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ORIGINAL ARTICLE

Adiponectin gene variants, adiponectin isoforms and cardiometabolic risk in type 2 diabetic patients

Lydia Foucan[1,2]*, Suliya Maimaitiming[3,4], Laurent Larifla[1,5], Segho Hedreville[5], Jacqueline Deloumeaux[1,2], Marie-Odile Joannes[1], Anne Blanchet-Deverly[1], Fritz-Line Velayoudom-Céphise[1], Roberte Aubert[3], Roger Salamon[6], Jean-Paul Donnet[7], Frederic Fumeron[3,4]

[1]Research Group Clinical Epidemiology and Medicine, ECM/L.A.M.I.A EA 4540, University Hospital of Guadeloupe, University of Antilles and Guyane, [2]Department of Medical Information and Public Health, [5]Cardiology Unit, [7]Diabetology Unit, University Hospital of Pointe-à-Pitre, Guadeloupe, [3]INSERM, U695, [4]University Paris Diderot-Paris 7, UFR de Médecine Site Bichat Paris, and [6]Inserm U897, Bordeaux School of Public Health, Victor Segalen Bordeaux 2 University, Bordeaux, France

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***Correspondence**
Lydia Foucan Tel.: +590-590-89-15-34
Fax: +590-590-89-15-95
E-mail address: lfoucan@yahoo.fr, lydia.foucan@chu-guadeloupe.fr

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ABSTRACT

Aims/Introduction: The aim of the present study was to examine the associations of rs2241766 (+45T>G), rs1501299 (+276G>T), rs17300539 (–11391G>A) and rs182052 (–10069G>A) in the adiponectin (Ad) gene with adiponectin concentrations, and concomitantly the association of these variants with cardiometabolic risk in type 2 diabetic patients of African ancestry.

Materials and Methods: A cross-sectional study of 200 patients was carried out. Concentrations of total, high (HMW), middle (MMW) and low (LMW) molecular weight adiponectin isoforms were measured. The four polymorphisms were genotyped.

Results: Decreased values were noted for total Ad in overweight, dyslipidemia and coronary artery disease (CAD), for HMW in overweight and dyslipidemia, for MMW in CAD, for LMW in dyslipidemia and CAD, for the percentage HMW/total in overweight, and for MMW:HMW ratio in patients without hypertriglyceridemic waist (HTGW). Significant associations were noted between total Ad, HMW, and HMW/total Ad and rs182052 under a dominant model ($P = 0.04$, $P = 0.03$ and $P = 0.04$, respectively), and between MMW and rs17300539 ($P = 0.006$). No significant difference in adiponectin concentrations was noted according to rs2241766 and rs1501299 genotypes. Patients carrying the rs2241766 G allele (TG+GG) had an increased risk of HTGW (odds ratio [OR] 3.1; $P = 0.04$) and of CAD (OR 3.3; $P = 0.01$). The odds of having low total adiponectin concentrations (<25th percentile: 3.49 ng/mL) for carrying the rs182052A allele (AA+GA) was: OR 0.40; $P = 0.009$. The single-nucleotide polymorphism associated with adiponectin levels was not concomitantly associated with cardiometabolic risk factors.

Conclusions: Adiponectin concentrations and *ADIPOQ* variants are implicated in the pathophysiological process leading to cardiovascular diseases, but the genetic effects seem to be independent of adiponectin concentrations in our Afro–Caribbean diabetic patients.

INTRODUCTION

Adiponectin (Ad), an adipose tissue-derived peptide, is a determinant of insulin sensitivity that exerts anti-inflammatory and anti-atherogenic effects[1]. Decreased plasma Ad levels are associated with type 2 diabetes mellitus[2] and arteriosclerosis[2–5]. Ad circulates in human blood in multiple isoforms: high (HMW), middle (MMW) and low (LMW) molecular weight forms[6]. There is no consensus about the clinical or biological relevance of these forms, although HMW is considered the

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main determinant of insulin sensitivity. In addition, in type 2 diabetic patients, agents such as thiazolidinediones[7] or pioglitazone[8] could preferentially modify levels of certain isoforms.

Inconsistent results have been reported on the association between adiponectin concentrations and coronary artery disease (CAD)[9–11], especially when the multimeric forms were evaluated[12–14]. Thus, in a recent report, total Ad and LMW levels were not associated with myocardial infarction in non-diabetic men, whereas the MMW:HMW ratio correlated with incident myocardial infarction[12].

