

ACDC/Adiponectin and PPAR- γ Gene Polymorphisms: Implications for Features of Obesity

László B. Tankó,* Afshan Siddiq,† Cécile Lecoer,† Philip J. Larsen,‡ Claus Christiansen,* Andrew Walley,† and Philippe Froguel†§

Abstract

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Objective: The main purpose of this study was to investigate associations of single-nucleotide polymorphisms (SNPs) in the adipocyte C1q and collagen domain-containing (ACDC) gene and its regulator, the nuclear peroxisome proliferator-activated receptor (PPAR)- γ gene, with body fat mass and its topographical distribution in postmenopausal women.

Research Methods and Procedures: Participants were 1501 healthy women, 60 to 85 years old, who were genotyped for four SNPs in the ACDC gene (–11391G/A, –11377C/G, +45T/G, +276G/T) and the Pro12Ala SNP in the PPAR- γ gene. Total body fat mass and the central to peripheral fat mass ratio (CFM/PFM ratio) were measured using DXA. Adiponectin and homeostasis model assessment of insulin resistance were measured in 287 subjects.

Results: The –11377C/G SNP was associated with adiponectin ($p < 0.001$) and the CFM/PFM ratio ($p = 0.005$); the G allele being associated with low adiponectin and high CFM/PFM ratio. Similar associations of adiponectin ($p = 0.0001$) and the CFM/PFM ratio ($p = 0.002$) characterized the 1_2 (G_G) promoter haplotype (11391G/A_–11377C/G). Genotype variation of SNP Pro12Ala was associated

with total body fat mass ($p = 0.04$); women with GG being the most obese ($p = 0.01$). The Ala/Ala (GG) genotype of Pro12Ala SNP interacted with the CC genotype of SNP-11377C/G in the determination of BMI ($p = 0.001$), when analyzed using a codominant model.

Discussion: Polymorphisms in the ACDC gene are associated with body fat distribution, whereas the Pro12Ala polymorphism in PPAR- γ is associated with overall adiposity, apparently in interaction with an ACDC promoter SNP.

Key words: adiponectin, body fat distribution, genetics, DXA, postmenopausal women

Introduction

Adiponectin is an adipocyte-derived hormone, which is abundantly present in the plasma (1) and exhibits insulin sensitizing, anti-inflammatory, and antiatherogenic effects (2–4). In humans, adiponectin is encoded by the adipocyte C1q and collagen domain containing (ACDC)¹ gene at chromosome 3q27 (5). Several genetic studies have shown an association of single nucleotide polymorphisms (SNPs) in the ACDC gene with obesity, insulin sensitivity, type 2 diabetes, and coronary heart disease (5–12). Four ACDC SNPs (–11391 G/A, –11377 C/G, +45 T/G, and +276 G/T) and their haplotypes have been repeatedly associated with low plasma adiponectin (6,8,10), but the physiological mechanisms by which adiponectin deficiency favors metabolic diseases and atherogenesis is not completely understood.

Plasma adiponectin is inversely associated with the degree of obesity (13) and is particularly low in subjects with type 2 diabetes (14). Emerging evidence indicates that body

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*Center for Clinical and Basic Research, Ballerup, Denmark; †Section of Genomic Medicine, Hammersmith Campus, Imperial College London, United Kingdom; ‡Rheoscience, Rødovre, Denmark; and §CNRS 8090-Institute of Biology, Pasteur Institute, Lille, France. Address correspondence to László B. Tankó, Ballerup Byvej 222, 2750 Ballerup, Denmark. E-mail: lbt@cbr.dk

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¹ Nonstandard abbreviations: ACDC, adipocyte C1q and collagen domain containing; SNP, single nucleotide polymorphism; CFM, central fat mass; PFM, peripheral fat mass; PPAR, peroxisome proliferator activated receptor; TBFM, total body fat mass; HOMA_{IR}, homeostasis model assessment of insulin resistance; LD, linkage disequilibrium; HTR, Haplotype Trend Regression; THESIAS, Testing Haplotype Effects in Association Studies.

