Ther Adv Cardiovasc Dis
(2010) 4(4) 223–230
DOI: 10.1177/1753944710371483

Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population

Benedetta D. Chiodini, Claudia Specchia, Francesca Gori, Simona Barlera, Andria D'Orazio, Silvia Pietri, Luisa Crociati, Antonio Nicolucci, Monica Franciosi, Stefano Signorini, Paolo Brambilla, Maria Grazia Franzosi, on behalf of GISSI Prevenzione Investigators* and SiBioC-GISSI Prevenzione Group**

***Abstract*:**
Objective: While many studies have shown an association between the gene coding for adiponectin (ADIPOQ) and adiponectin levels, much more controversy surrounds its association with metabolic traits such as insulin resistance, obesity and type 2 diabetes. Furthermore, very few studies have looked into the relations between ADIPOQ variants and risk of cardiovascular disease. The present study assessed the influence of four common ADIPOQ Single Nucleotide Polymorphisms (SNPs), rs17300539 (−11391G→A), rs266729 (−11377C→G), rs2241766 (+45T→G) and rs1501299 (+276G→T) on the risk of myocardial infarction and type 2 diabetes.
Methods and Results: A large genetic association case-control study was conducted in 2008 Italians, including patients with myocardial infarction, type 2 diabetes, or both, and a reference group of healthy controls. Homozygotes TT for the rs1501299 (+276) had half the risk of either myocardial infarction alone or in association with type 2 diabetes when compared to the carriers of the G allele (OR = 0.58, $p = 0.01$, and OR = 0.55, $p = 0.006$ respectively). SNPs rs17300539 (−11391), rs266729 (−11377) and rs2241766 (+45) showed no significant association with any of the three case groups.
Conclusions: These results suggest that homozygotes TT for the adiponectin polymorphism rs1501299 (+276) are protected from the risk of myocardial infarction.

***Keywords*:** adiponectin, genetic association, myocardial infarction, type 2 diabetes

Introduction

Adiponectin is an adipokine, which is an adipocyte-secreted protein. It has several functions: it may act as an antidiabetic hormone, directly regulating energy homeostasis, glucose and lipid metabolism, and insulin sensitivity *in vitro* and *in vivo*. Through action on endothelial function, inhibition of vascular smooth muscle cell proliferation and the anti-inflammatory effects on the cellular components of the vascular wall, it can also have an anti-atherogenic effect [Arita *et al.* 2002]. Adiponectin could be a link between obesity and related atherosclerosis. Low serum adiponectin levels have been associated with a higher body mass index (BMI), an increased risk of insulin resistance, type 2 diabetes (T2D) and cardiovascular disease [Chen *et al.* 2005; Pischon *et al.* 2004]. Serum adiponectin seems to be even lower in diabetic patients with coronary artery disease (CAD) [Hotta *et al.* 2000].

The gene coding for adiponectin, ADIPOQ, is located on chromosome 3q27. This region has shown linkage to T2D [Mori *et al.* 2002;

*A complete list of GISSI-Prevenzione study committees, collaborators, and participating centers was published in GISSI-Investigators (1999) *Lancet* 354: 447–455.

**A complete list of SiBioC-GISSI Prevenzione Group Investigators was published in Chiodini *et al.* (2007) *Eur Heart J* 28: 1977–1983.

Correspondence to:
Claudia Specchia, PhD
Department of Biomedical Sciences and Biotechnologies, University of Brescia, Brescia, Italy and Department of Cardiovascular Research, Mario Negri Institute for Pharmacological Research Via La Masa 19, 20156 Milano, Italy
specchia@gissi.marionegri.it

Benedetta D. Chiodini, MD
Division of Genetics and Molecular Medicine, King's College London, London, UK and Department of Cardiovascular Research, Mario Negri Institute for Pharmacological Research, Milano, Italy

Francesca Gori, Biotech D
Simona Barlera, MSc
Silvia Pietri, Lab Techn
Luisa Crociati, Biol D
Maria Grazia Franzosi, Biol D
Department of Cardiovascular Research, Mario Negri Institute for Pharmacological Research, Milano, Italy