The Ad-encoding gene, *ADIPOQ*, is located on chromosome 3q27 within a region linked to type 2 diabetes mellitus, metabolic syndrome and CAD[15]. Several single-nucleotide polymorphisms (SNPs) have been identified, and genetic associations have been reported between these SNPs and insulin resistance, type 2 diabetes, and adiponectin levels in cross-sectional or prospective studies and in different populations[16–19]. Association studies between *ADIPOQ* SNPs and CAD also provided inconsistent results[20–23], but a recent meta-analysis showed that the associations between rs2241766 (+45T>G) and rs1501299 (+276G>T) in the *ADIPOQ* gene and cardiovascular disease were significant but weak, and that studies are still required to confirm these associations[24]. In our previous study in Afro–Caribbean patients with type 2 diabetes, rs2241766 was associated with CAD under a dominant model[13], as in Caucasian patients[23]. Studies on rs17300539 (−11391G>A) and rs182052 (−10069G>A) are scarce, particularly in type 2 diabetes mellitus patients and in individuals of African descent[18,25,26].

We made the assumption that there is a concomitant relationship between the following three parameters: (i) *ADIPOQ* variants; (ii) adiponectin concentrations; and (iii) risk factors; and we aimed in the present study to examine the associations of rs2241766 (+45T>G), rs1501299 (+276G>T), rs17300539 (−11391G>A) and rs182052 (−10069G>A) in the *ADIPOQ* gene with Ad isoforms, and concomitantly the association of these variants with cardiometabolic risk factors in Caribbean type 2 diabetic patients of African ancestry.

METHODS

In a cross-sectional study carried out in the French West Indies University Hospitals, we studied 200 volunteers with type 2 diabetes from the Departments of Cardiology and Endocrinology. The ethnic origin was defined by whether the patient defined him/herself as Afro–Caribbean. The exclusion criteria included previous history of kidney or inflammatory disease, pregnant women, patients treated with thiazolidinediones or pioglitazone and those of another ethnic background. The protocol was approved by the ethical committee. All participants gave their written informed consent. Data were collected between 2007 and 2009.

The individuals were interviewed by physicians using a standard questionnaire that provided information on age, sex, history of cardiovascular diseases and use of antihypertensive, antidiabetic or lipid-lowering treatments.

Height and weight were measured with participants standing without shoes and lightly clothed. Body mass index was calculated as weight divided by height squared (kg/m^2). These measurements were made by trained nurses and physicians. Blood pressure was measured according to a standardized protocol with an automatic sphygmomanometer. The retained values were the average of two or more readings. Blood samples were obtained from participants after overnight fasting. Plasma cholesterol and triglyceride concentrations were measured by enzymatic method (Boehringer–Mennheim). All usual blood analyses were carried out with standardized programs.

Circulating total and multimers (HMW, MMM and LMW) Ad concentrations were measured using an enzyme-linked immunosorbent assay kit (ALPCO-Bühlmann, Mulhouse, France). Treatment with two different proteases enabled the selective determination of HMW and HMW plus MMW, in addition to total Ad concentrations. The concentrations of MMW and LMW isoforms were then calculated by subtraction. This method has been validated against western blot analysis[6], and was used previously[27,28]. The percentage of HMW adiponectin to total adiponectin (HMW:total Ad) and the MMW:HMW ratio were calculated.

Genotyping

Genomic deoxyribonucleic acid was extracted from peripheral white blood cells by standard methods and stored at −20°C until analysis. Genotyping of the study population was carried out using a TaqMan allelic discrimination assay on an ABI PRISM 7000 sequence detector according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The conditions for TaqMan reaction were as follows: 50°C for 2 min, 95°C for 10 min, and 54 cycles of 95°C for 15 s and 60°C for 1 min. This method achieved a successful genotyping rate >95%.

Four SNPs were genotyped rs2241766 (+45T>G), rs1501299 (+276G>T), rs17300539 (−11391G>A) and rs182052 (−10069 G>A) of the *ADIPOQ* gene.

These polymorphisms were chosen because of different arguments from the literature: rs2241766 (+45T>G) and rs1501299 (+276G>T) are the most cited regarding the cardiovascular risk, the rs17300539 (−11391G>A) was highly associated with adiponectin levels, and the rs182052 (−10069G>A) seemed specially associated with diseases in populations of African origin.

