Brief Genetics Report

Common Polymorphisms in the Adiponectin Gene *ACDC* Are Not Associated With Diabetes in Pima Indians

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Adiponectin is an abundant adipose tissue–derived protein with important metabolic effects. Plasma adiponectin levels are decreased in obese individuals, and low adiponectin levels predict insulin resistance and type 2 diabetes. Two variants in the adiponectin gene *ACDC* have been previously associated with plasma adiponectin levels, obesity, insulin resistance, and type 2 diabetes. To determine the role of genetic variation in *ACDC* in susceptibility to obesity and type 2 diabetes in Pima Indians, we screened the promoter, exons, and exon-intron boundaries of the gene to identify allelic variants. We identified 17 informative polymorphisms that comprised four common (minor allele frequency >15%) linkage disequilibrium clusters consisting of 1–4 variants each. We genotyped one representative polymorphism from each cluster in 1,338 individuals and assessed genotypic association with type 2 diabetes, BMI, serum lipid levels, serum adiponectin levels, and measures of insulin sensitivity and secretion. None of the *ACDC* variants were associated with type 2 diabetes, BMI, or measures of insulin sensitivity or secretion. One variant, single nucleotide polymorphism (SNP)-12823, was associated with serum adiponectin levels ($P = 0.002$), but this association explained only 2% of the variance of serum adiponectin levels. Our findings suggest that these common *ACDC* polymorphisms do not play a major role in susceptibility to obesity or type 2 diabetes in this population. *Diabetes* 54:284–289, 2005

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CIR, corrected insulin response; ISI, insulin sensitivity index; SNP, single nucleotide polymorphism.

Adiponectin is an adipose tissue–derived protein with important metabolic effects (1). Plasma adiponectin levels are decreased in individuals with obesity (2), insulin resistance (3), and type 2 diabetes (2), and low plasma adiponectin levels predict the development of insulin resistance (4) and type 2 diabetes (5) in Pima Indians.

Recent genome-wide scans in humans have mapped a susceptibility locus for type 2 diabetes and the metabolic syndrome to chromosome 3q27, where the gene encoding adiponectin, *ACDC*, is located (6,7). Two single nucleotide polymorphisms (SNPs) in *ACDC* were found to be associated with type 2 diabetes in Japanese individuals (8). One of the SNPs (T→G in codon 276) was also associated with lower plasma adiponectin levels, although this relationship was limited to obese subjects (8). Another SNP (T→G at nucleotide 94: SNP 45) was found to be associated with obesity, insulin resistance, and dyslipidemia in a German population (9). A haplotype defined by these two SNPs has also been associated with components of the insulin resistance syndrome in Caucasians (10).

The *ACDC* gene spans 16 kb, contains three exons, and yields a 4.5-kb mRNA transcript. We screened ~8 kb of *ACDC* corresponding to 2.8 kb of promoter sequence, each exon, and flanking intronic sequence and identified 17 SNPs. These included five promoter, seven exonic, and five intronic SNPs. At the time of the present study, five SNPs (SNP-12823, SNP-12128, SNP 3187, SNP 3267, and SNP 3286) were confirmed in a public database (available at http://www.ncbi.nlm.nih.gov/SNP) and correspond to dbSNP entries rs860291, rs266730, rs1063537, rs2082940, and rs1063538, respectively.

The genotypic distribution among 100 individuals partitioned into high– and low–plasma adiponectin level extremes (following adjustment for percent body fat) allowed us to make certain assumptions regarding the amount of disequilibrium between SNPs. When genotypes at SNPs were in >90% concordance among the 100 individuals, we grouped them into clusters and only genotyped one representative SNP from each cluster. On this basis, the 17 SNPs identified in Pimas were divided into 8 clusters, each containing 1–4 SNPs (Fig. 1). Clusters 3, 7, and 8 consisted of SNPs with a minor allele frequency <15% and, as the goal of this study was to evaluate