Andria D'Orazio, Lab Techn
Antonio Nicolucci, MD
Monica Franciosi, MSc Biol
Consorzio Mario Negri Sud, Department of Clinical Pharmacology and Epidemiology, Santa Maria Imbaro, Italy

Stefano Signorini, MD
University Department of Laboratory Medicine, Hospital of Desio, Milano, Italy

Paolo Brambilla, MD
University Department of Laboratory Medicine, Hospital of Desio, Milano, Italy and University of Milano-Bicocca, Medical School DMS, Milano, Italy

Vionnet *et al.* 2000; Hegele *et al.* 1999], metabolic syndrome [Francke *et al.* 2001; Kissebah *et al.* 2000], cholesterol concentration in low-density lipoprotein (LDL) size fractions [Rainwater *et al.* 1999], and CAD [Chiodini and Lewis, 2003]. The ADIPOQ gene includes three exons and in Europeans is organized in two linkage dysequilibrium (LD) blocks ($D' > 0.8$ for pairs of consecutive SNPs) with a boundary point placed in the first intron [Heid *et al.* 2006].

Among several polymorphisms investigated, four SNPs, rs17300539 (−11391), rs266729 (−11377), rs2241766 (+45) and rs1501299 (+276), have been tested in many studies for their association with low adiponectin levels and metabolic traits, in particular T2D. Referring to the LD structure reported by Heid and colleagues, two of these SNPs, rs17300539 (−11391) and rs266729 (−11377), map in the first LD block and the other two, rs2241766 (+45) and rs1501299 (+276), are in the second, in exon 2 and intron 2, respectively. Although results have not always been consistent, many studies described an association between one or more of these allelic variants and adiponectin levels [Mackevics *et al.* 2006; Mousavinasab *et al.* 2006; Schwarz *et al.* 2006; Filippi *et al.* 2005; Jang *et al.* 2005; Lee *et al.* 2005; Pollin *et al.* 2005; Qi *et al.* 2005; Tanko *et al.* 2005; Vasseur *et al.* 2005, 2002; Fumeron *et al.* 2004; Menzaghi *et al.* 2004].

With regard to associations between the same four polymorphisms and metabolic traits such as BMI, insulin resistance (IR) and T2D, the literature offers various contradictory studies and different associations in different samples [Gable *et al.* 2006]. In a meta-analysis [Menzaghi *et al.* 2007] of the four genetic variants, the only evidence of a significant association was observed between rs1501299 (+276) and IR, and no significant association was found between any of the SNPs and risk of T2D. The surrogate index for insulin resistance $HOMA_{IR}$ (homeostasis model assessment of insulin resistance) was higher in rs1501299 (+276)-G allele carriers than TT subjects, indicating higher insulin sensitivity in carriers of allele T, which showed a marginal association with higher adiponectin levels.

The relations between the four adiponectin polymorphisms and cardiovascular outcomes have not been studied as much as adiponectin levels or metabolic traits, and have generally been weak [Hegener *et al.* 2006; Jang *et al.* 2006, 2005; Filippi *et al.* 2005]. A meta-analysis by Qi and colleagues considered four studies of subjects with T2D, and included a total of 827 diabetic CAD-positive cases and 1887 diabetic CAD-negative controls [Qi *et al.* 2006]. Evidence of association was only found for polymorphism rs1501299 (+276), where TT individuals had about half the cardiovascular risk of allele G carriers: a finding consistent with the association indicated by the meta-analysis by Menzaghi and colleagues for the same SNP and IR and adiponectin levels [Menzaghi *et al.* 2007]. A recent prospective association study could also not confirm a common role of variations in the adiponectin gene on the cardiovascular risk in a population of Europeans [Gable *et al.* 2007].

The aim of this study was to investigate the effect of the four genetic variants at the ADIPOQ gene, rs17300539 (−11391), rs266729 (−11377), rs2241766 (+45) and rs1501299 (+276), on the cardiovascular risk in four large, independent groups of patients with myocardial infarction (MI), T2D, or both, and a reference group of healthy controls.