Clinical Factors

Obesity was defined as body mass index ≥30 kg/m^2. Dyslipidemia was defined as having one of the following: high-density lipoprotein cholesterol concentration <1.04 mmol/L in men or <1.29 mmol/L in women, triglyceride concentration ≥1.69 m mol/L, low-density lipoprotein cholesterol concentration ≥3.40 mmol/L, or under lipid-lowering treatment combined with a history of blood lipid abnormality. The "hypertriglyceridemic waist" phenotype (HTGW), was defined as a waist circumference ≥90 cm in men or ≥85 cm in women, along

with a plasma triglyceride concentration ≥2.0 mmol/L in men or ≥1.5 mmol/L in women[29,30].

Pre-existing CAD was defined on evidence of previous acute myocardial infarction, coronary bypass surgery, coronary angioplasty or documented myocardial ischemia. The latter was established on the basis of a positive stress test (exercise testing, myocardial scintigraphy or stress echography).

Statistical Methods

The results are expressed as mean ± standard deviation for the continuous variables, and as number (percentage) for the categorical variables. Variables were compared between groups using analysis of covariance. Variables with a skewed distribution (triglycerides and Ad concentrations) were $\log_{10}$ transformed to approach a normal distribution.

We used logistic regression analysis to study the associations between *ADIPOQ* variants and the following five conditions: overweight, dyslipidaemia, HTGW, total Ad concentration <25th percentile (3.49 ng/mL) and pre-existing CAD. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The data were adjusted for age and sex.

The IBM SPSS statistical software package version 19.0 (IBM, Armonk, NY, USA) was used for the data analysis. All *P*-values were two-sided, and $P < 0.05$ was considered significant.

RESULTS

The clinical and biological characteristics of the patients are shown in Table 1 for the overall study sample. The sample comprised 81 (40.5%) men. The mean age was 64 ± 12 years. A total of 56% of the patients had dyslipidemia, 82% were overweight, 12% had HTGW and 25% had total Ad levels <25th percentile (3.49 ng/mL). A total of 54 patients (27%) had pre-existing coronary events and among them, 41 (76%) had had a previous acute myocardial infarction.

Table 1 | Characteristics of the type 2 diabetic patients

	n	
Age (years)	200	64 ± 12
Sex (male)	200	40.5%
BMI (kg/m^2)	200	29.4 ± 5.5
Overweight	200	81.5%
Hypertriglyceridemic waist phenotype	198	12.0%
Dyslipidemia	200	55.5%
Total adiponectin (μg/mL)	200	7.1 ± 5.7
HMW Ad (μg/mL)	200	3.1 ± 4.2
MMW Ad (μg/mL)	200	1.3 ± 1.2
LMW Ad (μg/mL)	200	2.6 ± 1.5
HMW:total Ad	200	0.4 ± 0.2
MMW:HMW ratio	200	1.2 ± 3.0

Data are presented as mean ± standard deviation or percentage. Ad, adiponectin; BMI, body mass index; HMW, high molecular weight; LMW, low molecular weight; MMW, middle molecular weight.

The levels of total adiponectin and the multimeric forms according to overweight, dyslipidemia, HTGW and pre-existing CAD are presented in Table 2.

Mean total adiponectin levels were the lowest in patients with overweight, dyslipidemia and CAD. This decrease in total Ad was associated with decreased levels of HMW and of the percentage HMW / total in patients with overweight, and with decreased levels of HMW and LMW in patients with dyslipidemia. MMW and LMW were lower in patients with CAD than in the others, but the difference in HMW levels was not statistically significant. The MMW:HMW ratio was higher in patients with HTGW than in the others ($P = 0.04$).

The genotype distributions in the study population was in Hardy–Weinberg equilibrium for rs2241766 ($P = 0.90$), rs1501299 ($P = 0.90$) and rs182052 ($P = 0.90$), but not for rs17300539 ($P = 0.02$).

Regarding the relationship between SNPs and adiponectin concentrations, total Ad, HMW and HMW:total Ad were significantly higher in patients carrying the rare allele rs182052 A (AA+GA) than in those with the GG genotype (Table 3). MMW concentration was lower in carriers of the rare allele of rs17300539 A (AA+GA) than in those with the GG genotype (0.6 ± 0.5 vs 1.3 ± 1.2; $P = 0.006$; data not shown). No significant difference in adiponectin concentrations was noted according to rs2241766 and rs1501299 genotypes.

No significant difference in genotype frequencies of the four SNPs studied was noted in patients with and without dyslipidemia or overweight. The frequency of carriers of the minor allele rs2241766 G (TG+GG) was higher in patients with CAD than without (21% vs 8.5%; $P = 0.01$), and in those with HTGW than in the others (25% vs 10%; $P = 0.04$; Table 4). For the rs2241766, serum triglycerides concentrations were significantly higher in participants carrying the minor allele GG+TG than in those with the TT genotype (1.7 ± 1.9 vs 1.1 ± 0.7 mmol/L; $P = 0.01$; Table 4).

