# A Haplotype at the Adiponectin Locus Is Associated With Obesity and Other Features of the Insulin Resistance Syndrome

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**Adiponectin is a protein secreted by adipocytes that modulates insulin action. To assess whether variants of this gene contribute to the prevalence of insulin resistance in Caucasians, we genotyped 413 nondiabetic individuals for two single nucleotide polymorphisms (SNPs) at this locus. The two SNPs (45T→G and 276G→T) were chosen because of their association with type 2 diabetes in Japanese. Whereas each polymorphism was significantly associated with some correlate of insulin resistance, the haplotype defined by the two together was strongly associated with many components of the insulin resistance syndrome. Homozygotes for the risk haplotype had higher body weight ($P = 0.03$), waist circumference ($P = 0.004$), systolic ($P = 0.01$) and diastolic ($P = 0.003$) blood pressure, fasting glucose ($P = 0.02$) and insulin ($P = 0.005$) levels, homeostasis model assessment (HOMA) for insulin resistance ($P = 0.003$), and total to HDL cholesterol ratio ($P = 0.01$). Homozygotes also had significantly lower plasma levels of adiponectin ($P = 0.03$), independent of sex, age, and body weight. In an independent study group of 614 Caucasians, including 310 with type 2 diabetes, the risk haplotype was confirmed to be associated with increased body weight ($P = 0.03$) but not with type 2 diabetes per se. We conclude that variability at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome, but given the nature of the two SNPs, the risk haplotype is most probably a marker in linkage disequilibrium with an as yet unidentified polymorphism that affects plasma adiponectin levels and insulin sensitivity. *Diabetes* 51: 2306–2312, 2002**

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CV, coefficient of variation; $HOMA_{IR}$, homeostasis model assessment for insulin resistance; IBW, ideal body weight; PPAR, peroxisome proliferator–activated receptor; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor; UTR, untranslated region.

Our view of adipose tissue has dramatically changed over the last decade. Once considered an inert energy depot, adipose tissue has emerged as an important endocrine organ regulating whole-body metabolism and other vital functions related to inflammation and immune responses (1,2). These actions are mediated by a number of molecules that are secreted by adipocytes and act in an autocrine, paracrine, or endocrine fashion (1,2). Among those identified to date are leptin, adipsin, tumor necrosis factor (TNF)-α, resistin, and adiponectin (3–10). In concert, these cytokines, or adipokines, are believed to adapt metabolic fluxes to the amount of stored energy (1,2). Dysregulation of this network has been implicated in the etiology of insulin resistance and other components of the insulin resistance syndrome (syndrome X), such as glucose intolerance, obesity, dyslipidemia, and high blood pressure (1,11).

Adiponectin, also known as APM1, Acrp30, or adipoQ, is an adipose tissue–specific protein of 247 amino acids that shares significant similarity with collagens VIII and X and complement protein C1q (7–10). Although adiponectin's functions are not completely understood, we know that its expression is reduced in the presence of obesity and increased by caloric restriction or treatment with insulin-sensitizing peroxisome proliferator–activated receptor (PPAR)-γ activators (12–14). In animal models of obesity and diabetes, administration of adiponectin or its globular domain produces weight loss and improves insulin sensitivity and glucose tolerance (2,15–17). These effects result from actions on skeletal muscle to increase fatty acid oxidation and on liver to increase sensitivity to the antigluconeogenesis effects of insulin (15–17).

With such profound effects on metabolism, genetic variability in adiponectin may be a determinant of insulin resistance. Results of two linkage studies are consistent with this hypothesis: a genome scan for insulin resistance loci in U.S. Caucasian families detected a signal at 3q27 (18), the locus of the adiponectin gene, and early-onset type 2 diabetes has been linked to the same position in French families (19). Moreover, an association study from Japan ties these linkage results directly to the adiponectin gene. Two single nucleotide polymorphisms (SNPs) in the adiponectin gene, a silent T to G substitution in exon 2 (45T→G) and a G to T substitution in intron 2 (276G→T),

were significantly associated with type 2 diabetes (20). Furthermore, a recent report described an association between SNP 45 and obesity in a German population (21).