Methods

Study population

We enrolled 503 patients with T2D among those recruited in the IGLOO cohort study (Impaired Glucose intolerance & Long-term Outcomes Observational Study) [Franciosi *et al.* 2005]. The other case groups of 503 MI only and 499 both MI and T2D were selected at random from the GISSI-Prevenzione study (GISSI-P) [GISSI-Investigators, 1999]. Details on the study design, eligibility criteria, and results of IGLOO and GISSI-P are reported elsewhere [Franciosi *et al.* 2005; GISSI-Investigators, 1999]. The GISSI-P and IGLOO studies were both approved by the local ethics committees of the participating hospitals. The control group comprised 503 nondiabetic subjects, not affected by CAD, without previous MI, dyslipidaemia or family history of CAD, recruited among blood donors belonging to the AVIS (Associazione Volontari Italiani Sangue) association. Written informed consent was obtained from each person.

Power calculation

The sample size was calculated on the basis of the results of the recent meta-analysis by Menzaghi

and colleagues, in which the homozygotes TT for the SNP rs1501299 (+276) had a lower cardiovascular risk than the carriers of other genotypes, with an odds ratio (OR) of 0.55 [Menzaghi *et al.* 2007]. Sample size calculations had shown that a sample size of 500 individuals was required in order to have 80% power for detecting a 50% decrease in risk in terms of OR associated with MI for homozygotes TT. This calculation assumed an allele frequency of 30% for SNP rs1501299 (+276)-T allele (Table 1) and a prevalence of MI in the Italian population of 1%. A significance level of alpha = 0.05 with a two-sided test was considered. The software QUANTO 1.2 was used (see http://hydra.usc.edu/gxe).

Genotyping

DNA was extracted from frozen EDTA-whole blood using a salting-out procedure [Miller *et al.* 1988]. All polymerase chain reactions (PCRs) were done in a 5 µl volume containing TaqMan universal PCR Master Mix, specific TaqMan® SNP Genotyping Assays, purchased from Applied Biosystems (Foster City, CA, USA), and 10 ng of genomic DNA. The 7900 Real-Time PCR System (ABI) was used for SNP genotyping. To ensure the quality of automatic allele calling, all samples were analysed in two replicates and the concordance rate was 100%.

Statistical analysis

Continuous variables were compared among groups using analysis of variance (ANOVA), and variable transformed as required. Differences in percentages were assessed by chi-squared test or Fisher's exact test, as appropriate. The pairwise LD among the four SNPs was assessed by the correlation coefficient r^2. For each SNP, a chi-squared test was performed to assess whether the observed genotype frequencies were in Hardy–Weinberg equilibrium (HWE) among controls. Frequency distributions by genotype and by minor allele were compared across groups. Genetic models were tested comparing a dominant, recessive or an additive model in terms of logit, i.e. a model of allelic association, with a model of genotype association by likelihood ratio test.

In order to quantify the association between each SNP genotypes and case groups, separate unconditional multiple logistic regression models were fitted, including age and sex as covariates. To avoid overadjustment, BMI, glucose, blood pressure and lipids were not considered in the model. We calculated 99% confidence intervals (CIs) to measure the statistical precision of the ORs. Homogeneity of risks among case groups was assessed using a Wald test with one degree of freedom [Hosmer and Lemeshow, 1989].

A p-value of $p < 0.0125$ was considered significant to adjust for multiple testing across the four SNPs.

Statistical analysis was performed using STATA 9.0 (see http://www.stata.com). HWE was tested using the *gtab* STATA package.

Results

The study population consisted of 2008 unrelated Italians. Age, sex, glucose, lipids, systolic and diastolic blood pressure (BP) and BMI are described in Table 2, separately for the case groups and the controls. The T2D population was slightly older than the other three groups, which were very homogeneous in terms of age. There were more males in all groups except T2D cases where the two sexes were more balanced. As expected, the control group had the highest high-density lipoprotein (HDL) cholesterol levels and the lowest triglycerides, the subjects with MI plus T2D having the lowest HDL and highest triglycerides.

Table 1. The four single nucleotide polymorphisms in the study population.

ID number	Position	Chromosomal position	HWE χ^2 test p-value[a]	Variant	Minor allele	Minor allele frequency
rs17300539	–11391	188,042,154	0.25	G→A	A	0.09
rs266729	–11377	188,042,168	0.72	C→G	G	0.22
rs2241766	+45	188,053,586	0.10	T→G	G	0.16
rs1501299	+276	188,053,817	0.02	G→T	T	0.30

[a]Evaluated in the control group. HWE, Hardy–Weinberg equilibrium.