fat distribution, i.e., relative presence of central to peripheral fat mass (CFM/PFM ratio), has more critical implications for women's cardiovascular morbidity/mortality than overall obesity per se (15–22). Indeed, numerous studies have shown reciprocal associations of central and peripheral fat compartments with adiponectin (23–26), insulin sensitivity (23,27,28), atherogenic lipid metabolites (29,30), and even with direct measures of atherosclerosis (23,27,31). However, it remains to be clarified whether body fat distribution is modulated by adiponectin secretion profile, or body fat distribution defines circulating adiponectin levels.

Further complexity is added by the fact that the *ACDC* gene is regulated by the nuclear peroxisome proliferator-activated receptor (*PPAR*)- γ , also suggested by its activation in response to *PPAR*- γ agonists (32). The antidiabetic effects of this group of drugs seem to involve up-regulation of adiponectin expression and a favorable impact on the topographical distribution of body fat mass (33–35). Moreover, the *PPAR*- γ Pro12Ala SNP allele was shown to be protective against type 2 diabetes, and modulation of fat accumulation was proposed to explain this effect (36,37). However, there is limited information on the specific interactions between these two genes in the determination of body fat accumulation and/or body fat distribution.

Therefore, the main aim of this study was to investigate independent associations of common SNPs in the *ACDC* and *PPAR*- γ genes with BMI and DXA measures of overall obesity (TBFM) and body fat distribution (CFM/PFM ratio) in a large community-based population of generally healthy postmenopausal Danish women, 60 to 85 years old. In addition, associations of the SNPs with circulating adiponectin levels and a surrogate marker of insulin resistance [homeostasis model assessment of insulin resistance ($HOMA_{IR}$)] were revisited in a subset of 287 subjects.

Research Methods and Procedures

Subjects

The study population was a community-based sample of 1501 generally healthy postmenopausal Danish women, 60 to 85 years old, who were invited to participate in a general health-check at the Center for Clinical and Basic Research. Those accepting the invitation underwent thorough examinations gathering information on skeletal and cardiovascular health in the community. Participants were 71.0 ± 5.2 years old, with a mean BMI and CFM/PFM ratio of 26.1 ± 3.8 kg/m² and 0.80 ± 0.22 , respectively. One-fifth of women reported current smoking, and 31.5% reported former smoking. One-fourth of women reported no weekly fitness activity. The prevalence of overt diabetes (defined by ongoing antidiabetic medication or fasting hyperglykemia according to World Health Organization criteria) and treated hyperlipidemia were only 2.3% and 2.8%, respectively.

To verify the previously described association of SNPs in the *ACDC* gene with circulating adiponectin in elderly women, circulating levels of this adipokine were measured in a subpopulation of 287 women. These women included lean, centrally, peripherally, and generally obese women providing a broad range of variation in TBFM (11.5 to 52.2 kg), CFM/PFM ratio (0.26 to 1.73), and adiponectin concentration (1.2 to 9.6 μ g/liter).

All women signed an approved informed consent form, and the study was carried out in accordance with the Helsinki Declaration II and Good Clinical Practice. The Local Ethical Committee approved the study protocol.

Phenotypes

Information was collected about age, height, weight, BMI, smoking habits, alcohol and coffee consumption, weekly fitness activity, prevalent diabetes, systolic and diastolic blood pressure, prevalent hypertension and hyperlipidemia, history of cardiovascular events, and related medications, as previously described in detail (27). TBFM was measured by DXA using a Hologic QDR4500 scanner (software version 9.03D; Hologic, Waltham, MA) and expressed in kilograms. Body fat mass was divided into two main compartments: 1) CFM, including the subcutaneous and visceral fat of the trunk, and 2) PFM, including subcutaneous fat mass of the four extremities. Body fat distribution was estimated by the calculated CFM/PFM ratio. BMI was calculated as weight (in kilograms) divided by squared height (in meters).

