# Genetic Variation in the Gene Encoding Adiponectin Is Associated With an Increased Risk of Type 2 Diabetes in the Japanese Population

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**An adipocyte-derived peptide, adiponectin (also known as GBP28), is decreased in subjects with type 2 diabetes. Recent genome-wide scans have mapped a diabetes susceptibility locus to chromosome *3q27*, where the adiponectin gene (*APM1*) is located. Herein, we present evidence of an association between frequent single nucleotide polymorphisms at positions 45 and 276 in the adiponectin gene and type 2 diabetes ($P = 0.003$ and $P = 0.002$, respectively). Subjects with the G/G genotype at position 45 or the G/G genotype at position 276 had a significantly increased risk of type 2 diabetes (odds ratio 1.70 [95% CI 1.09–2.65] and 2.16 [1.22–3.95], respectively) compared with those having the T/T genotype at positions 45 and 276, respectively. In addition, the subjects with the G/G genotype at position 276 had a higher insulin resistance index than those with the T/T genotype (1.61 ± 0.05 vs. 1.19 ± 0.12, $P = 0.001$). The G allele at position 276 was linearly associated with lower plasma adiponectin levels (G/G: 10.4 ± 0.85 μg/ml, G/T: 13.7 ± 0.87 μg/ml, T/T: 16.6 ± 2.24 μg/ml, $P = 0.01$) in subjects with higher BMIs. Based on these findings together with the observation that adiponectin improves insulin sensitivity in animal models, we conclude that the adiponectin gene may be a susceptibility gene for type 2 diabetes. *Diabetes* 51:536–540, 2002**

Insulin resistance is a fundamental element in the etiology of type 2 diabetes and is quite often associated with obesity (1). Adipose tissue, in addition to its function as the major energy reservoir, produces and secretes proteins such as adipsin (2), tumor necrosis factor-α (3), and leptin (4). The *APM1* (also known as *GBP28*) encodes adiponectin, which is abundantly expressed in adipose tissue (5–8). Plasma adiponectin levels have been reported to be reduced in patients with obesity (9), type 2 diabetes (10), and coronary artery diseases (10), all of which are closely related to insulin resistance. Moreover, it was recently demonstrated that treatment of a diabetic animal with adiponectin markedly improved insulin sensitivity (11) by reducing triglyceride accumulation in skeletal muscle, presumably because of promotion of β-oxidation of lipids. These observations suggest that adiponectin plays an important role in insulin sensitivity. A diabetes susceptibility locus has recently been mapped to chromosome *3q27* (12,13), where the adiponectin gene is located. We therefore hypothesized that genetic variation in the adiponectin gene may predispose humans to insulin resistance and type 2 diabetes.

## RESEARCH DESIGN AND METHODS

**Subject characteristics.** Nondiabetic subjects were recruited from an unselected population undergoing routine health checkups at the Hiroshima Atomic Bomb Casualty Council Health Management Center (Hiroshima, Japan). Inclusion criteria were as follows: *1*) >60 years of age, *2*) $HbA_{1c}$ values <5.8%, and *3*) no family history of type 2 diabetes in first- and second-degree relatives. The family histories of type 2 diabetes were assessed based on answers to a questionnaire that collected information on first- and second-degree relatives and their history of type 2 diabetes. The above criteria were chosen to enhance statistical power to detect associations. Whole blood samples were drawn in the fasting state, and fasting glucose, insulin, and $HbA_{1c}$ levels were measured. The remaining samples were sent to the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, where genomic DNA was extracted and plasma adiponectin levels were measured. The diabetic subjects were randomly recruited from patients attending the outpatient clinic of the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo. Diabetes was diagnosed in accordance with World Health Organization criteria (14). GAD antibody–positive samples were excluded from the present study. All subjects enrolled in this study were of full Japanese ethnicity, and there were no second-degree or closer relationships among the subjects. A total of 480 nondiabetic and 384 diabetic subjects were enrolled in the present study, and the clinical characteristics are shown in Table 1. The study was performed after obtaining

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

EH, Estimation Haplotype; HOMA, homeostasis model assessment; HOMA-IR, HOMA of insulin resistance; OR, odds ratio; SNP, single nucleotide polymorphism.