Table 2. Main characteristics of the case groups and the controls.

	MI $N=503$	T2D $N=503$	MI + T2D $N=499$	Controls $N=503$	*p*-value[b]
Age (years)[a]	56.5 (4.5)	62.8 (7.0)	56.6 (6.4)	54.7 (5.2)	<0.0001
Males (%)[a]	449 (89.3)	285 (56.7)	420 (84.2)	482 (95.8)	<0.001
Cholesterol HDL (mg/dL)[a]	41.2 (11.5)	50.9 (14.2)	38.8 (9.8)	57.3 (13.3)	<0.0001
Triglycerides (mg/dL)[a]	153.9 (66.5)	162.8 (100.4)	188.4 (100.8)	114.2 (66.7)	<0.0001
Fasting blood glucose (mg/dL)[a]	93.5 (15.6)	146.1 (43.1)	150.4 (50.8)	92.3 (11.3)	<0.0001
Systolic BP (mmHg)[a]	122.4 (15.3)	142.1 (15.4)	124.2 (14.6)	129.5 (10.9)	<0.0001
Diastolic BP (mmHg)[a]	76.7 (9.1)	84.9 (8.1)	76.6 (8.4)	83.6 (6.2)	<0.0001
BMI (kg/m^2)[a]	26.4 (3.4)	29.5 (4.4)	27.5 (3.8)	26.4 (3.3)	<0.0001

[a]Mean (standard deviation).
[b]Overall *p*-value.
MI, myocardial infarction; T2D, type 2 diabetes; HDL, high-density lipoprotein; BP, blood pressure; BMI, body mass index.

Table 3. Genotype and allele frequency distribution in case groups and controls.

Rs17300539 (−11391)	*N*	GG *N* (%)	GA *N* (%)	AA *N* (%)	*p*-value[a]	Minor allele %	*p*-value[a]
MI	503	411 (81.7)	87 (17.3)	5 (1.0)	0.72	9.6	0.65
T2D	503	403 (80.1)	96 (19.1)	4 (0.8)		10.3	
MI + T2D	499	416 (83.4)	78 (15.6)	5 (1.0)		8.8	
Controls	503	414 (82.3)	87 (17.3)	2 (0.4)		9.0	
rs266729 (−11377)	*N*	CC *N* (%)	CG *N* (%)	GG *N* (%)	*p*-value[a]	Minor allele %	*p*-value[a]
MI	503	295 (58.6)	177 (35.2)	31 (6.2)	0.15	23.8	0.02
T2D	503	322 (64.0)	159 (31.6)	22 (4.4)		20.2	
MI + T2D	499	288 (57.7)	176 (35.3)	35 (7.0)		24.6	
Controls	503	321 (63.8)	160 (31.8)	22 (4.4)		20.3	
rs2241766 (+45)	*N*	TT *N* (%)	GT *N* (%)	GG *N* (%)	*p*-value[a]	Minor allele %	*p*-value[a]
MI	503	358 (71.2)	136 (27.0)	9 (1.8)	0.004	15.3	0.07
T2D	503	370 (73.5)	117 (23.3)	16 (3.2)		14.8	
MI + T2D	499	321 (64.3)	168 (33.7)	10 (2.0)		18.8	
Controls	503	359 (71.4)	126 (25.0)	18 (3.6)		16.1	
rs1501299 (+276)	*N*	GG *N* (%)	GT *N* (%)	TT *N* (%)	*p*-value[a]	Minor allele %	*p*-value[a]
MI	503	259 (51.5)	203 (40.4)	41 (8.1)	0.05	28.3	0.01
T2D	503	245 (48.7)	206 (41.0)	52 (10.3)		30.8	
MI + T2D	499	271 (54.3)	189 (37.9)	39 (7.8)		26.7	
Controls	503	239 (47.5)	198 (39.4)	66 (13.1)		32.8	

[a]*p*-value refers to comparison of frequency distribution by genotype and by minor allele across groups.
MI, myocardial infarction; T2D, type 2 diabetes.