Here we show that SNPs 45T→G and 276G→T identify a haplotype that is associated with obesity and other features of the insulin resistance syndrome in Caucasians. We further demonstrate that this association may be mediated by an effect on circulating levels of adiponectin.

## RESEARCH DESIGN AND METHODS

**Nondiabetic individuals.** Four hundred thirteen unrelated, Caucasian residents of the Gargano area (east coast of Italy) were included in the study. Subjects were recruited among the employees of the hospital "Casa Sollievo della Sofferenza" (San Giovanni Rotondo, Italy) who had fasting plasma glucose <7 mmol/l at screening and were not taking any medications. The study and informed consent procedures were approved by the local research ethics committee. All study subjects were examined between 8:00 and 9:00 A.M. after an overnight fast. At that time, height, weight, waist and hip circumferences, and blood pressure were measured in duplicate, and a blood sample was drawn for biochemical measurements and DNA extraction. Height and weight were used to calculate BMI and percent ideal body weight (% IBW) (calculated by multiplying BMI by 4.39 for men and 4.76 for women) (22). In each subject (standing), waist circumference (the widest value between the lower rib margin and the iliac crest) was measured with a plastic measuring tape by the same investigator. Systolic and diastolic (disappearance of Korotkoff sound, phase V) blood pressure were measured in the sitting position with an appropriately sized cuff after a 5-min rest. Plasma glucose (mmol/l), serum insulin (pmol/l), and lipid profile (total serum cholesterol, HDL cholesterol, serum triglycerides) were measured using commercially available enzymatic kits as previously described (23). The insulin resistance index $HOMA_{IR}$ (homeostasis model assessment) was calculated as fasting serum insulin (pmol/l) × fasting plasma glucose (mmol/l)/135 (24).

Plasma levels of adiponectin were measured in 32 TG/TG homozygotes (16 men and 16 women equally sampled from the obese and lean strata) and 32 non-TG carriers matched for sex, age, and body weight. Adiponectin was measured by quantitative Western blotting. After SDS-PAGE of 2 μl plasma, proteins were transferred to BA83 nitrocellulose (Schleicher & Schuell), stained with Ponceau S solution, and then blocked in PBS or Tris-buffered saline with 0.1% Tween-20 and 5% nonfat dry milk. A rabbit anti-human adiponectin antibody, directed against the hypervariable region of the human protein (DQETTTQGPGV), was employed. This antibody was visualized with an $^{125}$I-derivatized secondary goat anti-rabbit antibody (New England Nuclear); a standardized human serum sample was also applied to each gel in four different concentrations. However, as no standard for recombinant human adiponectin protein was included, human plasma levels are expressed as relative units per milliliter rather than as absolute values. Blots were analyzed with a Phosphoimager (Molecular Dynamics) and quantitated with Imagequant software. The intra-assay coefficient of variation (CV), based on 10 replicates of the same sample blotted on the same membrane, was 9%. The interassay CV, based on replicates of the same samples on different blots, ranged from 10 to 20%, emphasizing the high level of reproducibility of the assay.

**Case-control study.** A total of 310 unrelated cases with type 2 diabetes and 304 unrelated nondiabetic control subjects were included in the study. All subjects were Caucasian. Type 2 diabetic subjects were randomly selected from a sample of Joslin Clinic patients aged 40–70 years who met the following criteria: *1*) diabetes that was diagnosed after age 35 years according to World Health Organization criteria and *2*) treatment with only diet or oral agents for at least 2 years after diagnosis. Blood samples for DNA extraction were drawn at the time of visits to the Joslin Clinic. Control subjects were unrelated, nondiabetic spouses of subjects with type 2 diabetes and nondiabetic parents of patients with type 1 diabetes who were enrolled in family studies underway at the Joslin Clinic. All control subjects had a negative history for type 2 diabetes, were not currently taking any glucose-lowering medications, and had fasting glucose <6.1 mmol/l or $HbA_{1c}$ <6.1%. Height and weight were used to calculate % IBW as described above. Control subjects were subdivided into two groups, at low and high risk of type 2 diabetes, based on whether % IBW was below or above the median value (116.4%). The study and informed consent procedures were approved by the Joslin Committee on Human Studies.