Adiponectin was measured using a highly sensitive radioimmunoassay (Linco Research, St. Charles, MI). Insulin resistance was estimated by $HOMA_{IR}$ [$HOMA_{IR} = \text{fasting insulin } (\mu\text{U/mL}) \times \text{fasting glucose (mM)} / 22.5$] (38).

Genotyping of SNPs

Blood samples were obtained from the subjects, and genomic DNA was isolated from peripheral blood leukocytes. SNP -11391, SNP +276, SNP +45, and SNP Pro12Ala of *PPAR*- γ were all genotyped using the fluorescent 5' nuclease Taqman method on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Alameda, CA). The conditions for Taqman reaction were as follows: 50 °C for 2 minutes, 95 °C for 10 minutes, and 95 °C for 15 seconds, 60 °C for 1 minute for 50 cycles. Taqman MGB probes were ordered from Applied Biosystems (Foster City, CA). Taqman assay could not be designed for the SNPs -11377, and, therefore, this SNP was genotyped using LightCycler (Roche Diagnostic, Basel, Switzerland) based on hybridization probes and fluorescence resonance energy transfer between fluorescein and LC Red 640 (Roche Diagnostics). Primers were designed using GenBank accession no. NM004797 for human *ACDC* mRNA. All primer sequences are available on request.

Statistical Analysis

Linkage disequilibrium (LD) was estimated using expectation-maximization algorithm as implemented in GOLD (39). Statistical analyses of SNPs (corrections and comparisons of means) were performed using the SPSS software (version 11.0; SPSS, Chicago, IL). Linear regression assessed the putative effects of the genetic variable on a given phenotype characteristic. We also tested the interaction between promoter SNPs of *ACDC* and Pro12Ala of *PPAR-γ* by this method. First, we compared the slope between two genotyped groups for each SNP of *ACDC* by predicting the BMI or adjusted CFM/PFM on the Pro12Ala genotype. Second, we looked at the prediction improvement of the regression model with and without the interaction parameter.

Differences between the genotypes in a codominant model were tested with one-way ANOVA. However, if some of the genotype groups had a sample size <30, the Kruskal-Wallis test was used. Differences between two groups of genotypes were tested using a parametric *t* test, unless sample size in one group fell below 30 individuals, in which case a Mann-Whitney test was used. Characteristics of the subgroup of 287 women were compared using one-way ANOVA combined with Scheffe test or by Kruskal-Wallis test, as appropriate.

The Haplotype Trend Regression (HTR) program (40) was used to test the association of haplotype frequencies (estimated by an expectation-maximization algorithm) with the corrected phenotype characteristics. The HTR program also enables running permutations and providing empirical *p* values after 1000 simulations. The permutations are performed by shuffling the phenotypic values between individuals. To obtain the empirical *p* value, the program computes the number of times the *p* value (overall or for each haplotype) is <5% and divides it by the number of permutations. These permutations allow correction for multiple SNPs testing.

The program THESIAS (Testing Haplotype Effects in Association Studies) was used to test the effect of a particular SNP in a haplotype on a quantitative phenotype among unrelated individuals. This program is based on the maximum likelihood model described by Tregouet et al. (41) and is linked to the stochastic EM (SEM) algorithm. To test for the influence of a given SNP in a haplotype background, we did all possible pairwise comparisons of haplotypes different only for a given SNP (e.g., 1_2_1_1 vs. 1_1_1_1). The log-likelihood obtained is compared with the one given by the test without SNP testing taking the square of their difference, which follows a χ^2 distribution by approximation. If the difference is significant, the SNP is considered to have a major effect in the association between the haplotype and the phenotype.