Genotyping was complete for all individuals. The characteristics of the four studied SNPs are reported in Table 1. Genotype frequencies of all SNPs resulted in HWE in the control group after correction for multiple testing. The independence of the SNPs was confirmed, according to the correlation coefficients $r^2 < 0.1$.

SNPs rs17300539 (−11391), rs266729 (−11377), genotypes and allele distribution did not differ significantly in the four groups (Table 3). Tests of the genetic model showed that SNP rs2241766 (+45) was consistent with a genotype association while there was evidence of a genetic recessive model for SNP rs1501299 (+276) ($p > 0.20$).

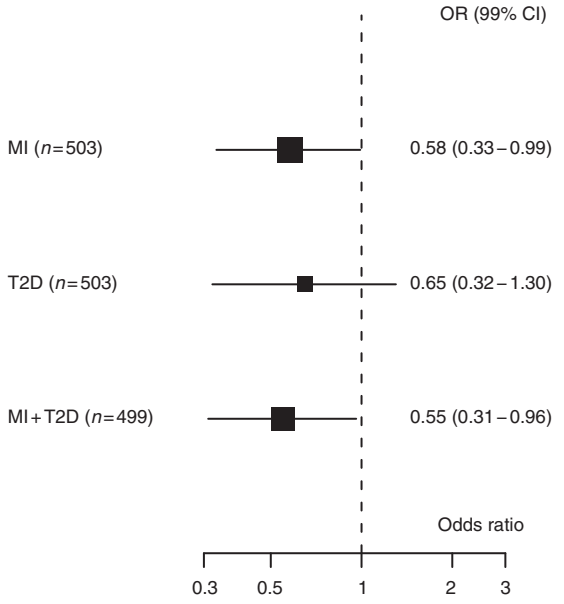

Figure 1. Association with myocardial infarction (MI) and type 2 diabetes (T2D) for single nucleotide polymorphism (SNP) rs1501299 (+276). Solid squares centred on the odds ratio (OR) estimate and scaled in proportion to sample size with 99% confidence interval (CI; horizontal bar) for subgroups comparing MI with or without a clinical history of diabetes, and T2D only. The reference group is non-MI, nondiabetics. The number of individuals in each subgroup (*n*) is shown.

SNPs rs2241766 (+45) genotype distribution ($p = 0.004$) and SNP rs1501299 (+276) T allele distribution ($p = 0.01$) differed significantly different between the groups. The associations of SNPs rs2241766 (+45) and rs1501299 (+276) between each case group and controls were tested by unconditional logistic regression, adjusting for variables such as sex and age. SNP rs2241766 (+45) genotype had no effect on MI and T2D, separately or combined. Evidence of association with MI was found for polymorphism rs1501299 (+276), where the homozygotes TT had about half the cardiovascular risk of allele G carriers (OR = 0.58, $p = 0.01$). Homozygotes TT for SNPs rs1501299 (+276) were similarly protected against MI and T2D combined (OR = 0.55, $p = 0.006$). No significant association was found with only T2D. However, comparing the homogeneity of genetic risks in the diagnostic groups, the OR of homozygotes TT for rs1501299 (+276) in the T2D+MI group was not significantly different from that in the T2D only group ($p = 0.38$). Figure 1 reports the adjusted OR and 99% CI.

Discussion

This large genetic association study included three case and one control groups of Italians, in order to investigate the relationship between four common adiponectin SNPs, rs17300539 (−11391), rs266729 (−11377), rs2241766 (+45) and rs1501299 (+276), and risk of MI alone or together with T2D. We also investigated the effect of the same polymorphisms on the risk of diabetes alone.

SNP rs1501299 (+276) was significantly associated with the risk of MI and the combination of MI and T2D. In particular, homozygotes TT appeared protected, having about half the risk of either the cardiovascular disease alone or with diabetes, compared with carriers of the G allele. Homozygotes TT also appeared to enjoy some protection from the risk of T2D, although the reduction was not statistically significant.

The observed deviation from HWE for SNP rs1501299 in the control group, although not statistically significant, should be taken into consideration in the evaluation of the association reported. We are confident to have no genotyping errors as HWE was satisfied in the other groups of cases, suggesting no systematic problem with the SNPs detection.