TABLE 1
Clinical characteristics of the subjects enrolled in the present study

| | Nondiabetic subjects | Type 2 diabetic subjects |
|---|---|---|
| *n* | 480 | 384 |
| Age (years) | 69.4 ± 8.79 | 58.7 ± 11.3 |
| Sex (M/F) | 285/195 | 267/117 |
| BMI (kg/m$^2$) | 23.9 ± 3.97 | 23.4 ± 3.98 |
| $HbA_{1c}$(%) | 5.19 ± 0.26 | 8.22 ± 1.93 |
| Fasting glucose (pmol/ml) | 5.16 ± 0.61 | 8.70 ± 2.91 |
| HOMA* | 1.57 ± 0.89 | 2.70 ± 2.26 |

Data are means ± SD. *The insulin resistance index was calculated by HOMA.

written informed consent from all of the nondiabetic and diabetic subjects, and it was approved by the Ethics Committee of the University of Tokyo.

**Biological measurements.** Insulin resistance was assessed by homeostasis model assessment (HOMA) (insulin resistance index = [fasting glucose (mmol/l) × fasting insulin (μU/ml)]/22.5 (15). Plasma adiponectin levels were determined as described below. A 100-μl volume of 441-fold diluted serum and standard samples was applied to a 96-well microtiter plate coated with mouse anti-adiponectin monoclonal antibody (6). The plate was incubated for an hour and then washed and incubated with the same mouse monoclonal antibody labeled with horseradish peroxidase. Next, the plate was washed and incubated with tetramethylbenzidine reagent. To stop the reaction, 0.36 N sulfuric acid solution was added, and the absorbance at 450 nm was measured.

**Screening for mutations or polymorphisms in the adiponectin gene.** A 16-kb region, including the adiponectin gene, was screened for mutations or polymorphisms by PCR direct sequencing. We performed mutation/polymorphism screening on 15 French white subjects and 15 Japanese (total 30) type 2 diabetic patients. Because power was sufficient to detect (with 80% probability) polymorphisms/mutations in which allele frequencies were as low as 0.026, the method used in the present study should be sufficiently powerful to detect relatively frequent polymorphisms that might be related to the pathogenesis of common type 2 diabetes. Genomic DNA was extracted with a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA). Sequencing was carried out using a Big Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA), and polymorphisms were designated according to their location relative to the A of the ATG of the initiator methionine (Met) of adiponectin (5).

**Genotyping of polymorphisms in the adiponectin gene.** The polymorphisms were typed by PCR direct sequencing or with a SNaPshot ddNTP Primer Extension Kit (Applied Biosystems) in 480 nondiabetic and 384 diabetic subjects. The chemistry of the primer extension method is based on the dideoxy single nucleotide extension of an unlabeled oligo-nucleotide primer. The primer is designed so that it binds to a complementary template one base adjacent to the polymorphism. DNA polymerase adds a single fluorescence-labeled ddNTP to its 3′ end. The conditions were as follows: 25 cycles of denaturation at 96°C for 5 s, annealing at 50°C for 5 s, and extension at 60°C for 30 s. The completed reaction mixture was then resolved on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sequences of the primers used for PCR direct sequencing are shown in an online Appendix at http://diabetes.diabetesjournals.org/.

**Statistical analysis.** The proportions of genotypes or alleles were compared by $\chi^2$ analysis. Odds ratios (ORs) and 95% CI, with adjustment for age and sex and for age, sex, and BMI, were calculated by logistic regression analysis. Differences in continuous parameter, such as BMI, among genotypes were evaluated by one-way ANOVA. We evaluated the influence of genotypes –3964, 45, and 276 on insulin resistance by using a general linear model to adjust for possible confounding factors such as age, sex, and BMI. The results obtained do not necessarily preclude an effect of age on insulin resistance but suggest the absence of a correlation between age and insulin resistance over the age of 60 years in our nondiabetic subjects. Frequencies of single nucleotide polymorphism (SNP)45-SNP276 haplotypes were estimated by using the Estimation Haplotype (EH) frequencies software program (ftp://linkage.rockefeller.edu/software/eh). Because the insulin resistance indexes and plasma adiponectin levels were not normally distributed in our data, these two values were log-transformed before analysis, and the values presented were back-transformed. First, to estimate the frequencies of each haplotype in the type 2 diabetic and nondiabetic groups separately, we used EH software. Next, we calculated the numbers for each haplotype by multiplying the estimated frequencies by twice the number of nondiabetic or type 2 diabetic subjects. Lastly, we generated data sets that reflected these numbers and subjected them to the logistic regression procedure. All of the statistical analyses except the estimations of haplotype frequency were performed using SAS for Windows, version 6.12 (SAS Institute, Cary, NC). A $P$ value of $<0.05$ was considered significant in the present study.