**SNP genotyping.** Genotypes were determined at positions 45 and 276 relative to the translation start site (corresponding to position 71 and 302 of GenBank NM_004797) by PCR followed by dot blotting and allele-specific hybridization. DNA fragments containing each SNP (250 bp for SNP 45T→G and 196 bp for 276G→T) were amplified by PCR from genomic DNA using primers 5′-TCTCTCCATGGCTGACAGTG-3′ and 5′-CCTTTCTCACCCTTCTCACC-3′ for SNP 45T→G and 5′-GGCCTCTTTCATCACAGACC-3′ and 5′-AGATGCAG CAAAGCCAAAGT-3′ for SNP 276G→T. PCR was performed on 30 ng DNA in 20 μl containing Tris HCl 10 mmol/l, pH 8.3, KCl 50 mmol/l, $MgCl_2$ 1.5 mmol/l, each dNTP 0.2 mmol/l, forward and reverse primers 0.4 μmol/l, *Taq* polymerase 0.035 U/μl (Applied Biosystems, Foster City, CA) for 30 cycles (60 s at 95°C, 45 s at 58°C, 45 s at 72°C) in an MJ Research thermal cycler. PCR fragments were dot-blotted on Nylon membranes in duplicate, hybridized with $^{32}$P-labeled allele-specific 17mers according to standard protocols, and autoradiographed overnight. Genotypes were inferred by comparing the autoradiograms of membranes hybridized with different allele probes. Because of PCR failure, genotypes could not be determined for 14 individuals at position 45 and 8 individuals at position 276. Genotypes for other polymorphisms at the adiponectin locus were similarly determined by PCR, dot blotting, and allele-specific hybridization (primer sequences and PCR conditions are available from the authors upon request).

**Data analysis.** Haplotypes at the 45 and 276 loci were inferred for each individual by maximum likelihood methods as previously described (25). Study subjects with phase-unknown genotype (i.e., heterozygotes at both SNPs) were assigned to the most likely haplotype phase (TT/GG). The conditional probabilities of this phase were 0.98 for Italian subjects, 1.0 for Joslin low-risk control subjects, 1.0 for Joslin high-risk control subjects, and 0.93 for Joslin patients. Multilocus haplotypes including all other SNPs were inferred by means of EHPlus software (26). Continuous variables were compared among genotype groups by ANOVA using the PROC GLM procedure of the SAS software package (SAS Institute, Cary, NC). Fasting insulin, $HOMA_{IR}$, and triglycerides were analyzed in the logarithms. All analyses were repeated after sequentially adding sex, current age, and % IBW as covariates. Genotype and allele frequencies were compared among study groups using $\chi^2$ tests.

## RESULTS

To assess whether adiponectin polymorphisms 45T→G and 276G→T contribute to insulin resistance, we genotyped 413 unrelated, nondiabetic Caucasian residents of the Gargano area (east coast of Italy) who had been characterized with respect to % IBW, waist circumference, blood pressure, fasting glucose and insulin, and lipid profile. Genotype distributions were in Hardy-Weinberg equilibrium at both loci, with T being the major allele at position 45 (T frequency = 0.802) and G the major allele at position 276 (G frequency = 0.701). T/T homozygotes at position 45 had significantly higher systolic blood pressure than T/G heterozygotes or G/G homozygotes (114 ± 13, 111 ± 11, and 109 ± 9 mmHg; $P = 0.02$) (Table 1). They also tended toward higher body weight, waist circumference, diastolic blood pressure, fasting blood glucose, serum insulin, and total cholesterol, although those differences were not statistically significant with this sample size (Table 1). A similar trend toward association with features of insulin resistance was observed for the G/G genotype at position 276 (Table 1). Statistical significance was reached in this case for $HOMA_{IR}$, an index of insulin resistance derived from fasting glucose and insulin (1.82, 1.59, and 1.47; $P = 0.05$) (24).