SNPs rs17300539 (−11391), rs266729 (−11377), rs2241766 (+45) had no effect on any of the diseases.

Unfortunately this study was unable to evaluate the relations between adiponectin SNPs and adiponectin levels. The MI and MI plus T2D groups included participants in the GISSI-Prevenzione study, for which no accurate and reliable data were available on adiponectin levels.

With regards to the relationship between the adiponectin SNPs and risk of CAD, very few studies have tried to answer this question. Some studies have looked at the risk of CAD in carriers of different allelic variants not affected by metabolic diseases, while others have only considered the CAD risk in T2D patients. Among the first group, an Italian investigation [Filippi *et al.* 2005] on 325 CAD-positive subjects and 270 controls found an association between the rs1501299 (+276) and both adiponectin levels and CAD, with carriers of the T allele at increased risk. These results seem to go in the opposite direction from a Korean study which

reported a protective effect of rs1501299 (+276) T allele with regard to several components of the metabolic syndrome and cardiovascular disease (e.g. IR, serum TG concentration and LDL particle size) [Jang *et al.* 2006, 2005]. In contradiction with both studies are the findings of a prospective, nested case–control investigation on 600 White men who subsequently suffered an atherothrombotic event, and 600 controls from the Physicians Health Study [Hegener *et al.* 2006], which showed an association between SNP rs266729 (−11377) and decreased risk of ischaemic stroke but no MI. A meta-analysis published in 2007 concerning SNPs in more than 2000 individuals [Menzaghi *et al.* 2007] found a significant association between SNPs rs17300539 (−11391) and rs1501299 (+276) and adiponectin levels and between SNP rs1501299 (+276) and IR. No significant global effects were observed between any of the SNPs rs17300539 (−11391), rs266729 (−11377), rs2241766 (+45) and rs1501299 (+276) and risk of T2D or higher BMI. However, diabetic individuals homozygous for allele T at SNP rs1501299 (+276) had about half the cardiovascular risk of allele G carriers. This very interesting finding was the outcome of a meta-analysis consisting of only four small studies, all including diabetic patients. Our results not only agree with the meta-analysis, but also show that individuals homozygous for allele T at SNP rs1501299 (+276) benefit from the same protection against the risk of MI regardless of whether they have diabetes. This suggests that SNP rs1501299 (+276) has an effect more on the risk of MI than T2D.

Although rs1501299 (+276) is located in an intronic region with no apparent biological function, this SNP may affect the expression level of the gene through some unknown mechanisms, or it may be in LD with undiscovered SNPs in the ADIPOQ gene or other genes with biological effects on IR [Jang *et al.* 2006]. This might also explain why the allele that raised the risk of CAD appeared to be the T allele in some studies [Filippi *et al.* 2005] and G allele in others [Jang *et al.* 2005; Qi *et al.* 2005; Bacci *et al.* 2004], and why the effects of SNP rs1501299 (+276) appear to be independent of circulating adiponectin and other markers related to lipids, inflammation and endothelial function [Qi *et al.* 2005].

Sometimes studies are unexpectedly unable to confirm previous findings, despite being well conducted. The reasons for the partially discrepant results are not known, and may reside in substantial differences between study populations. In fact, population specificity may consist of differences in LD block, haplotypes, population-specific interactions between genes and epigenetic modifications.

The present analysis has been performed on subjects of Italian origin only recruited from the GISSI-P study, which can be considered more genetically homogenous than the more unspecific ethnic group of Europeans. In this sense it is likely that LD is substantially different from previous investigations. The lack of LD between the four SNPs investigated in the present study is nevertheless supported by the results of Heid and colleagues that described that no notable correlation exists between any SNP of block 1 with a SNP of block 2 and that the four SNPs are not highly correlated each other as specified in the online appendix [Heid *et al.* 2006].

Conflict of interest statement

None declared.

References

Arita, Y., Kihara, S., Ouchi, N., Maeda, K., Kuriyama, H., Okamoto, Y. *et al.* (2002) Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 105: 2893–2898.