**GenBank accession numbers.** The GenBank accession numbers were as follows: human APM1 mRNA, NM004797; and human GBP28 gene, AB012163S1, 2, and 3.

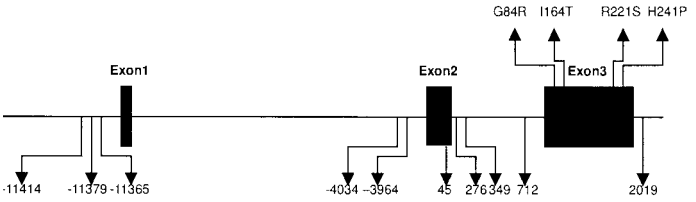


**FIG. 1. Schema of genomic structure and polymorphic variants of the adiponectin gene. The exon-intron organization of the gene is indicated by closed boxes. Arrows point to the positions of the polymorphic variants identified. Numbers indicate locations relative to the A of the ATG of the initiator Met of the adiponectin gene. Rare mutations with amino acid substitutions are also described.**

## RESULTS

**Detection of SNPs in the adiponectin gene.** We detected 10 relatively frequent polymorphisms in the adiponectin gene (Fig. 1) in two ethnic groups: French and Japanese. The allelic distributions were in the Hardy-Weinberg equilibrium for all of these polymorphisms. In addition to these polymorphisms, we found four rare mutations that resulted in substitutions of amino acids in exon 3 (G84R, I164T, R221S, and H241P) (Fig. 1). These missense mutations were quite rare (allele frequency <1%) and were detected in both type 2 diabetic and nondiabetic subjects.

**Associations between SNPs in the adiponectin gene and type 2 diabetes.** For SNPs at positions 45 and 276, statistically significant differences in the distribution of both genotypes ($P = 0.01$ and $P = 0.007$, respectively) and alleles ($P = 0.003$ and $P = 0.002$, respectively) between type 2 diabetic and nondiabetic subjects were detected (Table 2). The G allele was significantly more frequent than the T allele at positions 45 and 276 ($P = 0.003$ and $P = 0.002$, respectively) in type 2 diabetic subjects than in nondiabetic subjects. The subjects with the G/G genotype at position 276 were at increased risk for type 2 diabetes (OR 2.16, 95% CI 1.22–3.95) compared with those having the T/T genotype. The subjects with the G/T or G/G genotype at position 45 were also at significantly increased risk for type 2 diabetes (OR 1.41, 95% CI 1.06–1.88; OR 1.70, 95% CI 1.09–2.65, respectively) compared with those having the T/T genotype. No other SNPs were associated with increased risk of type 2 diabetes (Table A1 at http://diabetes.diabetesjournals.org). We estimated the frequency of the SNP45-SNP276 haplotypes among the type 2 diabetic and nondiabetic subjects (Table A2 at http://diabetes.diabetesjournals.org) and found significant differences in the distributions of the haplotypes between the 45T-276G and the 45T-276T haplotypes ($P = 0.029$). This finding is consistent with the data showing that the G allele of SNP276 is significantly associated with type 2 diabetes. However, the OR for diabetes associated with the G allele at SNP276 is roughly equal to that associated with the G

TABLE 2
Comparison of genotypic and allelic distribution of SNPs between type 2 diabetic and nondiabetic subjects

