Original Article

Interactions Between Noncontiguous Haplotypes in the Adiponectin Gene ACDC Are Associated With Plasma Adiponectin

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Adiponectin, an adipocyte protein important in insulin sensitization and cardioprotection, has a strong genetic component. We hypothesized that variants in the adiponectin gene (adipocyte collagen–domain containing [ACDC]) contribute to adiponectin levels in a biracial adolescent cohort. We genotyped 11 ACDC single nucleotide polymorphisms (SNPs) in 631 non-Hispanic white and 553 African-American unrelated adolescents in grades 5–12 randomly selected from the Princeton School District Study. ACDC SNPs −11,391 (A allele), −10,068 (G allele), and +276 (T allele) were associated with higher adiponectin, adjusting for sex, puberty stage, BMI *Z* score, and waist *Z* score. Contiguous two-SNP haplotypes of promoter variants −11,391/−10,068 were significantly associated with adiponectin levels in whites and African Americans ($P < 0.0001$ and 0.03, respectively). Extended haplotypes from the promoter through the second intron (−11,391 to +349) strongly associated with adiponectin in whites ($P = 6 \times 10^{-11}$) and African Americans ($P = 0.004$), but haplotypes of first intron SNPs −4,521 to −657 did not ($P > 0.2$). Noncontiguous haplotypes or interactions between two-SNP (−11,391/−10,068) and three-SNP (+45, +276, and +349) haplotypes predicted adiponectin better than either region alone. Variants of ACDC are associated with adiponectin levels in whites and African Americans. Interactions between noncontiguous ACDC haplotypes strongly influence adiponectin levels, suggesting nonadditive and potentially *cis* relationships between these regions.
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Adiponectin is an abundant adipocyte-derived protein that has important roles in insulin sensitization, cardioprotection, and anti-inflammatory processes. Low adiponectin levels have been associated with the development of several cardiovascular end points, including myocardial infarction and hypertension (1,2), and may also have direct cardioprotective effects on vascular tissue (3–5). In addition, low adiponectin levels are associated with poor plasma lipid profiles in adults and children (6–8) and precede insulin resistance and type 2 diabetes (9–11).

Adiponectin levels have a strong genetic component, with heritability estimated between 30 and 50% (12,13). The adiponectin protein is coded by the gene named adipocyte collagen–domain containing (ACDC). Linkage studies of adiponectin level have reported linkage peaks at the ACDC locus at chromosome 3q27 (14,15), which are reduced by the inclusion of ACDC SNPs in the linkage model (15). SNPs and haplotypes in ACDC are associated with adiponectin level (16,17), and some ACDC SNPs affect adiponectin expression or secretion (18,19). However, conflicting association results in various populations suggest a complex relationship between ACDC variation and phenotypic adiponectin levels.

Adiponectin levels are typically lower in African Americans than whites (20,21). Few studies have explored the genetic basis for adiponectin levels in African Americans. One genome scan in African Americans found high heritability for adiponectin levels ($h^2 = 0.82$) but no evidence of linkage near the ACDC locus (22). To date, only one study has reported on ACDC SNPs in African Americans in association with body fat and plasma lipids (23). Our objective, therefore, was to use a large, school-based cohort of adolescents to explore the associations of ACDC SNPs with adiponectin levels in non-Hispanic whites and African Americans.

RESEARCH DESIGN AND METHODS

Twelve-hundred thirty-six unrelated non-Hispanic white and African-American adolescents participating in the Princeton School District Study (Cincinnati, OH) were randomly selected for inclusion in the current study. Of 1,196 students with complete phenotypic data and adiponectin levels, 1,184 (631 white and 553 African American) had genotypic data for the majority of SNPs typed and were included in the analysis.

Details about the cohort assembly, data collection, and laboratory measurements are presented elsewhere (24). Briefly, students in grades 5–12 were invited to participate in the Princeton School District Study if they had no chronic disease, were not taking medications that affect carbohydrate metabolism, and were not pregnant. After a minimum 10-h overnight fast, venipuncture was performed; students completed medical history questionnaires; and height, weight, and waist circumference were measured in duplicate. Axillary hair was documented in boys as none, minimal, or adult distribution (25,26). Race/ethnicity was determined using self-report, with those reporting mixed or Hispanic race/ethnicity removed from the analysis.