Table 5 shows the adjusted ORs for overweight, dyslipidemia, HTGW, total Ad concentration <3.49 ng/mL (25th percentile) and pre-existing CAD for *ADIPOQ* variants. The frequent homozygous genotype was considered as the reference group. The rs182052 GG genotype was associated with an increased risk of low total Ad concentration, whereas the ORs were OR 0.4, 95% CI 0.2–0.9; $P = 0.03$ for the GA genotype, and OR 0.3, 95% CI 0.1–0.9; $P = 0.03$ for the AA genotype. The OR of low total Ad concentration for carrying the minor allele (AA+GA) was OR 0.4, 95% CI 0.2–0.8; $P = 0.009$ (data not shown). The rare allele rs2241766 G (GG+TG) was associated with an increased risk of CAD (OR 3.3, 95% CI 1.3–8.5; $P = 0.01$) and of HTGW (OR 3.1, 95% CI 1.1–9.5; $P = 0.04$).

DISCUSSION

Previous studies have reported associations between genetic variants of adiponectin and diabetes in Africans or African–Americans[18,25,26]. In the present study of Caribbean patients of African ancestry with type 2 diabetes mellitus, the rs2241766 in

Table 2 | Distribution of total adiponectin and multimeric forms according to overweight, dyslipidemia, hypertriglyceridemic waist, and pre-existing coronary artery disease

	Overweight			Dyslipidemia			HTGW			CAD		
	No 33	Yes 167	*P*	No 89	Yes 111	*P*	No 174	Yes 24	*P*	No 146	Yes 54	*P*
Total Ad (μg/mL)	9.8 ± 8.8	6.7 ± 4.8	**0.05**	7.7 ± 5.5	6.8 ± 5.8	**0.04**	7.2 ± 5.5	6.7 ± 58	0.62	7.4 ± 3.6	6.5 ± 5.7	**0.05**
HMW Ad (μg/mL)	5.5 ± 7.5	2.8 ± 3.2	**0.03**	3.4 ± 3.7	3.1 ± 4.6	**0.03**	3.1 ± 3.7	3.2 ± 4.6	0.80	3.2 ± 4.3	3.1 ± 3.9	0.17
MMW Ad (μg/mL)	1.7 ± 2.0	1.2 ± 0.9	0.25	1.4 ± 1.0	1.2 ± 1.3	0.09	1.3 ± 1.0	1.1 ± 1.3	0.37	1.3 ± 1.0	1.1 ± 1.6	**0.04**
LMW Ad (μg/mL)	2.6 ± 1.7	2.7 ± 1.5	0.31	2.9 ± 1.5	2.5 ± 1.5	**0.02**	2.6 ± 1.5	2.4 ± 1.5	0.64	2.8 ± 1.6	2.2 ± 1.2	**0.006**
HMW:total Ad	0.4 ± 0.2	0.3 ± 0.2	**0.02**	0.4 ± 0.2	0.3 ± 0.2	0.13	0.3 ± 0.2	0.4 ± 0.2	0.45	0.3 ± 0.2	0.4 ± 0.2	0.82
MMW:HMW ratio	0.7 ± 0.8	1.2 ± 3.3	0.34	0.8 ± 1.2	1.4 ± 3.9	0.16	1.0 ± 1.2	2.4 ± 3.9	**0.04**	1.0 ± 0.9	1.7 ± 5.6	0.11

The data are presented as mean ± standard deviation. Data were analyzed by ANCOVA after adjustment for age and sex. Significant *P*-values are presented in bold. Ad, adiponectin; CAD, coronary artery disease; HMW, high molecular weight; LMW, low molecular weight; MMW, middle molecular weight.