Results

SNP and Haplotype Analyses in the Entire Population

All SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). LD was calculated between the four SNPs of the *ACDC* gene. Very strong LD was observed between the promoter SNP -11391G/A and SNP -11377C/G ($D' = 0.99$) and between SNP +45T/G and SNP +276G/T ($D' = 1.00$). There was a fairly low LD between the two groups of SNPs: D' between SNP -11377 and +45 was 0.41.

The promoter SNP -11377C/G showed a significant association with increased CFM/PFM ratio ($p = 0.005$; Table 1). The Pro12Ala SNP in the *PPAR-γ* gene indicated a significant association with BMI ($p = 0.01$). Using a recessive model, women with the GG genotype had significantly higher BMI compared with those with CC + CG (28.2 ± 4.5 vs. 26.1 ± 3.8 kg/m², respectively; $p = 0.003$). Data were also analyzed after exclusion of subjects with prevalent diabetes ($n = 35$), which had no apparent influence on the results shown in Table 1.

In light of the results of the LD analysis, haplotype analysis was performed in two groups: 1) SNP -11391G/A combined with SNP -11377C/G (promoter haplotype) and 2) SNP +45T/G combined with SNP +276G/T. Regarding the promoter SNPs, the 1_1 (G_C) haplotype (frequency = 66%) was significantly associated with a decreased CFM/PFM ratio ($p < 0.001$), whereas the 1_2 (G_G) haplotype (frequency = 26%) was significantly associated with an increased CFM/PFM ratio ($p = 0.002$; Table 2). There were no significant associations of any of the +45T/G_+276G/T haplotypes with the CFM/PFM ratio (data not shown).

Haplotype analysis for the CFM/PFM ratio was also carried out using THESIAS and HTR software; both indicated statistically significant associations for the 1_2_1_1 (G_G_T_G) haplotype with respective *p* values of 0.005 and 0.004.

Potential interaction between *ACDC* promoter SNPs (-11377 and -11391) and the Pro12Ala SNP was also addressed in the entire population using linear regression analysis. The analysis did not reveal any apparent interaction between these SNPs in the determination of the CFM/PFM ratio (data not shown). However, a significant interaction was found between the Ala/Ala (GG) homozygous genotype of Pro12Ala SNP and the CC homozygous genotype of the -11377C/G *ACDC* SNP for the determination of BMI ($p = 0.001$), when analyzed using a codominant model (Table 3). It is to be added that only 18 individuals from the entire population possessed this specific genotype combination. The mean BMI for these individuals was 29.5 ± 4.7 kg/m² compared with 26.1 ± 3.8 kg/m², representing the mean of the entire population. Because of the small number of individuals with a GG genotype for Pro12Ala ($n = 31$) and AA genotype ($n = 4$) of the -11391G/A SNP, it was difficult to assess interactions between these two SNPs. The analysis testing a potential

Table 1. Association of the selected SNPs with overall obesity and body fat distribution in 1501 Danish white women

SNP		N	BMI	p	TBFM	p	CFM/PFM	p
-11391	GG	1205	26.1 ± 3.8	0.34	25.6 ± 7.1	0.79	0.80 ± 0.22	0.20
	GA	213	25.8 ± 3.6		25.2 ± 6.8		0.79 ± 0.20	
	AA	4	26.2 ± 3.3		25.9 ± 3.7		0.94 ± 0.03	
-11377	CC	782	25.9 ± 3.8	0.12	25.5 ± 7.1	0.36	0.78 ± 0.20	0.005
	CG	555	26.4 ± 3.8		25.7 ± 7.2		0.84 ± 0.23	
	GG	97	25.6 ± 3.7		24.7 ± 6.3		0.81 ± 0.21	
+45	TT	1116	26.1 ± 3.8	0.95	25.5 ± 7.1	0.39	0.81 ± 0.22	0.55
	TG	253	26.1 ± 3.7		25.5 ± 7.1		0.81 ± 0.21	
	GG	11	25.3 ± 3.9		27.1 ± 8.2		0.76 ± 0.25	
+276	GG	680	26.0 ± 3.8	0.72	25.3 ± 6.9	0.83	0.80 ± 0.22	0.63
	GT	578	26.2 ± 3.8		25.7 ± 7.3		0.81 ± 0.22	
	TT	119	25.9 ± 3.7		25.5 ± 6.7		0.79 ± 0.21	
Pro12Ala	CC	1088	26.1 ± 3.8	0.01	25.5 ± 7.0	0.07	0.81 ± 0.22	0.56
	CG	355	25.9 ± 3.9		25.4 ± 7.1		0.78 ± 0.21	
	GG	31	28.3 ± 3.3		29.5 ± 7.4		0.84 ± 0.19	