Bacci, S., Menzaghi, C., Ercolino, T., Ma, X., Rauseo, A., Salvemini, L. *et al.* (2004) The +276 G/T single nucleotide polymorphism of the adiponectin gene is associated with coronary artery disease in type 2 diabetic patients. *Diabetes Care* 27: 2015–2020.

Chen, M.P., Tsai, J.C., Chung, F.M., Yang, S.S., Hsing, L.L., Shin, S.J. *et al.* (2005) Hypoadiponectinemia is associated with ischemic cerebrovascular disease. *Arterioscler Thromb Vasc Biol* 25: 821–826.

Chiodini, B.D. and Lewis, C.M. (2003) Meta-analysis of 4 coronary heart disease genome-wide linkage studies confirms a susceptibility locus on chromosome 3q. *Arterioscler Thromb Vasc Biol* 23: 1863–1868.

Filippi, E., Sentinelli, F., Romeo, S., Arca, M., Berni, A., Tiberti, C. *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 <or = 50 years). *J Mol Med* 83: 711–719.

Franciosi, M., De Berardis, G., Rossi, M.C., Sacco, M., Belfiglio, M., Pellegrini, F. *et al.* (2005) Use of the

diabetes risk score for opportunistic screening of undiagnosed diabetes and impaired glucose tolerance: the IGLOO (Impaired Glucose Tolerance and Long-Term Outcomes Observational) study. *Diabetes Care* 28: 1187–1194.

Francke, S., Manraj, M., Lacquemant, C., Lecoeur, C., Lepretre, F., Passa, P. *et al.* (2001) A genome-wide scan for coronary heart disease suggests in Indo-Mauritians a susceptibility locus on chromosome 16p13 and replicates linkage with the metabolic syndrome on 3q27. *Hum Mol Genet* 10: 2751–2765.

Fumeron, F., Aubert, R., Siddiq, A., Betoulle, D., Pean, F., Hadjadj, S. *et al.* (2004) Adiponectin gene polymorphisms and adiponectin levels are independently associated with the development of hyperglycemia during a 3-year period: the epidemiologic data on the insulin resistance syndrome prospective study. *Diabetes* 53: 1150–1157.

Gable, D.R., Hurel, S.J. and Humphries, S.E. (2006) Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. *Atherosclerosis* 188: 231–244.

Gable, D.R., Matin, J., Whittall, R., Cakmak, H., Li, K.W., Cooper, J. *et al.* (2007) Common adiponectin gene variants show different effects on risk of cardiovascular disease and type 2 diabetes in European subjects. *Ann Hum Genet* 71: 453–66.

GISSI-Investigators (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardio. *Lancet* 354: 447–455.

Hegele, R.A., Sun, F., Harris, S.B., Anderson, C., Hanley, A.J. and Zinman, B. (1999) Genome-wide scanning for type 2 diabetes susceptibility in Canadian Oji-Cree, using 190 microsatellite markers. *J Hum Genet* 44: 10–14.

Hegener, H.H., Lee, I.M., Cook, N.R., Ridker, P.M. and Zee, R.Y. (2006) Association of adiponectin gene variations with risk of incident myocardial infarction and ischemic stroke: a nested case–control study. *Clin Chem* 52: 2021–2027.

Heid, I.M., Wagner, S.A., Gohlke, H., Iglseder, B., Mueller, J.C., Cip, P. *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: 375–384.

Hosmer Jr, D.W. and Lemeshow, S. (1989) Applied Logistic Regression, Wiley: New York.

Hotta, K., Funahashi, T., Arita, Y., Takahashi, M., Matsuda, M., Okamoto, Y. *et al.* (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20: 1595–1599.

Jang, Y., Lee, J.H., Chae, J.S., Kim, O.Y., Koh, S.J., Kim, J.Y. *et al.* (2005) Association of the 276G->T polymorphism of the adiponectin gene with cardiovascular disease risk factors in nondiabetic Koreans. *Am J Clin Nutr* 82: 760–767.

Jang, Y., Lee, J.H., Kim, O.Y., Koh, S.J., Chae, J.S., Woo, J.H. *et al.* (2006) 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* 55: 59–66.

Kissebah, A.H., Sonnenberg, G.E., Myklebust, J., Goldstein, M., Broman, K., James, R.G. *et al.* (2000) Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97: 14478–14483.