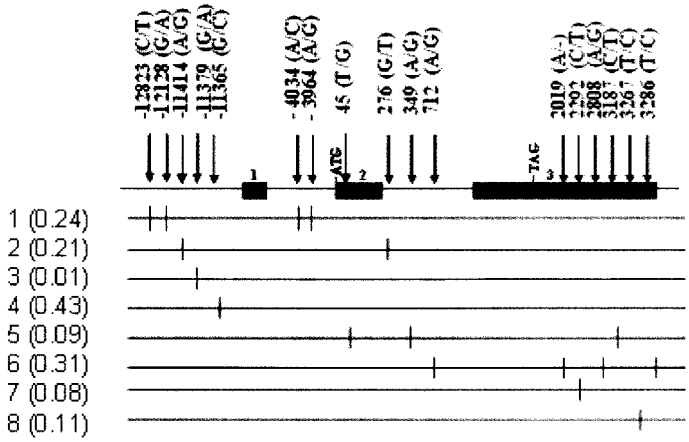

FIG. 1. Location of *ACDC* SNPs and corresponding linkage disequilibrium clusters. The three exons encoding the complete *ACDC* transcript are represented by ■ and introns by —. Variants are indicated by (↓), and the SNP identifier and base change are located above each arrow. Clusters of linkage disequilibrium are shown beneath the gene structure. Clusters 3, 7, and 8 consist of single SNPs. Minor allele frequency, calculated from 100 individuals, is given to the right of each cluster's numerical designation. The start codon (ATG) and stop codon (TAG) are found in exons 2 and 3, respectively.

relatively common variants, these SNPs were not evaluated further. Cluster 5 also had a minor allele that was relatively rare; however, we typed SNP 45 from this cluster because previous studies showed association with diabetes or related traits.

We genotyped SNPs representative of each nonrare linkage disequilibrium cluster corresponding to SNP-12823, SNP-11365, SNP 45, SNP 276, and SNP 3286 in 1,338 participants from a genome-wide linkage study (11). We first evaluated linkage disequilibrium, as quantified by D′, between pairs of these SNPs, as shown in Table 1. Each of the SNPs was in statistically significant linkage disequilibrium with each of the others, but the extent of association varied somewhat, with D′ ranging from 0.48 to 0.99. Furthermore, because the associated alleles often had different frequencies, the information associated with each SNP varied considerably.

We assessed the association of each SNP with type 2 diabetes and BMI. In a subgroup of 331 nondiabetic individuals who had undergone detailed physiologic studies (12), we also assessed the association with insulin sensitivity, measured by the hyperinsulinemic-euglycemic clamp and insulin secretion, as assessed by the acute insulin response to an intravenous glucose infusion. In the larger group of nondiabetic individuals, the insulin sensitivity index (ISI) and corrected insulin response (CIR) were analyzed. These are surrogate indexes of insulin sensitivity and insulin secretion, respectively, that are correlated with the more sophisticated measures (13) and that can be derived from an oral glucose tolerance test. ISI was calculated from the fasting serum insulin (I_0) and fasting plasma glucose (G_0) concentrations (ISI = 10^4/[I_0G_0]), while CIR was calculated from the 2-h postload insulin (I_2) and glucose (G_2) concentrations (CIR = I_2/G_2[G_2B70 mg/dl]) as described (14,15). The association of these SNPs with serum adiponectin levels, as well as levels of triglycerides and HDL choleseterol, was also assessed among nondiabetic individuals with normal renal function (16).

As shown in Table 2, none of the SNPs were associated with type 2 diabetes, BMI, ISI, CIR, or directly measured insulin sensitivity or secretion. There was a modest association of the G allele at SNP-11365 with lower HDL cholesterol levels. We also found that the G allele of SNP-12823 was significantly associated with lower adiponectin levels. Further adjustment for BMI did not substantially change these results. While statistically significant, the effect of this polymorphism accounted for only ~2% of the variance of serum adiponectin levels.

We identified six common *ACDC* haplotypes, none of which were significantly associated with type 2 diabetes, BMI, ISI, CIR, lipid levels, or directly measured insulin sensitivity or secretion (Table 3). We did observe an association between the A_G_T_C_C haplotype and serum adiponectin levels, but because this haplotype is the only one containing the A allele of SNP-12823, it essentially reflects the relationship between this variant and serum adiponectin previously found in the individual SNP analyses.