Data shown are means ± SD. *p* value was calculated using Kruskal-Wallis test for all SNPs apart from -11377 and +276 for which one-way ANOVA was performed. There was no correlation between the above phenotypes and age in this population. However CFM/PFM was corrected for BMI, whereas TBFM was corrected for BMI and smoking. Note that *N* does not always add up to 1501 because of some genotyping failures (success rate for genotyping was >92%). SNP, single nucleotide polymorphisms; TBFM, total body fat mass; CFM/PFM, central fat mass to peripheral fat mass ratio.

interaction between SNP +45T/G and the Pro12Ala variants for the determination of overall adiposity (TBFM) did not reveal statistically significant finding (data not shown). However, our analysis was again limited by the lack of subjects with the combination of the homogenous genotypes SNP +45GG and Pro12Ala Ala/Ala (GG).

Table 2. Independent associations of estimated promoter (SNP -11391G/A_-11377C/G) haplotypes in the *ACDC* gene with body fat distribution in 1397 postmenopausal Danish women 60 to 85 years old

Estimated haplotype	Estimated frequency	CFM/PFM ratio (mean)	<i>p</i>
1_1	0.662	-0.04	<0.001
1_2	0.261	0.10	0.002
2_1	0.07	0.08	0.22
2_2	0.0001	0.10	0.383

SNP, single nucleotide polymorphism; CFM/PFM, CFM/PFM, central fat mass to peripheral fat mass ratio; 1, wild type allele; 2, variant allele.

SNP and Haplotype Analysis in a Subpopulation (n = 287)

After observing the association of CFM/PFM with *ACDC* SNPs in the entire cohort, it was also important to study the associations of the SNPs with circulating adiponectin levels and the HOMA_{IR} index. After adjustment for the influence of age and BMI, adiponectin was inversely correlated with CFM/PFM ratio ($r = -0.36$, $p < 0.001$). There was also an inverse correlation between adiponectin levels and HOMA_{IR} ($r = -0.22$, $p < 0.001$) and a direct correlation between HOMA_{IR} and CFM/PFM ratio ($r = 0.25$, $p < 0.001$).

Similar to the finding in the entire study population, the promoter SNP -11377C/G showed a significant association with the CFM/PFM ratio ($p = 0.03$; Table 4). Unsurprisingly the association was less significant compared with the entire cohort, but it showed that there was some power to detect association even in this subgroup. SNP -11377C/G was also significantly associated with decreased adiponectin levels ($p < 0.001$), whereas SNP-11391G/A had a significant association with increased adiponectin levels ($p < 0.01$; Table 4). In a dominant model, women with CG or GG genotypes at SNP -11377 had significantly higher HOMA_{IR} compared with women with CC genotype (3.95 ±

Table 3. Interaction between SNP –11377C>G in the *ACDC* gene and the Pro12Ala variant of the *PPAR-γ* gene

	CC	CG	GG
CC (Pro/Pro)	26.02 ± 3.76 (563)	26.32 ± 3.77 (405)	25.59 ± 3.82 (68)
CG (Pro/Ala)	25.85 ± 3.99 (185)	26.38 ± 4.01 (130)	25.46 ± 3.07 (26)
GG (Ala/Ala)	29.52 ± 4.67 (18)	26.63 ± 4.11 (11)	26.10 ± 0.50 (2)
SNP	β	p	
–11377	0.003	0.67	
Pro12Ala	–0.003	0.76	
Interaction	0.13	0.001	

