.84 (1.61- 14.58)
Alleles	G A	360 (90) 40 (10)	390 (97.5) 10 (2.5)	<.001	4.33(2.14-8.79)

Table: 3. Association of the studied SNPs with clinical and biochemical features in IR cases

Variables	-11377C/G		p	−11391G/A		p
	CC+ CG	GG		GG+ GA	AA	
BMI(kg/m2)	22.9 ± 6.7	21.9 ± 5.9	NS	21.3 ± 4.5	22.9 ± 4.9	NS
Waist (cm)	84.4 ± 4.2	85.1 ± 4.9	NS	85.9 ± 8.9	86.4 ± 8.5	NS
Waist to hip ratio	0.71 ± 0.05	.72 ± 0.03	NS	.69 ± 0.04	.71 ± 0.01	NS
SBP (mmHg)	106.7 ± 10.8	125.3 ± 14. 1	.04	103.3 ± 14. 1	123.5 ± 13.8	.01
DBP (mmHg)	69.6 ± 11.7	99.9 ± 10.6	.01	68.0 ± 10.7	100.1 ± 11.6	.02
Glucose (mg/dl)	78.9 ± 4.3	84.3 ± 5.8	NS	84.3 ± 5.8	84.3 ± 5.8	NS
HOMA-IR	1.41± .82	4.5± .95	.04	4.5± .95	4.5± .95	.03
Cholesterol (mg/dl)	131.7 ± 35.4	137.0 ± 19.9	.46	134.1 ± 28.3	137.0 ± 38. 6	NS
Triglycerides (mg/dl)	100.9 ± 16.7	129.3 ± 12.5	.01	101.3 ± 12.5	130.3 ± 12.5	.01
HDL-C (mg/dl)	42.3 ±16.9	43.7 ±17.4	NS	44.7 ±19.5	42.7 ±18.5	NS
LDL-C (mg/dl)	85.5 ± 16.4	88.5 ± 19.8	NS	86.5 ± 20.1	79.5 ± 19.8	NS
Adiponectin (µg/mL	4.09±1.71	2.36 ±1.1	.02	4.06 ±1.2	2.29 ±1.4	.01

The genotype distributions of both SNPs were found to be in Hardy–Weinberg equilibrium in both cases and controls. Association of ADIPOQ variants with IR and metabolic parameters was observed. In the analysis of -11,391G/A, the frequency of the rare genotype (AA) was significantly higher in IR cases (OR: 4.33(2.14-8.79) than in controls ($P < 0.05$). This genotype was associated with a fourfold higher probability ($P < 0.01$). Similar findings for the 11,377C/G , the frequency of the rare genotype (GG) was significantly higher in IR (OR: 4.82, 95% CI: 2.14-8.79).

Table 3 shows the clinical and biochemical features in IR cases according to the two studied promoters SNPs. Regarding the SNPs -11,391G/A, cases with AA genotype had significantly higher HOMA-IR, SBP, DBP, TG levels and lower adiponectin concentration compared with those with the combined genotypes group GG+ GA genotypes . Similar findings were found in the SNPs -11,377C/G , cases with the GG genotype had significantly higher HOMA-IR, SBP, DBP, TG levels and lower adiponectin levels compared with those with the combined genotypes group CC+CG .

Insulin sensitivity is regulated by serum adiponectin .The susceptibility locus for type 2 diabetes is positioned on chromosome 3q27 and spans approximately 16 kilo bases (kb) of DNA and known as the Adiponectin gene. It was reported that there is a strong association between the common single nucleotide polymorphisms (SNPs) within the Adiponectin locus, especially SNPs −11391G/A, −11377C/ G and serum adiponectin concentrations (Filippi et al. 2004)(Arikoglu et al. 2014) (Filippi et al. 2004; Gonzales-Sanchez et al. 2005; Menzaghi et al. 2002; Schwarz et al. 2006; Supriyaprom et al. 2010; Vasseur et al. 2002, 2005; Yang et al. 2008). Consequently, several meta-analyses supported these findings (Dastani et al. 2012;

Heid et al. 2010; Richards et al. 2009).

Some studies reported the associations between the adiponectin encoding gene variants and insulin resistance, type 2 diabetes, and/or cardiovascular diseases (Vasseur et al. 2002)(Vazzana et al. 2012). The pathogenesis of insulin resistance are contributed by multiple mechanisms (Huang et al. 2004). It seems that the role of adipose tissue, obesity, and genetic factors is of great significance among these mechanisms.