Adiponectin has been recently identified as the most abundant of the known adipose tissue–derived proteins (1) with insulin-sensitizing and anti-inflammatory properties (1,17). Recently, the G allele of SNP 276 (G→T) was found to be associated with low plasma adiponectin levels in obese Japanese individuals (8). Moreover, individuals homozygous for the haplotype consisting of the G allele at position 276 and the T allele at position 45 had significantly lower adiponectin levels than those who did not carry the haplotype (10). In the present study, no association between SNPs 276 and 45 (or their haplotypes) and protein levels was found in Pima Indians, although another variant, SNP-12823, which is located within the *ACDC* promoter, was associated with serum adiponectin levels. However, it is worth noting that this SNP can only explain ~2% of the variance in serum adiponectin levels. Thus, the

TABLE 1
Linkage disequilibrium (D′) between pairs of SNPs in *ACDC*

	Frequency	SNP-12823	SNP-11365	SNP 45	SNP 276	SNP 3286
SNP-12823	0.76 [G/A]	—	−0.98	−0.97	−0.67	−0.93
SNP-11365	0.57 [G/C]	<0.01	—	−0.83	−0.48	−0.57
SNP 45	0.91 [T/G]	<0.01	<0.01	—	−0.97	0.99
SNP 276	0.79 [C/A]	<0.01	<0.01	<0.01	—	0.97
SNP 3286	0.69 [C/T]	<0.01	<0.01	<0.01	<0.01	—

The frequency of the more common allele, listed first in brackets, is shown. D′, which represents the degree of linkage disequilibrium between the SNPs expressed as a proportion of the maximum possible given the allele frequencies and the direction of association, is presented above the diagonal. D′ is calculated so that a positive value represents the association between the more common alleles at each SNP, while a negative value represents association between the more common allele at one SNP and the less common allele at the other. *P* values for the null hypothesis of no association between the alleles (D′ = 0.00) are presented below the diagonal.

TABLE 2
Association of SNPs in *ACDC* with diabetes, BMI, and other metabolic variables

SNP	Genotype	Asssociation with type 2 diabetes			Association with BMI			Association with serum adiponectin		
		Diabetes *n* (%)	Odds ratio (95% CI)	*P*	μ (kg/m^2)*	β (95% CI)†	*P*	m (mg/ml)*	β (95% CI)†	*P*
-12823	GG	601 (60.7)	—	—	572 (35.3)	—	—	316 (4.54)	—	—
	GA	412 (55.6)	—	—	398 (36.0)	—	—	240 (5.04)	—	—
	AA	43 (58.1)	1.19 (0.94–1.52)	0.15	41 (35.8)	0.00 (−0.02 to 0.02)	0.94	26 (5.64)	−0.11 (−0.18 to −0.04)	0.002
-11365	GG	279 (62.4)	—	—	273 (35.0)	—	—	156 (4.93)	—	—
	GC	520 (56.4)	—	—	501 (35.9)	—	—	300 (4.86)	—	—
	CC	188 (65.4)	0.91 (0.74–1.13)	0.41	176 (35.8)	−0.01 (−0.03 to 0.01)	0.31	86 (4.29)	0.06 (−0.01 to 0.12)	0.07
45	TT	846 (58.3)	—	—	808 (35.6)	—	—	462 (4.78)	—	—
	TG	177 (61.0)	—	—	171 (36.0)	—	—	100 (4.76)	—	—
	GG	5 (40.0)	0.90 (0.63–1.30)	0.59	5 (29.3)	0.00 (−0.04 to 0.05)	0.90	3 (6.45)	−0.01 (−0.11 to 0.09)	0.85
276	CC	620 (59.8)	—	—	595 (35.7)	—	—	331 (4.83)	—	—
	CT	333 (60.1)	—	—	318 (35.3)	—	—	179 (4.67)	—	—
	TT	50 (52.0)	1.09 (0.85–1.40)	0.52	46 (34.6)	0.00 (−0.02 to 0.03)	0.79	28 (4.67)	0.04 (−0.03 to 0.11)	0.29
3286	CC	505 (58.2)	—	—	485 (35.7)	—	—	273 (4.93)	—	—
	CT	459 (60.4)	—	—	438 (35.5)	—	—	255 (4.71)	—	—
	TT	94 (51.1)	1.05 (0.83–1.32)	0.69	90 (35.0)	0.00 (−0.03 to 0.02)	0.85	54 (4.64)	0.04 (−0.02 to 0.10)	0.15