This study was approved by the institutional review boards of Cincinnati Children's Hospital Medical Center and the University of Cincinnati. All

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ACDC, adipocyte collagen–domain containing; BIC, Bayesian information criterion; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism.

TABLE 1
Adiponectin SNP genotype and allele frequencies in non-Hispanic whites and African Americans

				Whites				African Americans			
				Genotype distribution*				Genotype distribution*			
Location†	dbSNP no.	Gene region	Alleles (1/2)*	11	12	22	Allele 2 frequency	11	12	22	Allele 2 frequency
−12,823	rs860291	Promoter	C/T	0.78	0.21	0.01	0.11‡	0.93	0.07	0	0.04
−11,391	rs17300539	Promoter	G/A	0.84	0.15	0.01	0.08‡	0.97	0.03	0	0.01
−10,068	rs182052	Intron 1	G/A	0.48	0.40	0.12	0.32	0.42	0.44	0.14	0.36
−4,521	rs822393	Intron 1	C/T	0.59	0.35	0.06	0.24‡	0.46	0.43	0.11	0.32
−4,041	rs822395	Intron 1	A/C	0.38	0.49	0.13	0.38‡	0.36	0.39	0.24	0.44§
−657	rs2036373	Intron 1	T/G	0.87	0.13	0.002	0.07	0.88	0.11	0.004	0.06
+45	rs2241766	Exon 1	T/G	0.85	0.13	0.02	0.08‡	0.91	0.09	<0.01	0.05
+276	rs1501299	Intron 2	G/T	0.54	0.39	0.07	0.27‡	0.42	0.47	0.11	0.35
+349	rs2241767	Intron 2	A/G	0.79	0.19	0.02	0.11‡	0.91	0.08	0.002	0.04
+712	rs3774261	Intron 2	G/A	0.37	0.50	0.13	0.38‡	0.19	0.50	0.31	0.56
+2,019	N/A	3′ UTR	A/Del	0.37	0.50	0.13	0.38‡	0.21	0.48	0.31	0.55

UTR, untranslated region. *1 and 2 denote alternate alleles; 11 homozygous for allele 1 and 12 heterozygous and 22 homozygous for allele 2. †Location relative to the transcription start site. ‡Significantly different allele frequency in whites compared with African Americans ($P < 0.001$). §P value for HWE <0.001; dropped from further analysis.

participants >18 years old and parents/guardian for those <18 years provided written informed consent, and individuals <18 years provided written assent.

Laboratory measurements. Plasma adiponectin levels were measured in duplicate using radioimmunoassay (Linco, St. Louis, MO), with a sensitivity of 0.5 μg/ml and intra- and interassay coefficients of variation of 5 and 15%, respectively.

Puberty staging. Pubertal status determination was described previously (24). Briefly, sex hormone cut points for testosterone and estradiol were established to distinguish prepuberty (Tanner I) from puberty (Tanner II–IV) using data from two large Cincinnati-based cohorts with full Tanner staging. Postpuberty was defined in girls with menarche duration ≥2 years and in boys with an adult distribution of axillary hair.

Calculated variables. BMI Z scores for age and sex were determined based on Centers for Disease Control and Prevention growth charts (27). Because nationally representative waist Z scores are not available, waist Z scores using age- and sex-specific means and standard deviations were derived from our cohort (n = 1,196).

SNP selection and genotyping. Eleven SNPs were selected to span the adiponectin gene ACDC, including several SNPs reported to be associated with adiponectin levels or metabolic phenotypes in other populations (16,17,28–30). We particularly focused on SNPs with a minor allele frequency >10%, although this was not achieved in all cases.

Blood samples were stored on wet ice immediately after collection, and buffy coats were stored at −80°C until processing. DNA was extracted using Gentra Puregene kits, and most SNPs were genotyped using TaqMan. Plates were read using an ABI 7900 machine with automatic allele calling, supplemented as necessary with manual allele calling, especially for SNPs of low minor allele frequency (<0.05). SNP +45 was genotyped using a PCR-based restriction fragment–length polymorphism protocol described by Zeitz et al. (31). Details about sample processing and genotyping protocols are included in Supplemental Table 1, which is detailed in the online appendix (available at http://diabetes.diabetesjournals.org).