Both obese children and morbidly obese adults have similar -11,377C and +276T allelic frequencies, suggesting that morbid adult and childhood obesity may share part of their genetic background(Bouatia-Naji et al. 2006). Moreover, data from other studies are in favor of the association of obesity risk alleles -11,377C and -276GT with low adiponectin levels (Vasseur et al. 2005)(Qi et al. 2012) (Pollin et al. 2005). The current study verified again that the adiponectin gene variants showed association with insulin resistance risk among Egyptian children. Decreased adiponectin levels were described in obese subjects. However, adiponectin levels in insulin-sensitive subjects are significantly higher than in insulin-resistant patients independent of their obesity status (Abbasi et al. 2004), which in consistent with the present results. The physiological contribution of adiponectin to weight gain may be mediated by its insulin-sensitizing action in adipose tissue. Insulin signaling in the adipocyte is important for lipid storage, and adipose tissue selective insulin receptor knockout mice are protected from obesity(Blüher et al. 2002).SNPs -11391 G/A, -11377 C/G and their haplotypes have been repeatedly associated with low plasma adiponectin (Vasseur et al. 2002)(Hara et al. 2002), but the physiological mechanisms by which adiponectin deficiency favors metabolic diseases and atherogenesis is not completely understood. As -11,377C and −11391G are the wild-type allele, its presence corresponds to the basal transcriptional activity of the promoter. Laumen et al. (Laumen et al. 2009) reported that SNPs −11426A/G, −11391G/A and −11377C/G within the Adiponectin promoter region were regulatory SNPs for Adiponectin promoter activity. They also established that the three SNPs led to a change in promoter activity and Adiponectin gene expression. The present data also found that −11391G/A and −11377C/G are linked to adiponectin levels. Previously, some studies found that the -11377G allele was distinctly associated with low plasma adiponectin level and IR resulting in T2DM risk(Ye et al. 2008). The present study found also low serum adiponectin level in −11391A and −11377G allele carriers as compared to those carried the wild-type alleles. Circulating adiponectin is inversely associated with the degree of obesity (Arita et al. 1999) and is particularly low in subjects with type 2 diabetes (Hotta et al. 2000). Emerging evidence indicates that body fat distribution, i.e., relative presence of central to peripheral fat mass, has more critical implications for women's cardiovascular morbidity/mortality than overall obesity per se. Indeed, numerous studies have shown reciprocal associations of central and peripheral fat compartments with adiponectin (Motoshima et al. 2002), insulin sensitivity(Tankó et al. 2003). The present study supports the significant effect of ADIPOQ variations, -11377 C/G and -11391 G/A on regulation of adiponectin levels. Adiponectin may play a role on not only mediating insulin resistance but also in cell dysfunction in the pathogenesis of diabetes(Retnakaran et al. 2005). Therefore, plasma/serum adiponectin levels and genomic DNA polymorphisms in the AdipoQ gene can be used as the biomarkers for early diagnosis and clinic prediction of diabetes, obesity, diabetic complications and other metabolic disorders.

CONCLUSION

In conclusion, the present cross-sectional study confirms the association between ADIPOQ variants and IR and low adiponectin concentrations among adolescents, suggesting the important role of these polymorphisms in the IR risk and regulation of adiponectin levels. This result might contribute to the formulation of therapy for insulin sensitivity. Both polymorphisms can be good early biomarkers of IR and in the prediction of metabolic complications among adolescents.

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REFERENCES

Abbasi, F. et al., 2004. Discrimination between obesity and insulin resistance in the relationship with adiponectin. Diabetes, 53(3), pp.585–590.

Arikoglu, H. et al., 2014. The Adiponectin variants contribute to the genetic background of type 2 diabetes in Turkish population. Gene, 534(1), pp.10–16.

Arita, Y. et al., 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochemical and biophysical research communications, 257(1), pp.79–83.

Berg, A.H., Combs, T.P. & Scherer, P.E., 2002. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in Endocrinology & Metabolism, 13(2), pp.84–89.

Blüher, M. et al., 2002. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Developmental cell, 3(1), pp.25–38.

Bouatia-Naji, N. et al., 2006. ACDC/adiponectin polymorphisms are associated with severe childhood and adult obesity. Diabetes, 55(2), pp.545–550.