Results shown are means ± SD; number of subjects in parentheses. Means of BMI stratified according to the genotype combinations of SNP-11377 C>G and Pro12Ala variants. Slopes and the significance of their departure from 0 in the linear regression (the dependent variable was lnBMI). There is a significant interaction between the SNP –11377 in the promoter of *ACDC* and Pro12Ala variant to increase BMI as indicated by the positive slope of the interaction parameter (β). SNP, single nucleotide polymorphism.

3.48 $\mu\text{U/mL} \times \text{mmol} \times 10^{-2}$, $n = 149$ vs. $3.12 \pm 1.90 \mu\text{U/mL} \times \text{mmol} \times 10^{-2}$, $n = 138$; $p = 0.03$). In this subpopulation, the variant T allele at SNP +276 was favorably associated with the CFM/PFM ratio; women with the TT genotype (0.71 ± 0.27 , $n = 22$) has significantly lower CFM/PFM ratios compared with those with GG and GT genotypes (0.82 ± 0.26 , $n = 260$; $p = 0.02$). None of the SNPs in the *ACDC* gene were associated with BMI in this subgroup (data not shown).

Haplotype analysis was also performed in the subgroup. When focusing on SNP –11391_–11377 haplotypes, the 2_1 (A_C) haplotype was associated with high adiponectin ($p = 0.006$ with 7000 simulations; frequency 7.1%), whereas the 1_2 (G_G) haplotype was associated with low adiponectin ($p = 0.0001$ with 7000 simulations; frequency, 27.7%; Table 5). Moreover, the latter haplotype was also associated with high CFM/PFM ratios ($p = 0.03$), whereas the 2_1 (A_C) haplotype had no apparent association with this measure of body fat distribution (Table 4). Haplotype analysis was also performed for SNP +45T/G and SNP +276G/T, and haplotype 1_2 (T_T; frequency = 28%) was significantly associated with high levels of adiponectin ($p = 0.021$; data not shown).

Discussion

To our knowledge, this is the first study that shows in a large genetically homogenous population of European postmenopausal women that SNPs in the *ACDC* and *PPAR-γ* genes are associated with direct measures of overall obesity and body fat distribution. In elderly women, the CFM/PFM ratio has more important implications for metabolic and cardiovascular risk than overall obesity per se (15–23,27,28). Thus, the independent association of a com-

mon *ACDC* SNP with increased CFM/PFM ratio through hypoadiponectinemia suggest a potential primary contribution of circulating adiponectin to body fat distribution in this population.

Associations with the CFM/PFM Ratio

Allele variations at SNP –11377 showed a significant association with the variation in the CFM/PFM ratio. Women with the variant GC or GG genotypes had increased CFM/PFM ratios and decreased serum adiponectin levels. Although the haplotype analysis including both promoter SNPs (–11391_–11377) did not add to this finding, the significant association of the 1_2 (G_G) haplotype with increased CFM/PFM, and decreased adiponectin levels further corroborated the apparent central role of the variant G allele at –11377. Collectively, these observations draw attention to the possibility that the variant G allele at the –11377 locus could be part of a genetic predisposition to the development of upper body obesity in women.