Statistical analyses. Analyses were conducted using SAS version 9.1 (Cary, NC) and Haplo.Stats version 1.2.0 (32). Continuous variables were analyzed for normality. Natural log transformations did not improve statistical deviations from normality in our large sample, so all variables were analyzed in original units.

Genetic analyses. All genetic analyses were conducted in whites and African Americans separately. Hardy-Weinberg equilibrium (HWE) was tested for each SNP, and linkage disequilibrium among SNPs was inferred using the Haploview (33) and EMLD programs (34). Haplotypes were estimated using the Haplo.Stats program. Contiguous haplotypes of arbitrary size were examined for global association with adiponectin level using Haplo.Stats, with the haplotype combination with the smallest global P value chosen.

Two different sections of the ACDC gene, the promoter and coding regions, have previously been reported as associated with adiponectin levels. Therefore, the relative contributions of these two physically distant regions of the ACDC gene to adiponectin levels were explored. Extended haplotypes of multiple SNPs spanning the gene were subdivided based on the linkage disequilibrium structure of the gene and the physical location of the SNPs within the gene. Thus, haplotypes from three different regions of ACDC (e.g., promoter, first intron, and coding region/second intron) were estimated. These regional haplotypes were examined for association with adiponectin level individually and jointly. In addition, an interaction between the promoter and coding region/second intron haplotypes was specifically considered by creating a "noncontiguous " haplotype excluding SNPs in the first intron.

Linear regression, including genetic data. SNPs were analyzed separately in whites and African Americans using SAS. All results are reported adjusting for sex, puberty stage, BMI Z score, and waist Z score, although very similar results were seen in unadjusted analyses (data not shown). All SNPs were analyzed under an additive genetic model, using a single variable coding homozygous wild types as +1, heterozygotes as 0, and homozygous variants as −1, which, when analyzed as a continuous variable, tests the linear trend in adiponectin by genotype. Genotype-specific Bonferroni-adjusted least squares means and 95% CIs were generated using general linear modeling, modeling genotype categorically. Because 11 SNPs were analyzed, a Bonferroni multiple correction was also applied across SNPs, which is conservative because it does not account for correlated SNP data. Thus, a P value of 0.0045 for each SNP-level association (Table 2) was considered significant after this correction.

Individual-level haplotype combinations were weighted with their Bayesian posterior probability from Haplo.Stats (determined separately by race) and included in linear regression models in SAS. To compare non-nested models of different haplotype combinations, only individuals with a posterior probability of 0.50 or more for all haplotypes of interest and full data for the component SNPs were included (n = 559 whites and 498 African Americans). Interactions between gene region-specific haplotypes were modeled by multiplying the posterior probabilities of two haplotypes. Models were arranged by adjusted R^2, and non-nested models were evaluated using the Bayesian information criterion (BIC). The model with the lowest BIC value was selected. BIC differences >10 indicate "very strong" evidence in favor of the model with the smaller BIC (35). Multicollinearity among variables within the same model was evaluated using variance inflation factors, with variance inflation factor ≤4.0 acceptably independent. Data are presented as least squares mean (95% CI) or β ± SE, with P values ≤0.05 considered significant.

RESULTS

The 11 ACDC SNPs satisfied HWE assumptions, except SNP −4,041 in African Americans ($P < 0.0001$), which was eliminated from further analysis in that group. SNP +45 was somewhat out of HWE in whites ($P = 0.03$) but was retained in the analysis because of its previous associations with metabolic outcomes. In addition, this finding may be due to chance, because after Bonferroni correction, this deviation from HWE was no longer significant. Allele frequencies differed significantly by race (Table 1), except for SNPs −10,068 and −657. Linkage disequilibrium was strong in the coding region/second intron in whites and African Americans (Supplemental Table 2, which is detailed in the online appendix). Whites also

TABLE 2
Genotypic association with adiponectin in non-Hispanic whites and African Americans