Table 3 | Distribution of total adiponectin and multimeric forms according to rs182052 genotypes of the *ADIPOQ* gene

rs182052 G>A (*n* = 189)	Genotypes				Dominant model
	AA 32	GA 77	GG 80	*P*	AA/GA v GG *P*
Total Ad (μg/mL)	7.3 ± 6.8	7.9 ± 6.0	6.4 ± 4.9	0.10	**0.04**
HMW Ad (μg/mL)	3.7 ± 6.6	3.7 ± 4.0	2.6 ± 3.2	0.09	**0.03**
MMW Ad (μg/mL)	1.2 ± 0.9	1.4 ± 1.4	1.3 ± 1.0	0.81	0.62
LMW Ad (μg/mL)	2.5 ± 1.0	2.9 ± 1.7	2.5 ± 1.6	0.52	0.29
HMW:total Ad	0.4 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.10	**0.04**
MMW:HMW ratio	1.0 ± 0.9	1.3 ± 4.6	1.1 ± 1.5	0.86	0.87

The data are presented as mean ± standard deviation. Data were analyzed by analysis of covariance after adjustment for age and sex. Significant *P*-values are presented in bold. Ad, adiponectin; HMW, high molecular weight; LMW, low molecular weight; MMW, middle molecular weight.

Table 4 | Frequencies of carrying the rare allele of rs2241766 in patients with coronary artery disease or hypertriglyceridemic waist phenotype and mean triglyceride levels according genotypes

rs2241766 T>G	CAD			HTGW			Triglycerides	
	No 141, %	Yes 51, %	*P*	No 167, %	Yes 24, %	*P*	mmol/L	*P*
TT (*n* = 169)	71.5	78.4	**0.01**	89	75	**0.04**	1.1 ± 0.7	**0.01**
GG/TG (*n* = 23)	8.5	21.6		10	25		1.7 ± 1.8	

The data are presented as column percentage for coronary artery disease (CAD) and hypertriglyceridemic waist (HTGW) and as mean ± standard deviation for triglyceride levels. Significant *P*-values are presented in bold.

ADIPOQ was associated with HTGW phenotype and pre-existing CAD. Associations were also noted between rs182052 and adiponectin levels, but this variant was not concomitantly associated with overweight, dyslipidemia, HTGW phenotype or CAD. In addition, the HMW isoform, which is considered the main determinant of insulin sensitivity, was associated with overweight and dyslipidemia, but not with pre-existing CAD, suggesting that all the multimeric forms do not have the same functions for all diseases.

Decreased levels of total Ad, HMW and percentage of HMW adiponectin to total adiponectin (HMW:total Ad) were associated with overweight in the present study population. The complex HMW:total Ad, also called adiponectin sensitivity index, was reported to correlate more strongly with insulin sensitivity than just total adiponectin in patients with type 2 diabetes mellitus[31]. The adipose tissue is considered as an active organ secreting proteins that have marked effects on cardiovascular disease and type 2 diabetes mellitus[32]. Adiponectin, secreted by adipose tissue, appears to protect against all stages of atherosclerotic plaque formation and progression, and plaque rupture and thrombosis[33]. Except for HMW level, the odds of CAD decreased for all Ad forms in the present study. Regarding cardiovascular events, there are conflicting reports. In a cohort of CAD patients, total Ad and its isoforms did not cor-

Table 5 | Adjusted adds ratios of overweight, dyslipidemia, hypertriglyceridemic waist, low total adiponectin concentration and pre-existing coronary artery disease in patients with type 2 diabetes for *ADIPOQ* polymorphisms

ADIPOQ	*N*	Overweight		Dyslipidemia		HTGW		Low total Ad levels		CAD	
		OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
rs2241766											
TT	169	1		1		1		1		1	
GG/TG	23	0.3 (0.1–0.9)	0.06	1.6 (0.6–4.0)	0.30	3.1 (1.1–9.3)	**0.04**	0.7 (0.3–2.9)	0.62	3.3 (1.3–8.7)	**0.01**
rs1501299											
GG	72	1		1		1					
GT	93	1.4 (0.6–3.6)	0.46	1.0 (0.5–1.9)	0.92	0.4 (0.1–1.2)	0.10	0.6 (0.3–1.2)	0.16	0.7 (0.2–1.4)	0.32
TT	29	0.5 (0.2–1.4)	0.17	1.7 (0.7–4.1)	0.24	0.8 (0.2–2.8)	0.69	0.8 (0.3–2.1)	0.60	1.1 (0.4–2.9)	0.87
rs17300539											
GG	182	1		1		1		1		1	
GA/AA	8	1.0 (0.1–9.0)	0.97	1.3 (0.3–5.7)	0.70	1.2 (0.1–10.5)	0.28	1.7 (0.4–8.1)	0.49	1.6 (0.3–7.1)	0.57
rs182052											
GG	80	1		1		1		1		1	
GA	77	1.0 (0.4–2.3)	0.92	1.1 (0.6–2.1)	0.76	2.2 (0.8–5.8)	0.12	0.4 (0.2–0.9)	**0.03**	1.4 (1.7–3.0)	0.31
AA	32	2.1 (0.5–7.9)	0.26	0.9 (0.4–2.1)	0.54	0.7 (0.1–3.5)	0.65	0.3 (0.1–0.3)	**0.03**	0.5 (0.2–1.6)	0.27