Much stronger associations with features of the insulin resistance syndrome were observed when the two SNPs were considered together as haplotypes (Table 2). The two polymorphisms were in linkage disequilibrium ($R = -0.31$, $P = 0.0001$), with estimated 45/276 haplotype frequencies of 0.300 for TT, 0.198 for GG, 0.501 for TG, and 0.001 for GT (Fig. 1). Homozygous carriers of the TG haplotype (i.e., individuals who were T/T at 45 and G/G at 276, solid line in Fig. 1) had higher fasting glucose and insulin levels than heterozygous carriers of the TG haplotype (TG/X, dashed line in Fig. 1) or noncarriers (X/X) ($P = 0.02$ and $P = 0.005$, respectively) (Table 2). The association was even stronger

TABLE 1
Clinical characteristics of nondiabetic subjects from San Giovanni Rotondo according to adiponectin genotypes at positions 45 and 276

| | SNP 45 | | | SNP 276 | | |
|---|---|---|---|---|---|---|
| | T/T | T/G + G/G | *P* | G/G | G/T + T/T | *P* |
| *n* | 260 (65.2) | 139 (34.8) | | 207 (51.1) | 198 (48.9) | |
| M/F | 98/162 | 46/93 | 0.45 | 82/125 | 67/131 | 0.37 |
| Age (years) | 38 ± 12 | 37 ± 11 | 0.55 | 38 ± 11 | 38 ± 12 | 0.90 |
| % IBW | 117 ± 21 | 115 ± 18 | 0.52 | 117 ± 20 | 115 ± 20 | 0.22 |
| Waist (cm) | 82.7 ± 12 | 80.2 ± 12 | 0.12 | 83.0 ± 12 | 80.7 ± 12 | 0.14 |
| Systolic blood pressure (mmHg) | 114 ± 13 | 111 ± 11 | 0.02 | 113 ± 12 | 113 ± 12 | 0.81 |
| Diastolic blood pressure (mmHg) | 76 ± 9 | 74 ± 8 | 0.09 | 76 ± 8 | 75 ± 8 | 0.15 |
| Fasting blood glucose (mmol/l) | 5.0 ± 0.5 | 4.9 ± 0.5 | 0.55 | 5.1 ± 0.5 | 4.9 ± 0.5 | 0.13 |
| Serum insulin (pmol/l) | 46 ± 26 | 44 ± 21 | 0.87* | 49 ± 27 | 42 ± 22 | 0.08* |
| $HOMA_{IR}$ | 1.72 ± 1.0 | 1.62 ± 0.9 | 0.80* | 1.82 ± 1.1 | 1.56 ± 0.9 | 0.05* |
| Cholesterol (mmol/l) | 5.09 ± 1.09 | 4.87 ± 0.91 | 0.09 | 5.04 ± 1.01 | 5.02 ± 1.06 | 0.67 |
| HDL cholesterol (mmol/l) | 1.37 ± 0.34 | 1.37 ± 0.30 | 0.92 | 1.34 ± 0.34 | 1.40 ± 0.32 | 0.22 |
| Total/HDL ratio | 3.9 ± 1.2 | 3.7 ± 1.1 | 0.15 | 4.0 ± 1.3 | 3.8 ± 1.1 | 0.12 |
| Triglycerides (mmol/l) | 1.06 ± 0.75 | 1.09 ± 0.74 | 0.81* | 1.16 ± 0.9 | 0.99 ± 0.53 | 0.22* |

Data are means ± SD. Significance tests were based on the comparison of three genotype groups. Because heterozygotes and homozygotes for the minor alleles were virtually identical for every variable, these groups were combined for presentation. Genotypes at positions 45 and 276 were available for 399 and 405 study subjects, respectively. *Significance was tested on log-transformed values.

for $HOMA_{IR}$ ($P = 0.003$) (Table 2). TG/TG homozygotes also had significantly higher body weight ($P = 0.03$), waist circumference ($P = 0.004$), systolic ($P = 0.01$) and diastolic ($P = 0.003$) blood pressure, and ratio of total to HDL cholesterol ($P = 0.01$) (Table 2). All differences remained significant after excluding individuals with ambiguous haplotype phase ($n = 43$) and adjusting for age and sex. Further adjustment for body weight decreased the magnitude of the associations, but insulin level ($P = 0.05$) and diastolic blood pressure ($P = 0.04$) were still significant, and $HOMA_{IR}$ ($P = 0.07$) and fasting blood glucose ($P = 0.07$) were almost significant. These results indicate that mechanisms independent of body weight contribute to the genotype effect in addition to any mechanisms that operate indirectly through the genotype's effect on body weight.