SNP	Genotype	Whites			African Americans		
		n	Adiponectin*	*P* value†	*n*	Adiponectin*	*P* value†
−12,823	CC	415	10.4 ± 0.2	0.4	436	8.7 ± 0.2	0.7
	CT	109	10.6 ± 0.4		35	9.1 ± 0.6	
	TT	6	12.0 ± 1.6		0	—	
−11,391	GG	516	10.2 ± 0.2	0.002	527	8.8 ± 0.2	0.11
	GA	94	11.9 ± 0.4		15	10.6 ± 0.9	
	AA	6	13.2 ± 1.6		0	—	
−10,068	GG	300	10.7 ± 0.2	0.03	229	9.2 ± 0.3	0.15
	GA	254	10.6 ± 0.3		242	8.6 ± 0.3	
	AA	72	9.1 ± 0.5		74	8.5 ± 0.4	
−4,521	CC	371	10.6 ± 0.2	0.19	253	9.1 ± 0.3	0.14
	CT	223	10.2 ± 0.3		237	8.7 ± 0.3	
	TT	38	9.6 ± 0.6		57	8.5 ± 0.5	
−4,041	AA	236	10.2 ± 0.3	0.8	N/A		
	AC	307	10.4 ± 0.2				
	CC	84	11.0 ± 0.4				
−657	TT	546	10.3 ± 0.2	0.12	486	8.9 ± 0.2	0.3
	TG	79	10.9 ± 0.4		60	8.5 ± 0.5	
	GG	1	7.9 ± 3.9		2	7.4 ± 2.6	
+45	TT	505	10.4 ± 0.2	0.15	486	8.9 ± 0.2	0.3
	TG	80	9.8 ± 0.4		47	8.1 ± 0.5	
	GG	9	10.0 ± 1.3		1	7.2 ± 3.6	
+276	GG	335	10.1 ± 0.2	0.05	229	8.6 ± 0.3	0.03
	GT	239	10.8 ± 0.3		253	9.0 ± 0.3	
	TT	47	10.6 ± 0.6		62	9.1 ± 0.5	
+349	AA	497	10.4 ± 0.2	0.5	500	8.9 ± 0.2	0.3
	AG	122	10.3 ± 0.4		48	8.5 ± 0.5	
	GG	9	9.9 ± 1.3		1	7.2 ± 3.6	
+712	GG	233	10.0 ± 0.3	0.17	103	8.7 ± 0.4	0.6
	GA	310	10.8 ± 0.2		273	9.0 ± 0.2	
	AA	82	10.4 ± 0.4		171	8.8 ± 0.3	
+2,019	AA	234	10.0 ± 0.3	0.17	113	8.4 ± 0.4	0.5
	A Del	310	10.8 ± 0.2		264	9.0 ± 0.2	
	Del Del	84	10.4 ± 0.4		168	8.7 ± 0.3	

Data are least squares means ± SE from general linear models, modeling genotype as a categorical variable. *Adjusted for sex, puberty stage, BMI *Z* score, and waist *Z* score. †*P* values for additive trend in adiponectin by genotype from regression analysis.

showed strong linkage disequilibrium in the 5′ promoter region, which was attenuated in African Americans.

Adiponectin SNPs are associated with adiponectin level. Among whites, the SNP −11,391 A allele (P = 0.002), −10,068 G allele (P = 0.03), and +276 T allele (P = 0.05) were associated with higher adiponectin levels in adjusted analyses (Table 2). In African Americans, the SNP −11,391 A allele (P = 0.11) and +276 T allele (P = 0.03) were also marginally or significantly associated with higher adiponectin level. After conservatively correcting for multiple comparisons, only the association between SNP −11,391 A allele in whites remained significant.

Haplotypes of promoter and coding region/second intron SNPs are associated with adiponectin level. Haplotypes of SNPs −11,391 and −10,068 (two-SNP haplotypes) were associated with adiponectin level in whites and African Americans (global $P = 2 \times 10^{-5}$ and 0.03, respectively). In adjusted regression analyses, the A/G haplotype was associated with higher adiponectin levels compared with the other two-SNP haplotypes in whites (β ± SE = 1.7 ± 0.4 μg/ml, $P < 0.0001$) and African Americans (β ± SE = 1.7 ± 1.0 μg/ml, P = 0.09).

Haplotypes of the coding region/second intron SNPs +45, +276, and +349 (three-SNP haplotypes) were associated with adiponectin level in whites but not in African Americans (global P = 0.001 and 0.4, respectively). In whites, the three-SNP haplotype combinations T/T/A (β ± SE = 0.66 ± 0.3 μg/ml, P = 0.04) and T/G/G (β ± SE = 1.4 ± 0.6 μg/ml, P = 0.02) were associated with higher adiponectin in adjusted analyses.