When addressed in the subpopulation, women with the variant TT genotype at the +276 locus had significantly lower CFM/PFM ratios compared with those with the GT or GG genotypes. These associations were independent of the previously described associations of the same locus with BMI: subjects with TT having lower BMI compared with those with GG genotype (9). However, the favorable association of the TT genotype could not be confirmed by results from the entire population. Furthermore, according to haplotype analysis including SNPs –11391, –11377, and +276, only the 1_2_1 (G_G_G) ($p = 0.002$) haplotype was associated with the CFM/PFM ratio. This finding does not exceed the significance of the association of the promoter haplotype 1_2 (G_G) alone ($p = 0.002$). Therefore, it

Table 4. Independent associations of SNP genotypes with selected phenotype characteristics in 287 postmenopausal Danish women 60 to 85 years old

SNP	N	CFM/PFM ratio	p	Adiponectin ($\mu\text{g/L}$)	p	HOMA _{IR} ($\mu\text{U/mL} \cdot \text{mmol} \cdot \text{l}^{-2}$)	p
-11391							
GG	241	0.82 \pm 0.26	0.68	4.07 \pm 1.52	<0.01	3.56 \pm 2.74	0.662
GA	42	0.79 \pm 0.24		4.73 \pm 1.71		3.16 \pm 1.76	
-11377							
CC	138	0.77 \pm 0.24	0.03	4.55 \pm 1.68	<0.001	3.12 \pm 1.90	0.115
CG	137	0.85 \pm 0.27		3.89 \pm 1.38		3.99 \pm 3.60	
GG	12	0.85 \pm 0.28		3.41 \pm 1.05		3.56 \pm 1.74	
+45							
TT	237	0.80 \pm 0.26	0.18	4.23 \pm 1.58	0.34	3.60 \pm 3.05	0.983
TG	40	0.86 \pm 0.24		4.12 \pm 1.46		3.19 \pm 1.64	
GG	4	0.87 \pm 0.21		3.03 \pm 0.43		3.15 \pm 2.10	
+276							
GG	143	0.83 \pm 0.28	0.06	4.10 \pm 1.58	0.18	3.71 \pm 3.02	0.122
GT	117	0.81 \pm 0.23		4.28 \pm 1.47		3.51 \pm 2.87	
TT	22	0.71 \pm 0.27		4.85 \pm 1.79		2.90 \pm 1.81	
Pro12Ala							
CC	211	0.82 \pm 0.26	0.33	4.17 \pm 1.59	0.84	3.82 \pm 4.2	0.83
CG	65	0.84 \pm 0.24		4.12 \pm 1.44		3.31 \pm 1.9	
GG	5	0.79 \pm 0.07		4.06 \pm 1.71		4.29 \pm 2.5	

Adiponectin was correlated with both age and BMI, the CFM/PFM ratio was correlated with BMI, and HOMA_{IR} was correlated with BMI and current smoking. Phenotypes were corrected accordingly. *p* values were calculated using Kruskal-Wallis test for all the SNPs, apart from SNP -11391, for which one-way ANOVA was used. Phenotype characteristics are defined by the original uncorrected mean \pm SD. SNP, single nucleotide polymorphism; CFM/PFM, central fat mass to peripheral fat mass ratio; HOMA_{IR}, homeostasis model assessment of insulin resistance.

Table 5. Independent associations of the estimated promoter SNP haplotypes (-11391_-11377) in the *ACDC* gene with selected phenotype characteristics in the subpopulation of 287 Danish white women

Estimated haplotype	Estimated frequency	Adiponectin (mean)	p	HOMA _{IR} (mean)	p	CFM/PFM (mean)	p
1_1	0.648	0.045	0.052	-0.047	0.11	-0.039	0.09
1_2	0.277	-0.243	0.0001	0.110	0.06	0.138	0.03
2_1	0.071	0.374	0.006	-0.075	0.63	-0.049	0.67
2_2	0.003	0.085	0.69	0.169	0.44	0.006	0.99

The mean values are obtained using each phenotype corrected for covariates; adiponectin was corrected for age and BMI, CFM/PFM ratio was corrected for age, and HOMA_{IR} was corrected for BMI and smoking habit. The mean allows determination of the direction of the association for each specific phenotype. SNP, single nucleotide polymorphism; HOMA_{IR}, homeostasis model assessment of insulin resistance; CFM/PFM, central fat mass to peripheral fat mass ratio; 1, wild-type allele; 2, variant allele.

seems unlikely that SNP +276 has critical implications for the association of the *ACDC* gene with body fat distribution.