| Polymorphisms | Genotypes [*n* (%)] | | | *P* | Alleles [*n* (%)] | | *P* |
|---|---|---|---|---|---|---|---|
| −11414 | A/A | A/G | G/G | 0.37 | A | G | 0.59 |
| T2DM | 219 (57.0) | 143 (37.2) | 22 (5.7) | | 581 (75.7) | 187 (24.3) | |
| NDM | 275 (57.3) | 187 (40.0) | 18 (3.7) | | 737 (76.8) | 223 (23.2) | |
| −11379 | G/G | G/A | A/A | 0.50 | G | A | 0.51 |
| T2DM | 367 (95.6) | 17 (4.4) | 0 (0) | | 751 (97.8) | 17 (2.2) | |
| NDM | 454 (94.5) | 26 (5.5) | 0 (0) | | 934 (97.3) | 26 (2.7) | |
| −11365 | C/C | C/G | G/G | 0.25 | C | G | 0.10 |
| T2DM | 233 (60.7) | 127 (33.1) | 24 (6.2) | | 592 (77.3) | 174 (22.7) | |
| NDM | 265 (55.2) | 178 (37.1) | 37 (7.7) | | 706 (73.8) | 250 (26.1) | |
| −4034 | A/A | A/C | C/C | 0.19 | A | C | 0.21 |
| T2DM | 327 (85.2) | 56 (14.6) | 1 (0.2) | | 710 (92.4) | 58 (7.6) | |
| NDM | 425 (88.5) | 52 (10.8) | 3 (0.7) | | 902 (94.0) | 58 (6.0) | |
| −3964 | A/A | A/G | G/G | 0.03 | A | G | 0.49 |
| T2DM | 334 (87.0) | 50 (13.0) | 0 (0) | | 718 (93.5) | 50 (6.5) | |
| NDM | 429 (86.4) | 47 (9.8) | 4 (0.8) | | 905 (94.3) | 55 (5.7) | |
| 45 | T/T | T/G | G/G | 0.01 | T | G | 0.003 |
| T2DM | 164 (42.7) | 169 (44.0) | 51 (13.3) | | 497 (64.7) | 271 (35.3) | |
| NDM | 251 (52.3) | 183 (38.1) | 46 (9.6) | | 685 (71.4) | 275 (28.6) | |
| 276 | G/G | G/T | T/T | 0.007 | G | T | 0.002 |
| T2DM | 224 (58.3) | 142 (37.0) | 18 (4.7) | | 590 (76.8) | 178 (23.2) | |
| NDM | 236 (49.2) | 203 (42.3) | 41 (8.5) | | 675 (70.3) | 285 (29.7) | |
| 349 | A/A | A/G | G/G | 0.39 | A | G | 0.25 |
| T2DM | 172 (44.8) | 169 (44.0) | 43 (11.2) | | 513 (66.8) | 255 (33.2) | |
| NDM | 237 (49.4) | 192 (40.0) | 51 (10.6) | | 666 (69.4) | 294 (30.6) | |
| 712 | A/A | A/G | G/G | 0.42 | A | G | 0.98 |
| T2DM | 126 (32.8) | 206 (53.7) | 52 (13.5) | | 458 (59.6) | 310 (40.4) | |
| NDM | 168 (35.0) | 237 (49.4) | 75 (15.6) | | 573 (59.7) | 387 (40.3) | |
| 2019 | D/D | D/I | I/I | 0.69 | D | I | 0.41 |
| T2DM | 138 (35.9) | 186 (48.4) | 60 (15.7) | | 462 (60.2) | 306 (39.8) | |
| NDM | 182 (37.9) | 232 (48.3) | 66 (13.8) | | 596 (62.1) | 364 (37.9) | |

Numbers indicate the location of the SNP relative to the A of the ATG of the initiator Met of the adiponectin gene. D, deletion of nucleotide A at position 2019; I, insertion of nucleotide A at position 2019; NDM, nondiabetic subjects; T2DM, type 2 diabetic subjects. df = 2 and 1 for the distribution of genotypes and alleles, respectively.

allele at SNP45 (Table A2), suggesting that a combination of SNP45 and SNP276 was responsible for the increased risk of type 2 diabetes or that both were in linkage disequilibrium with an unidentified and truly relevant SNP. Because SNP45 is a silent polymorphism, the former is less likely, and a more extensive search for SNPs in the intronic and promoter region, which we already searched, is needed to investigate the latter possibility.

**Effect of SNPs on insulin resistance and BMI.** We next tested whether these SNPs had any effect on insulin resistance. Because treatment for type 2 diabetes may affect plasma insulin levels, we evaluated the effect of polymorphisms on clinical variables in nondiabetic subjects. After adjusting for possible confounding effects of age, sex, and BMI, we found significant associations between SNPs at positions −4034, −3964, and 276 and the insulin resistance index (Table 3). No other SNPs were associated with HOMA of insulin resistance (HOMA-IR) (Table 3). There were significant individual differences in HOMA among the three SNP276 genotypes (G/G: 1.61 ± 0.05, G/T: 1.45 ± 0.05, and T/T: 1.19 ± 0.12, $P = 0.002$, $r^2 = 0.28$) (Fig. 2*A*). There were no differences in BMI according to SNP genotypes (data not shown).