Contiguous haplotypes encompassing SNPs from the promoter through the second intron, however, were best associated with adiponectin levels in whites (global $P = 6 \times 10^{-11}$ for SNPs −11,391 to +349) and African Ameri-

TABLE 3
Model comparisons including no genetics, SNPs, or haplotypes in non-Hispanic whites and African Americans

Model structure	Whites (n = 559)*			African Americans (n = 498)*		
	Genetic variables	Adjusted R^2	BIC	Genetic variables	Adjusted R^2	BIC
Base model, no genetics†	—	0.178	1,500	—	0.124	1,297
Models of individual haplotypes and SNPs						
Best +45/+276/+349 (3-SNP) haplotypes only	T/T/A T/G/G	0.187	1,496‡	T/T/A	0.129	1,295‡
Best −11,391/−10,068 (2-SNP) haplotypes only	A/G	0.199	1,487‡	A/G G/A	0.131	1,295
Best additive SNPs only	SNP −11,391 SNP −10,068	0.205	1,484‡	SNP −10,068 SNP +276	0.134	1,293‡
Models of haplotype combinations and interactions						
Best 2-SNP and 3-SNP haplotypes	A/G T/G/G G/G/G	0.207	1,484	G/A T/T/A	0.134	1,293
Best 2-SNP * 3-SNP haplotype interactions	A/G * T/G/G A/G * T/T/A	0.214	1,477‡	A/G * T/G/G A/G * T/T/A G/A * T/G/A G/G * G/G/G	0.142	1,290‡
Best model including 5-SNP haplotypes	A/G G/G/G A/G/T/G/G	0.217	1,477	G/G/G A/G/T/T/A G/A/T/G/A G/G/G/G/G	0.154	1,284‡

Higher adjusted R^2 and lower BIC values indicate better model fit to the data. *n with complete data for all analyses, for comparable non-nested models by race. †Base model includes sex, puberty stage, BMI Z score, and waist Z score. ‡Model superior to previous model (BIC difference ≥2).

cans (global P = 0.004 for SNPs −11,391 to +45). For consistency, the extended haplotype −11,391 to +349 was analyzed in whites and African Americans.

Interactions between promoter and coding region/second intron haplotypes significantly impact adiponectin levels. To explore the critical regions of the extended haplotype, we divided this haplotype into three sections based on linkage disequilibrium structure and physical location of SNPs in the gene: *1*) promoter SNPs −11,391/−10,068 (two-SNP haplotypes), *2*) first intron SNPs −4,521 to −657, and *3*) coding region/second intron SNPs +45/+276/+349 (three-SNP haplotypes). The first intron haplotypes were not significantly associated with adiponectin in either whites or African Americans (global $P > 0.2$). We then explored the associations of the two-SNP and three-SNP haplotypes with adiponectin levels.

To test whether each haplotype was individually associated with adiponectin and whether haplotypes improved the model over simple SNP models, we compared the BIC values from various models. Table 3 shows that inclusion of three-SNP haplotypes improved the fit over the base (nongenetic) model in whites and African Americans. The two-SNP haplotype models were superior to the three-SNP haplotype models in whites but not African Americans. In whites and African Americans, models including the best additive SNPs significantly further improve the model fit over either the two-SNP or three-SNP haplotype models. Thus, the two-SNP and three-SNP haplotypes are somewhat important in explaining adiponectin level, but they are not superior to additive individual SNP models.

TABLE 4
Linear regression models of adiponectin level in non-Hispanic whites and African Americans

	Whites		African Americans	
	β	P value	β	P value
Intercept	10.5 ± 0.5	<0.0001	9.8 ± 0.6	<0.0001
Sex*	2.3 ± 0.3	<0.0001	1.5 ± 0.3	<0.0001
Puberty*	−1.4 ± 0.5	0.005	−0.9 ± 0.6	0.10
Post-puberty*	−1.4 ± 0.5	0.007	−0.8 ± 0.5	0.15
BMI Z score	−1.1 ± 0.3	<0.0001	−0.8 ± 0.3	0.02
Waist Z score	−0.2 ± 0.3	0.5	−0.5 ± 0.3	0.10
Haplotypes†				
A/G	1.4 ± 0.4	0.002		
G/G/G	−0.9 ± 0.5	0.06	3.4 ± 1.5‡	0.02
A/G/T/G/G	5.5 ± 1.7	0.001		
G/G/G/G/G			−5.8 ± 1.9‡	0.002
A/G/T/T/A			2.0 ± 1.2	0.10
G/A/T/G/A			−1.2 ± 0.4	0.002
Model-adjusted R^2	0.217		0.154	
Model BIC	1,477		1,284	