Low total adiponectin (Ad): total Ad concentration <25th percentile (3.49 ng/mL). Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for age and sex. Significant *P*-values are presented in bold. CAD, coronary artery disease; HTGW, hypertriglyceridemic waist.

relate with severity of CAD[34]. Inoue *et al.*[14] suggested that serum HMW adiponectin levels might serve as a predictor of future cardiovascular events in patients with CAD, whereas Baessler *et al.*[12] observed, in a prospective study, that neither total Ad nor LMW were significantly different in patients with or without incident myocardial infarction. However, these authors found a decreasing association of the MMW:HMW ratio with incident myocardial infarction[12].

The relevance of the different Ad multimers is not fully known, and ethnic variations in adiponectin isoform distribution has been evocated[17,35]. The clinical importance of measuring, not only the total Ad levels, but the level of each Ad isoform separately[6] has been highlighted. However, little is known about production and secretion of the different Ad isoforms. According to some authors, MMW and LMW could proceed from sources other than adipose tissue in contrast to HMW that might be secreted primarily by the adipose tissue with differences in adiponectin secretion between subcutaneous and visceral adipose tissue[36–37]. This could partly explain the observed ethnic differences in the relationship between adiponectin isoforms and risk factors[17], as fat distribution also differs between ethnic groups. In the present diabetic patients who had a high prevalence of overweight (81.5%), our results show that HMW is not the isoform most broadly related to risk factors. In fact, HMW was not associated with CAD, whereas a strong link was found between LMW and CAD. We also noted a stronger relationship between LMW and dyslipidemia than between HMW and dyslipidemia. The ethnicity of our study population probably had an impact on these relationships. Indeed, in our Afro–Caribbean population, as in other populations of African descent, the abdominal subcutaneous fat is generally more common than the abdominal visceral fat, and could have an impact on the secretion of HMW.

In the present study, the rs2241766 was associated with pre-existing CAD and with HTGW, which is an optimal screening tool to identify subjects with metabolic syndrome and at high risk of cardiovascular disease[29]. Conversely, the rs1501299, rs17300539 and rs182052 were not significantly associated with CAD. However, some authors reported, in a large cohort of diabetic men, significant associations between *ADIPOQ* rs1501299, decreased cardiovascular risk and increased plasma Ad level[38]. In a recent study of type 2 diabetics in a Saudi population, there was no association between this *ADIPOQ* variant (rs1501299) and CAD risk, but a significant association with rs2241766[20]. A recent meta-analysis showed that the associations between variants in the *ADIPOQ* and cardiovascular disease were weak, and highlighted the need to confirm the associations[24].

Slightly higher plasma triglyceride and insulin levels were reported in the rs2241766 European G allele carriers[16]. Triglyceride levels were also the highest in our Afro–Caribbean patients carrying this rs2241766 G allele. Thus, it appears that in addition to the association with CAD, rs2241766 is also associated in our population to major metabolic syndrome features, such as triglyceride levels and HTGW.

Studies analyzing the relationships between rs182052 and cardiometabolic risk factors are scarce. The association found in the present study between rs182052 and adiponectin levels is particularly interesting because, in the literature, associations between this polymorphism and diseases have been described mainly in populations of African ancestry. In a study of African–Americans, rs182052 was associated with type 2 diabetes mellitus under a dominant model, and the presence of the minor allele (A/A or G/A) was associated with earlier onset of type 2 diabetes and diabetic nephropathy[25]. In the present study, the rs182052 minor allele was associated with a decrease of the odds of low total Ad

(OR 0.40), but not with overweight, dyslipidemia or pre-existing CAD. These results are supported by those of An *et al.*[39] who evaluated approximately 40 *ADIPOQ* SNPs in two Hispanic and African–American cohorts, and found no consistent evidence of an association between SNPs associated with plasma adiponectin levels, and an association with glucose homeostasis phenotypes in either cohort[39]. Furthermore, in the African–American cohort, there were seven *ADIPOQ* SNPs associated with adiponectin levels, but only one SNP, rs17300539, was associated concomitantly with fasting glucose and plasma Ad levels[39]. Several factors or mechanisms could interfere in the association between *ADIPOQ* SNPs, adiponectin levels and cardiometabolic diseases. In fact, a local production of adiponectin by cardiomyocytes was previously shown[40], and the involvement of both adenosine monophosphate-activated protein kinase and cyclooxygenase-2–dependent mechanisms in the adiponectin protection of the heart from ischemia-reperfusion injury was suggested[41].