**Relationship between SNP276 and plasma adiponectin levels.** We hypothesized that SNP276 might affect the expression and plasma levels of adiponectin. Overall, the plasma adiponectin levels tended to be lower in the subjects with the G allele (G/G: 14.2 ± 0.56 μg/ml, G/T: 15.5 ± 0.61 μg/ml, T/T: 17.3 ± 1.35 μg/ml, $P = 0.08$). Because an interaction between BMI and genotype at position 276 was observed ($P = 0.036$), we performed a subset analysis by subdividing the subjects into groups according to the tertiles of their BMI. As shown in Fig. 2*B*, there were no differences in plasma adiponectin levels among the SNP276 genotypes (G/G: 19.1 ± 0.97 μg/ml, G/T: 18.2 ± 1.15 μg/ml, T/T: 18.8 ± 2.14 μg/ml, $P = 0.84$) in the lean subgroup. In the intermediate subgroup, there was a tendency toward lower plasma adiponectin levels among the SNP276 genotypes (G/G: 12.8 ± 0.95 μg/ml, G/T: 15.1 ± 1.00 μg/ml, T/T: 16.0 ± 2.32 μg/ml, $P = 0.09$). In the obese subgroup, there were significant differences in adiponectin levels among the SNP276 genotypes (G/G: 10.4 ± 0.85 μg/ml, G/T: 13.7 ± 0.87 μg/ml, T/T: 16.6 ± 2.24 μg/ml, $P = 0.01$) (Fig. 2*B*). When subset analysis was performed by subdividing subjects by either median BMI values or quartiles of BMI values, those with the G/G genotype were found to have significantly lower plasma adiponectin levels than the subjects with the T/T genotype among those in the upper half or the highest quartile of BMI values. No other SNPs were associated with plasma adiponectin levels (data not shown).

TABLE 3
Genotype of the polymorphisms and the insulin resistance index of nondiabetic subjects

| Polymorphism | Genotype | | | *P* |
|---|---|---|---|---|
| −11414 | A/A | A/G | G/G | |
| | 1.57 ± 0.05 | 1.43 ± 0.06 | 1.29 ± 0.19 | 0.073 |
| −11379 | G/G | G/A | A/A | |
| | 1.51 ± 0.03 | 1.38 ± 0.15 | — | 0.400 |
| −11365 | C/C | C/G | G/G | |
| | 1.52 ± 0.04 | 1.50 ± 0.06 | 1.43 ± 0.12 | 0.802 |
| −4034 | A/A | A/C | C/C | |
| | 1.51 ± 0.04 | 1.43 ± 0.11 | 2.65 ± 0.44 | 0.029 |
| −3964 | A/A | A/G | G/G | |
| | 1.50 ± 0.04 | 1.52 ± 0.11 | 2.48 ± 0.38 | 0.038 |
| 45 | T/T | T/G | G/G | |
| | 1.52 ± 0.05 | 1.48 ± 0.06 | 1.56 ± 0.11 | 0.787 |
| 276 | G/G | G/T | T/T | |
| | 1.61 ± 0.05 | 1.45 ± 0.05 | 1.19 ± 0.11 | 0.002 |
| 349 | A/A | A/G | G/G | |
| | 1.49 ± 0.05 | 1.48 ± 0.06 | 1.66 ± 0.11 | 0.338 |
| 712 | A/A | A/G | G/G | |
| | 1.47 ± 0.06 | 1.49 ± 0.05 | 1.63 ± 0.09 | 0.312 |
| 2019 | D/D | D/I | I/I | |
| | 1.52 ± 0.06 | 1.56 ± 0.05 | 1.31 ± 0.09 | 0.072 |

The insulin resistance index was calculated with HOMA. D, deletion of nucleotide A at position 2019; I, insertion of nucleotide A at position 2019. df = 5.