Data are β estimates ± SE (μg/ml). *Reference categories are boys and prepuberty. †Two-SNP haplotypes include −11,391/−10,068; three-SNP haplotypes include +45/+276/+349; five-SNP haplotypes include −11,391/ −10,068/+45/+276/+349. Each haplotype is modeled compared with all other haplotypes of the same type. ‡In African Americans, haplotypes G/G/G and G/G/G/G/G are moderately collinear (variance inflation factor ≈ 5.0).

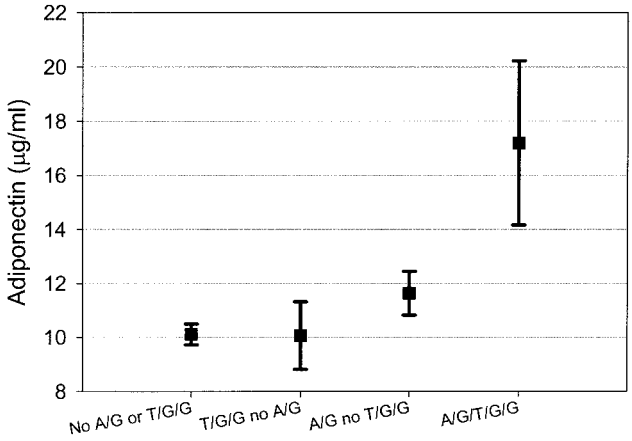

FIG. 1. Adiponectin by ACDC haplotype. Values are least squares means (error bars = 95% CI), adjusted for sex, puberty stage, BMI *Z* score, and waist *Z* score. Number of individuals per group were 480 (no A/G or T/G/G), 36 (T/G/G no A/G), 90 (A/G no T/G/G), and 6 (A/G/T/G/G). Individuals with A/G and T/G/G haplotypes but not the five-SNP A/G/T/G/G haplotype ($n = 2$) were not included in the analysis. The adjusted adiponectin level (least squares means ± SE) for those two individuals was 14.5 ± 7.3 μg/ml.

We then explored the combined effect of the two groups of haplotypes, either modeled independently as haplotypic interactions or as a noncontiguous five-SNP haplotype. Compared with the best additive SNP models, we found no improvement in model fit when including the two-SNP and three-SNP haplotypes as independent predictors. However, substantial improvement in the model fit was seen when interactions were modeled between the two-SNP and three-SNP haplotypes. Model fit further improved by modeling five-SNP noncontiguous haplotypes in African Americans but not in whites. Thus, individuals with specific haplotypes in both the promoter and coding region/second intron of ACDC experience a greater impact on adiponectin levels than individuals with either haplotype alone. Table 4 presents the final best models, which include the five-SNP haplotypes in whites and African Americans.

To further explore the nonadditive relationship, we focused on the rare A/G/T/G/G haplotype in whites. Compared with individuals lacking the two-SNP A/G and the three-SNP T/G/G haplotypes ($n = 480$), the T/G/G haplotype alone ($n = 36$) did not alter adiponectin level ($P = 0.9$), but the A/G haplotype alone ($n = 90$) was associated with higher adiponectin ($P = 0.0006$). Moreover, individuals with the A/G/T/G/G haplotype ($n = 6$) had significantly higher levels than those with either the A/G ($P = 0.004$) or the T/G/G ($P = 0.0008$) haplotypes alone or with neither haplotype ($P < 0.0001$; Fig. 1).

DISCUSSION

Adiponectin levels have a strong genetic basis, and linkage studies have identified that the adiponectin locus ACDC on chromosome 3q27 contributes to phenotypic adiponectin levels. We therefore explored the association of specific SNPs and haplotypes within ACDC with phenotypic adiponectin levels in a biracial adolescent cohort.