To determine whether this association might be mediated by differences in adiponectin expression, we measured plasma adiponectin concentrations in 32 TG/TG homozygotes (16 men and 16 women sampled equally from obese and lean strata) and 32 non-TG carriers matched for sex, age, and body weight. There was a marked sex difference, with women having higher adiponectin levels than men (41.8 ± 12 vs. 28.8 ± 10 U/ml). However, within each sex, TG/TG homozygotes had lower levels than non–TG carriers (39.1 ± 14 vs. 44.4 ± 11 U/ml in women and 25.6 ± 8 vs. 32.1 ± 11 in men). In a multivariate analysis, sex and TG genotype were both significant determinants of plasma adiponectin concentration ($P = 0.0001$ and $P = 0.03$, respectively). The association between TG haplotype and low adiponectin levels

TABLE 2
Clinical characteristics of nondiabetic subjects from San Giovanni Rotondo according to carrying status of adiponectin 45-276 haplotype TG

| | Haplotype 45-276 | | |
|---|---|---|---|
| | TG/TG | TG/X + X/X | *P* |
| *n* | 106 (27.1) | 285 (72.9) | |
| M/F | 44/62 | 109/176 | 0.38 |
| Age (years) | 39 ± 12 | 37 ± 11 | 0.32 |
| % IBW | 120 ± 22 | 114 ± 19 | 0.03 |
| Waist (cm) | 84.9 ± 11 | 80.6 ± 12 | 0.004 |
| Systolic blood pressure (mmHg) | 116 ± 12 | 112 ± 12 | 0.01 |
| Diastolic blood pressure (mmHg) | 78 ± 9 | 75 ± 8 | 0.003 |
| Fasting blood glucose (mmol/l) | 5.1 ± 0.5 | 4.9 ± 0.5 | 0.02 |
| Serum insulin (pmol/l) | 53 ± 32 | 42 ± 21 | 0.005* |
| $HOMA_{IR}$ | 2.01 ± 1.3 | 1.56 ± 0.8 | 0.003* |
| Cholesterol (mmol/l) | 5.15 ± 1.09 | 4.99 ± 1.02 | 0.11 |
| HDL cholesterol (mmol/l) | 1.32 ± 0.36 | 1.39 ± 0.32 | 0.13 |
| Total/HDL ratio | 4.1 ± 1.3 | 3.8 ± 1.1 | 0.01 |
| Triglycerides (mmol/l) | 1.22 ± 0.98 | 1.02 ± 0.63 | 0.06* |

Data are means ± SD. X denotes any haplotype other than TG. Significance tests were based on the comparison of three genotype groups (TG/TG vs. TG/X vs. X/X). Because TG/X heterozygotes and X/X homozygotes were virtually identical for every variable, these groups were combined for presentation. Genotypes were available at both positions for 391 study subjects. *Significance was tested on log-transformed values.

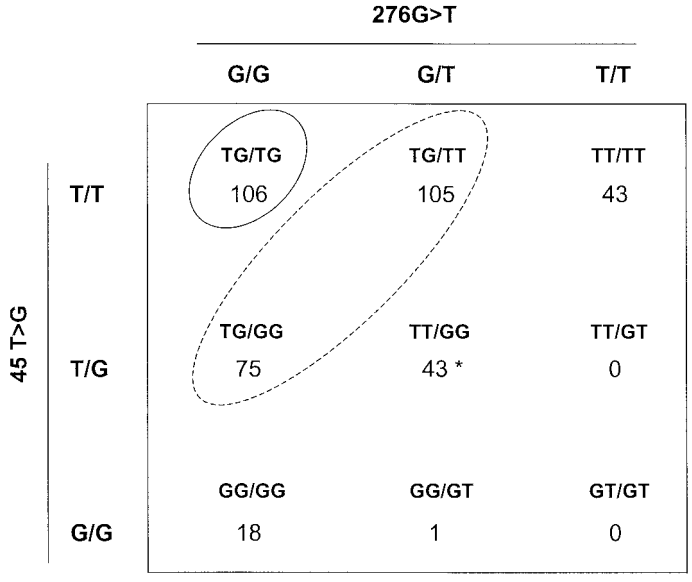


**FIG. 1. Distribution of 45-276 haplotypes in Italian subjects. Genotypes at positions 45 and 276 are shown outside the square. Haplotype phases are indicated in bold for each of the nine possible genotype combinations. The solid line encloses the genotype combination with phase TG/TG, and the dashed line, the combinations with phase TG/X. For doubly heterozygous individuals (indicated by the asterisk), the odds were 49:1 for phase TT/GG as opposed to TG/GT.**

remained significant after adjusting for body weight and insulin level.