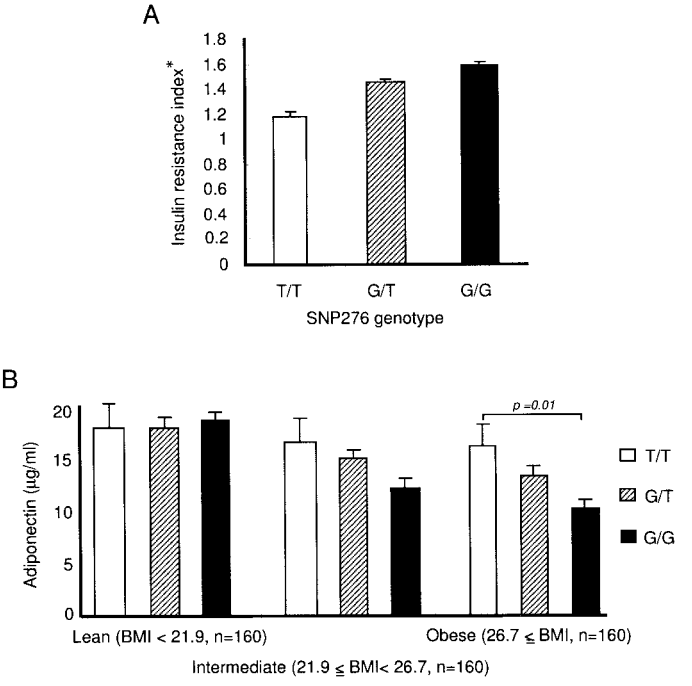


**FIG. 2. Effect of the position 276 genotype on insulin resistance and plasma adiponectin levels. Data are means ± SE. *A*: Effect of position 276 genotype on insulin resistance. There were significant differences in HOMA-IR between individuals among the three genotypes of SNP276. The insulin resistance index was calculated by HOMA. *B*: Effect of position 276 genotype on plasma adiponectin levels. In the lean subgroup (BMI <21.9 = lower tertile, *n* = 160), there were no differences in plasma adiponectin levels among the SNP276 genotypes. In the intermediate subgroup (21.9 ≤ BMI < 26.7, *n* = 160), there was a tendency toward lower plasma adiponectin levels in subjects with the G/G genotype compared with subjects with the T/T genotype. In the obese subgroup (BMI ≥ 26.7 = upper tertile, *n* = 160), the G allele at position 276 was linearly associated with lower plasma adiponectin levels.**

## DISCUSSION

The present study provides evidence that adiponectin is a novel susceptibility gene for type 2 diabetes in the Japanese population. We screened the adiponectin gene extensively for SNPs and found SNP276 to be associated with type 2 diabetes. SNP276 was also associated with insulin resistance, indicating that this SNP is related to the pathogenesis of type 2 diabetes.

A major question concerns the mechanism by which SNP276 influences insulin sensitivity and the risk for type 2 diabetes. SNP276 is in an intron of the adiponectin gene. It is noteworthy that intronic SNPs in *CAPN10* (16) or *COL1A1* (17) affect the expression levels of these genes. Recently, it was demonstrated that replenishment of adiponectin ameliorates insulin resistance in animal models of type 2 diabetes in which the animals were maintained on a high-fat diet (13). Administration of physiological doses of adiponectin reduced triglyceride accumulation in skeletal muscle and liver by enhancing fatty acid combustion and energy dissipation in skeletal muscle (13,18). Thus, decreased or deficient adiponectin presumably causes insulin resistance, thereby leading to the development of type 2 diabetes. In fact, subjects with the G/G genotype of SNP276, a putative at-risk genotype, had lower plasma adiponectin levels and higher insulin resistance than those with the T/T genotype, a putative not-at-risk genotype (Fig. 2*B*). Taken together with the fact that SNP276 is in an intron of the adiponectin gene, these results suggest that SNP276 may affect insulin resistance, which may be mediated through alteration of expression levels of adiponectin and in turn decreasing plasma concentrations. Because direct evidence that SNP276 affects the expression levels of adiponectin could not be obtained in this study, further study will be needed, such as a functional study or direct measurement of the expression levels of adiponectin in white adipose tissue derived from subjects with the SNP276 genotypes studied herein. However, we cannot explain at present why a relationship between SNP276 and insulin resistance was observed among the subjects as a whole regardless of their degree of obesity, whereas the association between adiponectin levels and SNP276 was seen only in relatively obese individuals.