We report that both promoter and coding region/second intron SNPs are associated with adiponectin level in non-Hispanic whites and African Americans. Consistent with the current study, several (16,17,28,36–38), but not all (30,39), studies have found the T allele of SNP +276 associated with higher adiponectin. Higher adiponectin has previously been associated with the SNP −11,391 A allele in French whites (17,40,41). Our results confirm this finding in whites and also replicate it among African Americans. We also report a novel association of the SNP −10,068 G allele with higher adiponectin levels in our white subset.

In addition, we report a novel within-gene interaction involving haplotypes in the promoter and coding region/second intron of ACDC impacting the level of plasma adiponectin. Previous attempts to explore the relationships among ACDC SNPs in their association with adiponectin levels have yielded conflicting information. Although the T/G haplotype of SNPs +45/+276 is associated with lower plasma adiponectin level (16), conditioning on the wild-type G allele at SNP −11,391 eliminated an association between SNPs +45 or +276 and adiponectin (17). We also failed to find any association between the +45/+276/+349 haplotype T/G/G and adiponectin level in those lacking the promoter −11,391/−10,068 A/G haplotype. However, whereas Vasseur et al. (17) explored the relationship among ACDC regions by subsetting on promoter-region genotype, we formally tested interactions between promoter and coding region/second intron haplotypes of ACDC. Because the strongest interactions were between the variant SNP −11,391 A allele and the +45/+276/+349 haplotype, we suspect that conditioning on the promoter-region wild-type −11,391 G allele would not reveal the interaction we report. Our analyses suggest that haplotypic associations with adiponectin may be dependent on the interaction between the promoter and coding region/second intron haplotypes, rather than the additive effects of each gene region alone.

To our knowledge, none of the SNPs included in the associated haplotypes is functional, although reports suggest that two of these SNPs may affect ACDC transcription. The SNP +45 has been shown to have differential transcription from its two alleles (19). However, the previous study reported higher transcription from the +45 G allele, whereas we found higher plasma adiponectin levels among those carrying the T allele. In addition, although the SNP −11,391 does not disrupt a transcription factor binding site, it does lie near a putative region of gene regulation (17). Further study will be necessary to determine the functional variants in the ACDC gene and the conditions under which variants in this gene may interact to alter adiponectin levels.

One way to explain nonlinear interactions between gene regions is functional SNPs acting in *cis* to affect ACDC gene expression. Although there are no other reports of interactions or *cis* effects within the ACDC gene, there is ample evidence that other genes, including those involved in the metabolic pathways of adiponectin, have *cis*-regulating elements and within-gene interactions that influence phenotypic gene expression (42,43). We could not directly test the relative importance of interaction (e.g., having both haplotype combinations) versus *cis* regulation (e.g., having both haplotype combinations on the same chromosome), either from a functional or an epidemiologic standpoint. However, the possibility for such *cis* relationships between the promoter and coding region/second intron of ACDC is consistent with our findings and should be explored further.

This study has several limitations. First, the frequencies for some of the SNPs and haplotypes most associated with adiponectin level were exceedingly small or nonexistent in African Americans, limiting our power. Furthermore, ge-

notypes were not available for 16 and 6% of individuals for SNPs −12,823 and +45, respectively. No race, sex, or adiposity differences were noted between typed and nontyped individuals (data not shown); however, missing data may reduce the power to detect differences by genotype. Second, although our data strongly supports a nonlinear interaction between the promoter and coding region/second intron haplotypes, we cannot prove that our results are dependent on the *cis* organization of the variants, and further functional studies will be necessary to explore this issue. Third, we reported that the SNP +45 is out of HWE in whites, and this may impact our findings. However, this finding may be due to chance, because after Bonferroni correction for the 11 SNPs tested, it is no longer significant. Finally, the relative contribution of these haplotypes is somewhat low, accounting for ~2–4% of the variance in adiponectin levels. Thus, although these haplotypes are significant independent predictors of adiponectin level, they should not be viewed as the primary determinants of it.

In summary, the current study provides novel data on the role of adiponectin gene ACDC SNPs in whites and African Americans, such that specific variants and haplotypes may interact to influence adiponectin levels. This analysis suggests that there is not a single SNP in ACDC responsible for modulating adiponectin levels. Rather, there may be multiple SNPs working in conjunction with each other and with the environment to influence adiponectin. More importantly, this interactive relationship is present in two racially distinct groups, suggesting a novel and complex genetic underpinning to adiponectin levels.

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