To investigate whether the association between TG haplotype and insulin resistance had an impact on the risk of diabetes, we genotyped 310 Joslin Clinic patients with type 2 diabetes and 304 nondiabetic control subjects subdivided into two groups according to body weight, one at lower risk of diabetes (% IBW below the median, $n$ = 151) and one at higher risk (% IBW above the median, $n$ = 153) (Table 3). Consistent with the findings in the Italian population, the TG haplotype was significantly more frequent in high-risk than in low-risk control subjects (0.618 vs. 0.517, $P = 0.03$) (Table 4). However, no further increase in haplotype frequency was observed in patients with overt diabetes (0.590), indicating that this haplotype was associated with obesity and insulin resistance rather than with the development of hyperglycemia (Table 4). Consistent with these findings, the TG haplotype was more frequent in obese than in lean type 2 diabetic patients (0.600 vs. 0.556), although the difference was not significant with this sample size. The haplotype was not associated with age at diabetes diagnosis or type of treatment.

While this work was in progress, the sequence of a large part of the adiponectin gene was screened for polymorphisms in Japanese and Caucasian subjects (27). Eight additional polymorphisms were identified in the 5′ flanking region (−11414, −11379, −11365), introns (−4041, −3964, 349, 712), and 3′ untranslated region (UTR) (2019) (26). Seven of these SNPs (those with frequency >5% in Caucasians) were genotyped in our populations, and linkage disequilibrium with the TG/X haplotype system was almost complete for two, moderate for four, and weak for the remaining one (Table 5). Only those in tight linkage disequilibrium with the TG haplotype (712 and 2019) showed association with insulin resistance (Table 5). Altogether, the nine SNPs defined six extended haplotypes having a frequency >5% (Fig. 2). Three did not contain the TG haplotype and were not associated with insulin resistance. None of the three containing the TG haplotype (1, 4, and 5 in Fig. 2) were more strongly associated with insulin resistance than the TG haplotype as a whole (data not shown).

| Haplotype | −11379 | −11365 | −4034 | −3964 | 45 | 276 | 349 | 712 | 2019 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | G | C | A | A | **T** | **G** | A | G | Ins |
| 2 | G | C | A | A | T | T | A | A | Del |
| 3 | G | C | A | A | G | G | G | A | Del |
| 4 | G | C | C | A | **T** | **G** | A | G | Ins |
| 5 | G | G | A | A | **T** | **G** | A | G | Ins |
| 6 | A | C | C | A | T | T | A | A | Del |

**FIG. 2. Common (>5%) adiponectin haplotypes in Italian subjects. The 45-276 TG haplotype is indicated in bold. The six haplotypes shown in the figure account for 70% of the haplotypes at this locus.**

## DISCUSSION

The cluster of metabolic abnormalities known as the insulin resistance syndrome or syndrome X is responsible for a large proportion of cardiovascular morbidity and mortality in the Western world (11). Prospective twin studies and segregation analyses in families strongly suggest that this syndrome is genetically determined (28,29). The genetic background of insulin resistance is likely to be polygenic, but the genes that are involved are mostly unknown (30). Our data indicate that adiponectin may be

TABLE 3
Clinical characteristics of nondiabetic individuals and patients with type 2 diabetes from the Boston area

| | Nondiabetic subjects % IBW < median | Nondiabetic subjects % IBW > median | Patients with type 2 diabetes | $P$ |
|---|---|---|---|---|
| $n$ | 151 | 153 | 310 | |
| M/F | 87/64 | 72/81 | 167/143 | 0.17 |
| Age (years) | 61 ± 16 | 63 ± 16 | 61 ± 6 | 0.14 |
| Age at diagnosis (years) | — | — | 47 ± 7 | |
| % IBW | 105 ± 8 | 135 ± 21 | 143 ± 32 | |
| Treatment (%) | | | | |
| Diet only | — | — | 7.4 | |
| Oral agents | — | — | 25.2 | |
| Insulin | — | — | 67.4 | |

Data are means ± SD unless noted otherwise. Median % IBW among nondiabetic subjects was 116.4.