Our present findings are consistent with recent reports of susceptibility loci for type 2 diabetes (11) and the quantitative trait loci for insulin resistance (12) being mapped to chromosome *3q27*, where the adiponectin gene is located. Moreover, the results of affected sib-pair analysis of a Japanese population suggested linkage with type 2 diabetes in the same chromosomal region (Y.M. S.O., C.D., K.Y., Céline Populaire, Cécile Lecoeur, Vincent Vatin, Emmanuelle Durand, K.H., T.O., K.T., P.B., T.K., P.F., unpublished data). The estimated relative risk of developing disease for siblings (λ-s) derived from the closest marker to the adiponectin gene was calculated to be 1.66. Based on this result, adiponectin is one of the positional candidate genes in this region that might explain this linkage. Further study is needed to clarify the relationship between this linkage and the observed association with the adiponectin gene.

The potential bias due to population stratification because of selection of case and control subjects from

geographically different areas might be raised as a possible limitation of this study. However, it should be noted that the Japanese population is relatively homogenous, which allowed the use of this approach in this study. Nevertheless, the distributions of SNPs at the whole-genome level should be investigated in different geographical areas before excluding a bias due to population stratification, which may have affected the results. A family-based association study, such as the transmission disequilibrium test, should also be performed.

It may be argued that Bonferroni's correction should be applied to the significance thresholds to avoid type 1 error due to multiple comparisons. If Bonferroni's correction were strictly adopted, the thresholds of significance in each comparison would be 0.00125 (a total of 40 comparisons in this study). After the correction, the alleles of SNP45 and SNP276 would be only marginally associated with type 2 diabetes. Thus, although there is a chance of type 1 error due to multiple comparisons, based on the fact that the comparisons we made were not totally independent of each other because of linkage disequilibrium (Table A3 at http://diabetes.diabetesjournals.org), we believe that this correction may be too conservative. Moreover, this study was designed and carried out based on the fact that the chromosomal region *3q27*, where the adiponectin gene is located, is linked to type 2 diabetes (11, Y.M., S.O., C.D., K.Y., Céline Populaire, Cécile Lecoeur, Vincent Vatin, Emmanuelle Durand, K.H., T.O., K.T., P.B., T.K., P.F., unpublished data) and the metabolic syndrome (12). Finally, adiponectin has been functionally implicated in the regulation of β-oxidation of lipids in the skeletal muscle (13,18) and insulin sensitivity.

In summary, SNPs in the adiponectin gene are associated with insulin resistance and type 2 diabetes, which might be mediated through alterations in the expression level and plasma concentration of adiponectin. We conclude that a variation in the adiponectin gene might play an important role in the pathogenesis of type 2 diabetes.

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## REFERENCES

1. Reaven GM: Pathophysiology of insulin resistance in human disease. *Physiol Rev* 75:473–486, 1995
2. White RT, Damm D, Hancock N, Rosen BS, Lowell BB, Usher P, Flier JS, Spiegelman BM: Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. *J Biol Chem* 267:9210–9213, 1992
3. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM: Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 95:2409–2415, 1995
4. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
5. Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: cDNA cloning and expression of a novel adipose specific collagen-like factor apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 221:286–289, 1996
6. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M: Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. *J Biochem (Tokyo)* 120:803–812, 1996
7. Hu E, Liang P, Spiegelman BM: AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 271:10697–10703, 1996
8. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* 270:26746–26749, 1995
9. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific: protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 257:79–83, 1999
10. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y: Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 20:1595–1599, 2000
11. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome *3q27-qter* and independent replication of a type 2-diabetes locus on chromosome 1q21–24. *Am J Hum Genet* 67:1470–1480, 2000
12. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, Comuzzie AG: Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97:14478–14483, 2000
13. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Abe M, Tanaka J, Ohsumi J, Ide T, Murakami K, Tsuboyama-KN, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: Replenishment of the fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 7:941–946, 2001
14. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183–1197, 1997
15. Matthews DR, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
16. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Lindner TH, Mashima H, Schwarz PE, del Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
17. Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FE, Grant SF, Hofman A, van Leeuwen JP, Pols HA, Ralston SH: Relation of alleles of the collagen type Iα1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women. *N Engl J Med* 338:1016–1021, 1998
18. Fruebis J, Tsao TS, Javorschi S, Reed DE, Erickson MR, Yen FT, Bihain BE, Lodish HF: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A* 98:2005–2010, 2001