SNP	Genotype	Association with HDL cholesterol			Association with ISI			Association with CIR		
		μ (mM)	β (95% CI)	*P*	μ (SD)	β (95% CI)	*P*	μ (SD)	β (95% CI)	*P*
-12823	GG	116 (1.19)	—	—	351 (0.03)	—	—	281 (0.06)	—	—
	GA	87 (1.17)	—	—	263 (−0.03)	—	—	222 (0.07)	—	—
	AA	12 (1.16)	0.04 (−0.04 to 0.11)	0.33	28 (0.17)	0.03 (−0.10 to 0.16)	0.16	26 (0.19)	−0.13 (−0.28 to 0.01)	0.07
-11365	GG	60 (1.16)	—	—	168 (−0.01)	—	—	142 (0.01)	—	—
	GC	106 (1.18)	—	—	324 (0.01)	—	—	276 (0.01)	—	—
	CC	26 (1.33)	−0.08 (−0.15 to −0.01)	0.03	100 (0.04)	0.00 (−0.12 to 0.12)	0.97	74 (0.02)	0.00 (−0.16 to 0.15)	0.96
45	TT	168 (1.19)	—	—	515 (0.01)	—	—	424 (0.04)	—	—
	TG	40 (1.15)	—	—	108 (−0.01)	—	—	92 (−0.10)	—	—
	GG	2 (1.20)	0.03 (−0.07 to 0.13)	0.60	2 (0.37)	0.03 (−0.17 to 0.22)	0.80	0	0.09 (−0.15 to 0.34)	0.45
276	CC	123 (1.17)	—	—	372 (0.02)	—	—	307 (0.05)	—	—
	CT	67 (1.18)	—	—	194 (−0.03)	—	—	159 (−0.03)	—	—
	TT	8 (1.37)	−0.04 (−0.13 to 0.05)	0.36	30 (0.11)	0.02 (−0.12 to 0.16)	0.83	24 (−0.20)	0.10 (−0.06 to 0.26)	0.21
3286	CC	102 (1.16)	—		311 (0.02)	—	—	256 (0.06)	—	—
	CT	92 (1.14)	—		274 (−0.01)	—	—	226 (0.07)	—	—
	TT	21 (1.17)	−0.01 (−0.08 to 0.05)	0.70	57 (0.00)	0.02 (−0.09 to 0.14)	0.70	46 (−0.29)	0.13 (−0.01 to 0.28)	0.07

SNP	Genotype	Association with triglycerides			Association with insulin secretion			Association with insulin sensitivity		
		m (mM)*	β (95% CI)†	*P*	m (pmol/l)*	β (95% CI)†	*P*	m (mg · kg^{-1} · min^{-1})*	β (95% CI)†	*P*
-12823	GG	113 (1.30)	—	—	124 (1,115)	—	—	181 (3.58)	—	—
	GA	82 (1.25)	—	—	82 (1,300)	—	—	130 (3.53)	—	—
	AA	11 (1.25)	0.03 (−0.09 to 0.15)	0.63	7 (1,250)	−0.05 (−0.27 to 0.17)	0.66	10 (3.46)	−0.01 (−0.08 to 0.06)	0.85
-11365	GG	57 (1.32)	—	—	36 (1,480)	—	—	63 (3.54)	—	—
	GC	100 (1.25)	—	—	115 (1,200)	—	—	166 (3.54)	—	—
	CC	26 (1.11)	0.08 (−0.04 to 0.19)	0.19	39 (1,025)	0.10 (−0.05 to 0.24)	0.19	60 (3.62)	−0.01 (−0.07 to 0.04)	0.61
45	TT	160 (1.25)			174 (1,150)			266 (3.56)		
	TG	39 (1.43)			28 (1,375)			40 (3.42)		
	GG	2 (1.25)	−0.09 (−0.25 to 0.07)	0.28	3 (1,535)	−0.12 (−0.33 to 0.09)	0.27	3 (5.22)	−0.02 (−0.13 to 0.08)	0.66
276	CC	118 (1.32)			130 (1,235)			188 (3.56)		
	CA	63 (1.17)			58 (1,150)			96 (3.48)		
	AA	9 (1.83)	−0.01 (−0.16 to 0.13)	0.87	10 (1,020)	0.03 (−0.12 to 0.18)	0.66	19 (3.48)	0.04 (−0.03 to 0.11)	0.24
3286	CC	96 (1.28)			109 (1,175)			159 (3.56)		
	CT	87 (1.17)			90 (1,160)			138 (3.53)		
	TT	22 (1.66)	−0.05 (−0.15 to 0.05)	0.34	15 (1,265)	−0.04 (−0.18 to 0.11)	0.62	25 (3.60)	0.01 (−0.06 to 0.07)	0.84

Odds ratios for diabetes and differences (β) between genotypes (GT) for continuous variables are adjusted for age, sex, ethnicity, and birth year in generalized estimating equation models that allow for the effect of sibship. Odds ratios or differences are analyzed under an additive model and are expressed per copy of the more common allele (the allele listed first). Means (μ) for continuous variables are adjusted for age and sex. *Represents geometric mean. †Difference (β) is expressed in terms of the natural logarithm of the relevant variable. Analysis of insulin secretion is restricted to those with normal glucose tolerance.