Comuzzie, A.G. et al., 2001. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. The Journal of Clinical Endocrinology & Metabolism, 86(9), pp.4321–4325.

Filippi, E. et al., 2004. Association of the human adiponectin gene and insulin resistance. European Journal of Human Genetics, 12(3), pp.199–205.

Filippi, E. et al., 2005. 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≤ 50 years). Journal of molecular medicine, 83(9), pp.711–719.

Hara, K. et al., 2002. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes, 51(2), pp.536–540.

Heid, I.M. et al., 2006. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes, 55(2), pp.375–384.

Hermann, T.S. et al., 2006. Quinapril treatment increases insulin-stimulated endothelial function and adiponectin gene expression in patients with type 2 diabetes. The Journal of Clinical Endocrinology & Metabolism, 91(3), pp.1001–1008.

Hoffstedt, J. et al., 2004. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. The Journal of Clinical Endocrinology & Metabolism, 89(3), pp.1391–1396.

Hotta, K. et al., 2000. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arteriosclerosis, thrombosis, and vascular biology, 20(6), pp.1595–1599.

Huang, K. et al., 2004. Plasma adiponectin levels and metabolic factors in nondiabetic adolescents. Obesity research, 12(1), pp.119–124.

Keskin, M. et al., 2005. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics, 115(4), pp.e500–e503.

Laumen, H. et al., 2009. Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes, 58(4), pp.984–991.

Matthews, D.R. et al., 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia, 28(7), pp.412–419.

Menzaghi, C., Trischitta, V. & Doria, A., 2007. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes, 56(5), pp.1198–1209.

Motoshima, H. et al., 2002. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. The

Journal of Clinical Endocrinology & Metabolism, 87(12), pp.5662–5667.

Ouchi, N. et al., 2000. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-κB signaling through a cAMP-dependent pathway. Circulation, 102(11), pp.1296–1301.

Pajvani, U.B. & Scherer, P.E., 2003. Adiponectin: systemic contributor to insulin sensitivity. Current diabetes reports, 3(3), pp.207–213.

Pollin, T.I. et al., 2005. Linkage of plasma adiponectin levels to 3q27 explained by association with variation in the APM1 gene. Diabetes, 54(1), pp.268–274.

Qi, L. et al., 2005. The+ 276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. Diabetes, 54(5), pp.1607–1610.

Qi, Q. et al., 2012. Genetic variants, plasma lipoprotein (a) levels, and risk of cardiovascular morbidity and mortality among two prospective cohorts of type 2 diabetes. European heart journal, 33(3), pp.325–334.

Retnakaran, R. et al., 2005. Adiponectin and beta cell dysfunction in gestational diabetes: pathophysiological implications. Diabetologia, 48(5), pp.993–1001.

Schäffler, A. et al., 2004. Identification of influencing variables on adiponectin serum levels in diabetes mellitus type 1 and type 2. Experimental and clinical endocrinology & diabetes, 112(7), pp.383–389.

Tankó, L.B. et al., 2003. Peripheral adiposity exhibits an independent dominant antiatherogenic effect in elderly women. Circulation, 107(12), pp.1626–1631.

Tomas, E. et al., 2002. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: Acetyl–CoA carboxylase inhibition and AMP-activated protein kinase activation. Proceedings of the *National Academy of Sciences*, 99(25), pp.16309–16313.

Vasseur, F. et al., 2005. Hypoadiponectinaemia and high risk of type 2 diabetes are associated with adiponectin-encoding (ACDC) gene promoter variants in morbid obesity: evidence for a role of ACDC in diabesity. *Diabetologia*, 48(5), pp.892–899.

Vasseur, F. et al., 2002. 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. *Human molecular genetics*, 11(21), pp.2607–2614.

Vazzana, N. et al., 2012. Endogenous secretory RAGE in obese women: association with platelet activation and oxidative stress. *The Journal of Clinical Endocrinology & Metabolism*, 97(9), pp.E1726–E1730.

Yamauchi, T. et al., 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature medicine*, 8(11), pp.1288–1295.

Yamauchi, T. et al., 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature medicine*, 7(8), pp.941–946.

Ye, F. et al., 2008. Association of SNPs in the proximal promoter region of the APM1 gene with obesity and type 2 diabetes. *Journal of Xian Jiaotong University (Medical Sciences)*, 29, pp.74–76.