Associations with Overall Obesity

Overall obesity was associated with the Pro12Ala variants. *PPAR-γ* is an established regulator of adipocyte differentiation nurturing the notion that SNPs in this gene may have direct implications for body fat accumulation. In support, our findings—in line with similar observations in French and Finnish populations (42,43)—indicated a linear association between the dose of the variant G allele and the degree of overall obesity as estimated not only by BMI but also by direct DXA measures of TBFM. Similar associations with direct measures of overall adiposity were not detected for SNPs in the *ACDC* gene.

A recent study by Stumvoll et al. (9) reported significantly higher BMIs in non-diabetic subjects with GG + GT genotypes at the 45T/G SNP locus of the *ACDC* gene compared with those with TT genotype (25.5 vs. 24.1 kg/m²). This finding could not be confirmed by the presence analysis, possibly because this study involved elderly women only, whereas the aforementioned study included men and women in their 30s. Despite this negative finding, we found interesting gene–gene interactions between the –11377C/G promoter SNP of the *ACDC* gene and the Pro12Ala SNP of the *PPAR-γ* gene. The CC genotype of SNP –11377 interacted with the homozygous Ala12Ala genotype in the associations with BMI. Although several limitations hampered the proper assessment of interactions of the Pro12Ala SNP with the other SNPs, no evidence for further interactions was apparent in our data set. Two recently published studies had similar conclusions regarding potential interactions of the Pro12Ala SNP with either the SNP +45T/G only or with haplotypes including +45T/G and +276G/T in the modulation of anthropometric measures of obesity, including BMI (44,45). While the potential involvement of the –11391 G/A or –11391G/A_–11377C/G haplotypes awaits further analyses in even larger samples, our findings draw attention to an interaction between the *PPAR-γ* gene with the promoter region of the *ACDC* gene, with apparent implications for the modulation of BMI.

The fact that the CC genotype of –11377C/G SNP was associated with high levels of adiponectin as well suggests that the –11377 CC + Ala12Ala genotype combination might have implications for the genetic modulation of insulin sensitivity. Because of the small number of individuals with this combination ($n = 18$) in the entire cohort and the limited availability of the HOMA_{IR} phenotype, our analysis did not have sufficient statistical power to detect supporting evidence for this notion. However, the Ala12Ala genotype was shown to have likely implications for the development of obesity (46), circulating adiponectin (47), and modula-

tion of insulin sensitivity (48–50). These may have consequences for cardiovascular risk suggested by a protective association of the Ala12Ala genotype with atherosclerosis (51). Cooperative interactions between the *ACDC* and *PPAR-γ* genes in the modulation of insulin sensitivity were shown in a recent family-based association study that revealed significant interactions between SNP +45T/G of the *ACDC* gene and the Ala12 allele (52) in a Taiwanese population. A study undertaken in 555 Italian subjects could not find evidence for a significant interaction between the Pro12Ala SNP and the +45T/G_+276G/T haplotype of the *ACDC* gene in the modulation of insulin sensitivity as estimated by the HOMA_{IR} index, although limitations of the relatively small sample size for interactions could contribute to the negative findings (47). Further analysis of interactions between the *ACDC* and *PPAR-γ* genes—including the effects of the –11377 CC + Ala12Ala genotype combination—in the modulation of insulin sensitivity seems warranted, when considerably larger samples or a pooled data set generated by existing and future studies will be available.

In conclusion, these findings seem to nurture the notion that SNPs in the *ACDC* gene have important implications for the variation in body fat distribution and circulating adiponectin in postmenopausal women. The common Pro12Ala SNP of *PPAR-γ* seems associated with body fat accumulation (overall obesity), apparently in interaction with an *ACDC* promoter SNP. Prospective studies are awaited to clarify the implications of these genes and their interactions for overall metabolic and cardiovascular risk in postmenopausal women.

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