TABLE 3
Association of common haplotypes in *ACDC* with diabetes, BMI, and other metabolic variables

Haplotype	Composition	Frequency	Diabetes		BMI (log kg/m^2)		Serum adiponectin (log μg/ml)	
			Odds ratio (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
I	G_G_T_C_C	0.07	1.91 (1.12–3.27)	0.086	−0.01 (−0.05 to 0.02)	0.932	−0.09 (−0.24 to 0.05)	0.706
II	G_G_T_A_T	0.16	0.90 (0.66–1.23)	0.971	−0.01 (−0.04 to 0.02)	0.993	−0.01 (−0.09 to 0.07)	1.000
III	G_G_G_C_T	0.09	0.91 (0.62–1.33)	0.992	−0.03 (−0.07 to 0.02)	0.753	0.02 (−0.10 to 0.14)	1.000
IV	G_C_T_C_C	0.39	1.06 (0.84–1.32)	0.993	0.01 (−0.01 to 0.03)	0.976	−0.04 (−0.11 to 0.04)	0.845
V	G_C_T_A_T	0.05	0.88 (0.49–1.57)	0.996	−0.01 (−0.06 to 0.05)	1.000	−0.11 (−0.26 to 0.05)	0.600
VI	A_G_T_C_C	0.23	0.84 (0.64–1.10)	0.668	0.01 (−0.02 to 0.04)	0.943	0.10 (0.03–0.17)	0.036

Haplotype	Composition	Frequency	HDL cholesterol (mmol/l)		ISI (SD units)		CIR (SD units)	
			β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
I	G_G_T_C_C	0.07	−0.09 (−0.30 to 0.11)	0.893	−0.07 (−0.36 to 0.21)	0.992	−0.03 (−0.31 to 0.25)	1.000
II	G_G_T_A_T	0.16	0.06 (−0.04 to 0.15)	0.731	−0.08 (−0.21 to 0.06)	0.812	−0.06 (−0.26 to 0.14)	0.983
III	G_G_G_C_T	0.09	−0.03 (−0.14 to 0.08)	0.981	0.00 (−0.23 to 0.22)	1.000	−0.07 (−0.32 to 0.18)	0.986
IV	G_C_T_C_C	0.39	0.06 (−0.02 to 0.13)	0.532	0.05 (−0.08 to 0.17)	0.962	0.00 (−0.16 to 0.16)	1.000
V	G_C_T_A_T	0.05	0.03 (−0.16 to 0.23)	0.999	0.14 (−0.16 to 0.45)	0.896	−0.22 (−0.57 to 0.13)	0.706
VI	A_G_T_C_C	0.23	−0.06 (−0.04 to 0.01)	0.449	0.00 (−0.14 to 0.13)	1.000	0.15 (−0.01 to 0.31)	0.282

Haplotype	Composition	Frequency	Triglycerides (log mmol/l)		Insulin secretion (log pmol/l)		Insulin sensitivity (log mg · kg^{-1} · min^{-1})	
			β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
I	G_G_T_C_C	0.07	0.08 (−0.18 to 0.33)	0.983	0.08 (−0.31 to 0.47)	0.997	−0.03 (−0.17 to 0.11)	0.996
II	G_G_T_A_T	0.16	0.01 (−0.15 to 0.17)	1.000	−0.04 (−0.26 to 0.18)	0.998	−0.06 (−0.13 to 0.02)	0.481
III	G_G_G_C_T	0.09	0.14 (−0.04 to 0.31)	0.497	0.04 (−0.24 to 0.32)	1.000	0.06 (−0.07 to 0.18)	0.903
IV	G_C_T_C_C	0.39	−0.10 (−0.22 to 0.02)	0.449	−0.06 (−0.18 to 0.07)	0.911	0.03 (−0.03 to 0.09)	0.916
V	G_C_T_A_T	0.05	0.09 (−0.30 to 0.47)	0.996	0.05 (−0.31 to 0.40)	1.000	−0.05 (−0.19 to 0.08)	0.946
VI	A_G_T_C_C	0.23	−0.01 (−0.14 to 0.12)	1.000	0.05 (−0.14 to 0.23)	0.993	0.02 (−0.05 to 0.10)	0.998