TABLE 4
Adiponectin genotype and allele distributions in nondiabetic individuals and patients with type 2 diabetes from the Boston area

| | Nondiabetic subjects % IBW < median | Nondiabetic subjects % IBW ≥ median | Patients with type 2 diabetes | *P* |
|---|---|---|---|---|
| *n* | 151 | 153 | 310 | |
| SNP 45 | | | | |
| T/T | 0.662 | 0.784 | 0.781 | |
| T/G | 0.311 | 0.183 | 0.197 | |
| G/G | 0.027 | 0.033 | 0.023 | 0.04* |
| Allele T | 0.818 | 0.876 | 0.879 | 0.03† |
| SNP 276 | | | | |
| G/G | 0.503 | 0.549 | 0.510 | |
| G/T | 0.391 | 0.379 | 0.400 | |
| T/T | 0.106 | 0.072 | 0.090 | 0.83* |
| Allele G | 0.699 | 0.734 | 0.710 | 0.52† |
| Haplotype 45-276 | | | | |
| TG/TG | 0.305 | 0.412 | 0.371 | |
| TG/X | 0.424 | 0.412 | 0.439 | |
| X/X | 0.271 | 0.176 | 0.190 | 0.15* |
| Haplotype TG | 0.517 | 0.618 | 0.590 | 0.03† |

Data are proportions. Median % IBW among nondiabetic subjects was 116.4. *4 df; †2 df.

one of the genes playing an important role. We have identified a haplotype at this locus that is strongly associated with high insulin levels in healthy individuals. The association extends to other features of the insulin resistance syndrome, such as increased waist circumference, higher blood pressure, and a higher ratio of total to HDL cholesterol. These effects appear to be mediated by both body weight–dependent and –independent mechanisms and are consistent with the recent finding of linkage with insulin resistance traits on 3q27, where the adiponectin gene is located (18).

Of particular importance is the observation that the same haplotype is associated with lower circulating levels of adiponectin, independent of body weight or insulin levels. This finding offers an entirely new interpretation of the relationship between plasma adiponectin levels and obesity. Hypoadiponectinemia is a feature of obesity and correlates with indexes of insulin resistance, such as fasting and 2-h insulin levels (12,31). The low level of adiponectin has been interpreted as the consequence of increased adiposity and/or insulin resistance. Our results suggest that, instead, hypoadiponectinemia is a primary, genetically determined defect contributing to the etiology of obesity and insulin resistance. This notion is in agreement with the recent report of decreased adiponectin levels early in the course of obesity in longitudinal studies of obesity-prone monkeys (32). An alternative explanation of our findings is that the haplotype is in linkage disequilibrium with a coding variant that decreases adiponectin immunoreactivity to the assay antibody.

The contribution to type 2 diabetes is less clear, consistent with the complexity of this disorder. Type 2 diabetes can be viewed as the result of two different pathophysiological defects, namely insulin resistance and a failure of the β-cell to compensate for insulin resistance by appropriately increased insulin secretion (28,33). Different sets of genes may be involved in these two processes. The observation of similar haplotype frequencies in type 2 diabetic patients and nondiabetic obese control subjects, which were significantly higher than in lean control subjects, suggests than the TG haplotype confers an increased risk of type 2 diabetes only through its association with obesity and insulin resistance and not through a diabetogenic effect on β-cell function.