Lee, Y.Y., Lee, N.S., Cho, Y.M., Moon, M.K., Jung, H.S., Park, Y.J. *et al.* (2005) Genetic association study of adiponectin polymorphisms with risk of Type 2 diabetes mellitus in Korean population. *Diabet Med* 22: 569–575.

Mackevics, V., Heid, I.M., Wagner, S.A., Cip, P., Doppelmayr, H., Lejnieks, A. *et al.* (2006) The adiponectin gene is associated with adiponectin levels but not with characteristics of the insulin resistance syndrome in healthy Caucasians. *Eur J Hum Genet* 14: 349–356.

Menzaghi, C., Ercolino, T., Salvemini, L., Coco, A., Kim, S.H., Fini, G. *et al.* (2004) Multigenic control of serum adiponectin levels: evidence for a role of the APM1 gene and a locus on 14q13. *Physiol Genomics* 19: 170–174.

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

Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 16: 1215.

Mori, Y., Otabe, S., Dina, C., Yasuda, K., Populaire, C., Lecoeur, C. *et al.* (2002) Genome-wide search for type 2 diabetes in Japanese affected sib-pairs confirms susceptibility genes on 3q, 15q, and 20q and identifies two new candidate loci on 7p and 11p. *Diabetes* 51: 1247–1255.

Mousavinasab, F., Tahtinen, T., Jokelainen, J., Koskela, P., Vanhala, M., Oikarinen, J. *et al.* (2006) Common polymorphisms (single-nucleotide polymorphisms SNP + 45 and SNP + 276) of the adiponectin gene regulate serum adiponectin concentrations and blood pressure in young Finnish men. *Mol Genet Metab* 87: 147–151.

Pischon, T., Girman, C.J., Hotamisligil, G.S., Rifai, N., Hu, F.B. and Rimm, E.B. (2004) Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 291: 1730–1737.

Pollin, T.I., Tanner, K., O'Connell, J.R., Ott, S.H., Damcott, C.M., Shuldiner, A.R. *et al.* (2005) Linkage

of plasma adiponectin levels to 3q27 explained by association with variation in the APM1 gene. *Diabetes* 54: 268–274.

Qi, L., Doria, A., Manson, J.E., Meigs, J.B., Hunter, D., Mantzoros, C.S. *et al.* (2006) Adiponectin genetic variability, plasma adiponectin, and cardiovascular risk in patients with type 2 diabetes. *Diabetes* 55: 1512–1516.

Qi, L., Li, T., Rimm, E., Zhang, C., Rifai, N., Hunter, D., Doria, A. *et al.* (2005) The +276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. *Diabetes* 54: 1607–1610.

Rainwater, D.L., Almasy, L., Blangero, J., Cole, S.A., VandeBerg, J.L., MacCluer, J.W. *et al.* (1999) A genome search identifies major quantitative trait loci on human chromosomes 3 and 4 that influence cholesterol concentrations in small LDL particles. *Arterioscler Thromb Vasc Biol* 19: 777–783.

Schwarz, P.E., Towers, G.W., Fischer, S., Govindarajalu, S., Schulze, J., Bornstein, S.R. *et al.* (2006) Hypoadiponectinemia is associated with progression toward type 2 diabetes and genetic variation in the ADIPOQ gene promoter. *Diabetes Care* 29: 1645–1650.

Tanko, L.B., Siddiq, A., Lecoeur, C., Larsen, P.J., Christiansen, C., Walley, A. *et al.* (2005) ACDC/adiponectin and PPAR-gamma gene polymorphisms: implications for features of obesity. *Obes Res* 13: 2113–2121.

Vasseur, F., Helbecque, N., Dina, C., Lobbens, S., Delannoy, V., Gaget, S. *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. *Hum Mol Genet* 11: 2607–2614.

Vasseur, F., Helbecque, N., Lobbens, S., Vasseur-Delannoy, V., Dina, C., Clement, K. *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: 892–899.

Vionnet, N., Hani, E.H., Dupont, S., Gallina, S., Francke, S., Dotte, S. *et al.* (2000) Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am J Hum Genet* 67: 1470–1480.