Haplotypes are defined by alleles at each SNP in the following order: *ACDC*-12823, *ACDC*-11365, *ACDC*-SNP 45, *ACDC*-SNP 276, and *ACDC*–3286. Odds ratios for diabetes or haplotypic differences (β) for continuous variables are calculated under an additive model and expressed per copy of the relevant haplotype. All parameters are calculated using generalized estimating equation models that adjust for age, sex, ethnicity, and birth year, which accounts for the correlation among siblings. *P* values are calculated using a Bonferroni correction to account for the fact that six different haplotypes have been examined.

present results suggest that none of these SNPs in *ACDC* have major effects on serum adiponectin concentrations.

In genome-wide linkage analyses in the present group of individuals we found that while 39% of the variance in serum adiponectin is potentially due to genetic factors (i.e., heritability = 0.39), there was no evidence for linkage on chromosome 3q27, where *ACDC* is located (16). Instead, significant linkage (logarithm of odds = 3.0 at 18 cM) was seen on chromosome 9p and modest evidence for linkage (logarithm of odds = 1.0 at 124 cM) was found on chromosome 3q13, ~70 cM centromeric to *ACDC*. Comuzzie et al. (18) have identified two major loci influencing adiponectin expression in European Americans on chromosomes 5 and 14 and modest evidence for linkage (logarithm of odds = 1.3) on 3q27 near *ACDC*.

Although some studies have reported suggestive evidence for the linkage of obesity with 3q27 (7), genome-wide linkage analyses of BMI in Pima Indians did not show strong evidence for linkage to the *ACDC* region (11). Furthermore, in the present study we found no evidence for association between *ACDC* SNPs and obesity in Pima Indians. These findings are consistent with results obtained in a Japanese population showing no evidence for a significant association with BMI (8). However, the role of *ACDC* SNPs in mediating susceptibility to obesity has been inconclusive. One study has reported an association between the variant G allele of SNP 45 and obesity in a German population (9), while results derived from other populations have found that the T allele at this position was associated with a higher body weight (10). Although the factors underlying the disparities among these studies are not yet known, it is possible that different environmental exposures, different patterns of linkage disequilibrium among populations, and sampling variation may play a role in the reported findings. It is also worth noting that the variant allele of SNP 45 is relatively rare and as such may produce spurious associations.

Low plasma adiponectin levels predict the development of type 2 diabetes in Pimas (5), and this has been confirmed in a German population (19). Linkage of the region near *ACDC* with type 2 diabetes has been reported in French and Japanese populations (6,20) and with fasting insulin levels in European Americans (7), thereby suggesting that a locus in this region may influence insulin sensitivity and risk for diabetes. In the Pimas, however, there is little evidence for linkage with either type 2 diabetes (11) or measures of insulin sensitivity (12) in this region. Because we have also not detected an association between *ACDC* SNPs and insulin sensitivity or type 2 diabetes in Pima Indians, it is likely that this gene does not play a major role in conferring increased susceptibility to these disorders in this population.