The biology of this association is unclear. Several studies of animal models have shown that adiponectin is a potent insulin enhancer, regulating energy homeostasis and glucose tolerance (15–17). Mice fed a high-fat diet experienced profound weight loss when chronically treated with a proteolytic fragment of adiponectin (15). Our findings of association with circulating adiponectin levels suggest that SNPs 45T→G and 276G→T may act

TABLE 5
$HOMA_{IR}$ of nondiabetic subjects from San Giovanni Rotondo according to genotypes for additional SNPs in the adiponectin gene

| | | | $HOMA_{IR}$ | | | |
|---|---|---|---|---|---|---|
| SNP | q | *R* | p/p | p/q | q/q | *P* |
| –11379G>A | 0.114 | –0.24* | 1.71 ± 1.0 | 1.66 ± 0.9 | 1.84 ± 1.6 | 0.99 |
| –11365C>G | 0.198 | 0.33* | 1.65 ± 0.9 | 1.80 ± 1.2 | 1.81 ± 0.9 | 0.69 |
| –4034A>C | 0.335 | 0.18* | 1.60 ± 0.9 | 1.77 ± 1.1 | 1.80 ± 1.1 | 0.27 |
| –3964A>G | 0.168 | 0.09† | 1.70 ± 1.0 | 1.70 ± 1.1 | 1.39 ± 0.5 | 0.75 |
| 349A>G | 0.197 | –0.46* | 1.73 ± 1.1 | 1.64 ± 0.9 | 1.67 ± 0.8 | 0.86 |
| 712A>G | 0.500‡ | 0.92* | 1.49 ± 0.8 | 1.63 ± 0.9 | 2.02 ± 1.2 | 0.001 |
| 2019del/insA | 0.500§ | 0.92* | 1.50 ± 0.8 | 1.64 ± 0.9 | 2.01 ± 1.2 | 0.003 |

Data are means ± SD unless noted otherwise. q, minor allele frequency; *R*, standardized coefficient of linkage diseqeuilibrium with the 45-276 TG haplotype system. *$P < 0.0001$; †$P < 0.025$; ‡q = allele G; §q = allele ins.

through decreased adiponectin expression, which may in turn cause increased body weight and insulin resistance. On the other hand, neither polymorphism affects known regulatory regions, one being a synonymous substitution and the other being located in an intron. Furthermore, in other populations, the association with obesity or type 2 diabetes at position 45 is with allele G (20,21), whereas in our study the association with insulin resistance involves allele T. Thus, these variants are probably markers of one or more haplotypes containing a causal polymorphism affecting plasma adiponectin levels. Differences among populations in the linkage disequilibrium structure at this locus may result in association of the disease haplotype with different SNP alleles in different populations. Calpain 10 is an example of such a situation (34,35).

As far as the identity of the causal variant is concerned, it is interesting to note that in the two populations that we studied, the TG haplotype is in almost complete linkage disequilibrium with an A insertion in the 3′ UTR (SNP 2019). This region plays a pivotal role in the control of gene expression by binding proteins that regulate mRNA processing, translation, or degradation (36). Alterations of protein-binding elements in the 3′ UTR have been implicated in the etiology of several disorders including myotonic dystrophy, α-thalassemia, neuroblastoma, and insulin resistance itself (36–38). Whereas the 2019 insertion is not placed in known *cis*-acting domains such as the adenylate/uridylate-rich elements, it may disrupt other regulatory elements that are postulated to exist in this region (39). Further studies examining the relation between mRNA stability and 2019 genotype will tell whether this variant is indeed responsible for decreased adiponectin levels and insulin resistance or is instead an innocent bystander.

Finally, the possible shortcomings must be considered of our evidence that variation in the adiponectin gene is associated with obesity and insulin resistance. The chief question is the possibility of a false-positive finding arising from the multiple comparisons made in the study. For example, 11 measures of insulin resistance were compared across genotypes. However, the 11 measures were not interpreted as independent comparisons but as alternative manifestations of a single factor. Reflecting this, an alternative analysis is to examine all of the associations simultaneously in a multiple logistic analysis between genotypes. Using this approach, the *P* value was still highly significant ($P = 0.01$). The other point where multiple comparisons are an issue is the haplotype analysis, where there were three choices for the risk haplotype. If the *P* values for the associations with the TG haplotype are multiplied by 3 (Bonferroni inequality), many of the variables remain significant ($P = 0.012$ for waist circumference, $P = 0.03$ for systolic and $P = 0.009$ for diastolic blood pressure, $P = 0.015$ for insulin levels, $P = 0.009$ for $HOMA_{IR}$, and $P = 0.03$ for the total to HDL cholesterol ratio). Thus, it seems highly unlikely that these findings of association are type 1 errors.


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