The cross-sectional design, the small sample size and the small number of *ADIPOQ* variants studied were limitations of the present study. However, its strength resides in the availability of data on the concentrations of total Ad and isoforms (HMW, MMW and LMW), concomitantly with genotyping of four polymorphisms of *ADIPOQ* in type 2 diabetic patients of African descent. In fact, because of the high cost and the laborious nature of adiponectin assays, most studies have focused on total adiponectin and HMW rather than on all the multimeric forms distribution. In addition, the exclusion of patients receiving thiazolidinediones or pioglitazone in the present study, minimized the potential impact of drugs on the association between adiponectin and risk factors.

In summary, adiponectin concentrations and *ADIPOQ* polymorphisms are implicated in the pathophysiological process leading to cardiovascular diseases in our Afro–Caribbean patients, but the SNP associated with adiponectin levels was not concomitantly associated with cardiometabolic risk factors. These genetic effects seem to be independent of adiponectin concentrations.

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REFERENCES

1. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res* 2005; 96: 939–949.
2. Daimon M, Oizumi T, Saitoh T, *et al.* Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in the Japanese Population: the Funagata study. *Diabetes Care* 2003; 26: 2015–2020.
3. Kumada M, Kihara S, Sumitsuji S, *et al.* Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003; 23: 85–89.
4. Pischon T, Girman CJ, Hotamisligil GS, *et al.* Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004; 291: 1730–1737.
5. Guerre-Millo M. Adiponectin: an update. *Diabetes Metab* 2008; 34: 12–18.
6. Ebinuma H, Miyazaki O, Yago H, *et al.* A novel ELISA system for selective measurement of human adiponectin multimers by using proteases. *Clin Chim Acta* 2006; 372: 47–53.
7. Tonelli J, Li W, Kishore P, *et al.* Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes* 2004; 53: 1621–1629.
8. Bodles AM, Banga A, Rasouli N, *et al.* Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. *Am J Physiol Endocrinol Metab* 2006; 291: E1100–E1105.
9. Kanaya AM, Wassel Fyr C, Vittinghoff E, *et al.* Serum adiponectin and coronary heart disease risk in older Black and White Americans. *J Clin Endocrinol Metab* 2006; 91: 5044–5050.
10. Kizer JR, Barzilay JI, Kuller LH, *et al.* Adiponectin and risk of coronary heart disease in older men and women. *J Clin Endocrinol Metab* 2008; 93: 3357–3364.
11. Lawlor DA, Davey Smith G, Ebrahim S, *et al.* Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. *J Clin Endocrinol Metab* 2005; 90: 5677–5683.
12. Baessler A, Schlossbauer S, Stark K, *et al.* Adiponectin multimeric forms but not total adiponectin levels are associated with myocardial infarction in non-diabetic men. *J Atheroscler Thromb* 2011; 18: 616–627.
13. Foucan L, Ezourhi N, Maimaitiming S, *et al.* Adiponectin multimers and ADIPOQ T45G in coronary artery disease in Caribbean type 2 diabetic subjects of African descent. *Obesity (Silver Spring)* 2010; 18: 1466–1468.
14. Inoue T, Kotooka N, Morooka T, *et al.* High molecular weight adiponectin as a predictor of long-term clinical outcome in patients with coronary artery disease. *Am J Cardiol* 2007; 100: 569–574.
15. Vionnet N, El Hani H, Dupont S, *et al.* 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* 2000; 67: 1470–1480.
16. Fumeron F, Aubert R, Siddiq A, *et al.* 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* 2004; 53: 1150–1157.
17. Lara-Castro C, Doud EC, Tapia PC, *et al.* Adiponectin multimers and metabolic syndrome traits: relative adiponectin resistance in African Americans. *Obesity (Silver Spring)* 2008; 16: 2616–2623.
18. Ukkola O, Santaniemi M, Rankinen T, *et al.* Adiponectin polymorphisms, adiposity and insulin metabolism: HERITAGE family study and Oulu diabetic study. *Ann Med* 2005; 37: 141–150.
19. Vasseur F, Helbecque N, Dina C, *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: 2607–2614.