RESEARCH DESIGN AND METHODS

Subjects who participated in this study included 1,338 Pima individuals (332 nuclear families and 112 extended pedigrees) who were previously selected for linkage studies (11) from participants in ongoing longitudinal studies of obesity and type 2 diabetes conducted in the Gila River Indian Community. Among the siblings in the linkage study, there were 1,080 (496 men and 584 women) who were typed for at least one of the *ACDC* polymorphisms. The prevalence of type 2 diabetes in these participants was 59%. The mean (±SD) age was 38.7 ± 13.7 years, mean BMI was 36.6 ± 8.2 kg/m^2, and mean serum adiponectin was 5.46 ± 2.69 μg/ml. A subset of 331 of these individuals had participated in detailed physiologic studies, including measurement of directly assessed insulin sensitivity at physiologic insulin concentrations by the hyperinsulinemic-euglycemic clamp and measurement of insulin secretion by the acute insulin response calculated at 3–5 min after a 25-g intravenous glucose bolus (see Pratley et al. [12] for further details). In 639 individuals, insulin and glucose concentrations had been measured during an oral glucose tolerance test at their last nondiabetic examination, and these measures were used to calculate surrogate measures of insulin sensitivity and secretion, ISI, and CIR (13–15). Serum adiponectin was measured in stored sera from most of these participants (16,21). Since adiponectin concentrations are strongly influenced by the presence of diabetes and renal function (21), these analyses were restricted to nondiabetic participants with normal renal function (n = 580). In those who had been examined since 1992 (n = 212), serum HDL cholesterol and triglycerides were measured. This study was approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases and the Tribal Council of the Gila River Indian Community. All subjects provided signed informed consent before participation.

***ACDC* genomic screening and SNP genotyping.** *ACDC* was screened using denaturing high-performance liquid chromatography and amplicons, which yielded denaturing high-performance liquid chromatography heteroduplexes, which were validated by direct sequencing in the 50 individuals with the highest serum adiponectin levels and the 50 individuals with the lowest adiponectin levels as described (22).

We genotyped SNP-12823, SNP-11365, SNP 45, and SNP 276 using pyrosequencing according to the manufacturer's recommendations (Pyrosequencing, Uppsala, Sweden) with minor modifications as described (23). SNP 3286 was genotyped by PCR restriction fragment–length polymorphism by first amplifying a PCR fragment of 397 bp and digesting it overnight at 37^1 with *Nsp I* (New England Biolabs, Beverly, MA). In the presence of the C allele, the PCR product was restricted into two fragments of 95 and 302 bp, while the T allele yielded an uncut fragment. Sequence information for all genotyping primers is available upon request.

Statistical analyses. Haplotype frequencies for pairs of SNPs were estimated with a pedigree-based maximum likelihood method implemented in the ILINK program (24). The degree of linkage disequilibrium between alleles for each pair of loci was expressed as D′, which represents the proportion of the maximum possible allelic association given the allele frequencies and the direction of association. The likelihood ratio test was used to assess statistical significance of the observed allelic associations.

Association between alleles at each SNP and prevalence of diabetes was assessed by logistic regression with control for the effects of age, sex, birth year, and ethnicity (i.e., regardless of whether the participant was of full Pima heritage). The proportion of participants who were full Pima/Tohono O'odham was 79%; 94% of the remaining subjects were >50% Pima/Tohono O'odham. To account for familial relationships (i.e., sibship), these analyses were conducted using generalized estimating equations as previously described (23). The association of each SNP with type 2 diabetes was analyzed under an additive model in which the logarithm of the odds ratio is expressed as a function of the number of copies of the more common allele. Additional analyses were conducted under models in which the effect of the common allele was assumed to be dominant or recessive; as the conclusions of these models were identical to those of the additive models, only results for the additive models are presented here. For analysis of continuous variables (e.g., BMI, lipids, serum adiponectin concentrations, and measures of insulin sensitivity and secretion), linear regression analyses were conducted in an analogous fashion to the logistic models for type 2 diabetes. The generalized estimating equations were fit using PROC GENMOD of SAS (SAS Institute, Cary, NC).

A modification of the zero-recombinant haplotyping technique (25) was used to analyze the associations of haplotypes in the five genotyped SNPs in the region with the four different traits. First, the EH algorithm was used to estimate haplotype frequencies. There were six common haplotypes with frequencies >0.05, which accounted for 98% of the observed haplotypes among the five SNPs. Next, the MLINK program (23) was used to estimate the probability that each individual carried each possible haplotype given these frequencies, the genotypes of the individual, and the genotypes of other individuals in the pedigree. As only four SNPs were necessary to assign individuals to these haplotypes, SNP 3286 was omitted from the likelihood calculations. The probabilities derived from MLINK were then utilized in logistic or linear regression analyses to evaluate the association of each of the common haplotypes with the traits of interest. Since this approach requires the assumption of no recombination across the region, individuals whose genotypes produced obligate recombinations (n = 9) were deleted from these analyses. These analyses were conducted with six different common haplotypes; therefore, a corrected P value (P_{corr}) was calculated using a Bonferroni correction [$P_{corr} = 1 - (1 - P)$] (5) to account for multiple comparisons.

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