20. Al-Daghri NM, Al-Attas OS, Alokail MS, *et al.* Adiponectin gene variants and the risk of coronary artery disease in patients with type 2 diabetes. *Mol Biol Rep* 2011; 38: 3703–3708.
21. Bacci S, Menzaghi C, Ercolino T, *et al.* The +276 G/T single nucleotide polymorphism of the adiponectin gene is associated with coronary artery disease in type 2 diabetic patients. *Diabetes Care* 2004; 27: 2015–2020.
22. Chiodini BD, Specchia C, Gori F, *et al.* Adiponectin gene polymorphisms and their effect on the risk of myocardial infarction and type 2 diabetes: an association study in an Italian population. *Ther Adv Cardiovasc Dis* 2010; 4: 223–230.
23. Lacquemant C, Froguel P, Lobbens S, *et al.* The adiponectin gene SNP+45 is associated with coronary artery disease in type 2 (non-insulin-dependent) diabetes mellitus. *Diabet Med* 2004; 21: 776–781.
24. Zhang H, Mo X, Hao Y, *et al.* Association between polymorphisms in the adiponectin gene and cardiovascular disease: a meta-analysis. *BMC Med Genet* 2012; 13: 40.
25. Bostrom MA, Freedman BI, Langefeld CD, *et al.* Association of adiponectin gene polymorphisms with type 2 diabetes in an African American population enriched for nephropathy. *Diabetes* 2009; 58: 499–504.
26. Olckers A, Towers GW, van der Merwe A, *et al.* Protective effect against type 2 diabetes mellitus identified within the ACDC gene in a black South African diabetic cohort. *Metabolism* 2007; 56: 587–592.
27. Kaser S, Tatarczyk T, Stadlmayr A, *et al.* Effect of obesity and insulin sensitivity on adiponectin isoform distribution. *Eur J Clin Invest* 2008; 38: 827–834.
28. Koenen TB, van Tits LJ, Holewijn S, *et al.* Adiponectin multimer distribution in patients with familial combined hyperlipidemia. *Biochem Biophys Res Commun* 2008; 376: 164–168.
29. Blackburn P, Lemieux I, Lamarche B, *et al.* Hypertriglyceridemic waist: a simple clinical phenotype associated with coronary artery disease in women. *Metabolism* 2012; 61: 56–64.
30. Lemieux I, Pascot A, Couillard C, *et al.* Hypertriglyceridemic waist: a marker of the atherogenic metabolic triad (hyperinsulinemia; hyperapolipoprotein B; small, dense LDL) in men? *Circulation* 2000; 102: 179–184.
31. Pajvani UB, Hawkins M, Combs TP, *et al.* Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004; 279: 12152–12162.
32. Fujimoto WY, Boyko EJ, Hayashi T, *et al.* Risk factors for type 2 diabetes: lessons learned from Japanese Americans in Seattle. *J Diabetes Invest* 2012; B3: B212–B224.
33. Szmitko PE, Teoh H, Stewart DJ, *et al.* Adiponectin and cardiovascular disease: state of the art? *Am J Physiol Heart Circ Physiol* 2007; 292: H1655–H1663.
34. Rizza S. Adiponectin isoforms are not associated with the severity of coronary atherosclerosis but with undiagnosed diabetes in patients affected by stable CAD. *Nutr Metab Cardiovasc Dis* 2009; 19: 54–60.
35. Retnakaran R, Hanley AJ, Connelly PW, *et al.* Low serum levels of high-molecular weight adiponectin in Indo-Asian women during pregnancy: evidence of ethnic variation in adiponectin isoform distribution. *Diabetes Care* 2006; 29: 1377–1379.
36. Delaigle AM, Jonas JC, Bauche IB, *et al.* Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. *Endocrinology* 2004; 145: 5589–5597.
37. Ma H, Gomez V, Lu L, *et al.* Expression of adiponectin and its receptors in livers of morbidly obese patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2009; 24: 233–237.
38. Qi L, Li T, Rimm E, *et al.* The +276 polymorphism of the APM1 gene, plasma adiponectin concentration, and cardiovascular risk in diabetic men. *Diabetes* 2005; 54: 1607–1610.
39. An SS, Hanley AJ, Ziegler JT, *et al.* Association between ADIPOQ SNPs with plasma adiponectin and glucose homeostasis and adiposity phenotypes in the IRAS Family Study. *Mol Genet Metab* 2012; 107: 721–728.
40. Pineiro R, Iglesias MJ, Gallego R, *et al.* Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Lett* 2005; 579: 5163–5169.
41. Shibata R, Sato K, Pimentel DR, *et al.* Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med* 2005; 11: 1096–1103.