The Common Polymorphisms (Single Nucleotide Polymorphism [SNP] +45 and SNP +276) of the Adiponectin Gene Predict the Conversion From Impaired Glucose Tolerance to Type 2 Diabetes

The STOP-NIDDM Trial

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Adiponectin is an adipose tissue–specific protein with insulin-sensitizing and antiatherogenic properties. Therefore, the adiponectin gene is a promising candidate gene for type 2 diabetes. We investigated the single nucleotide polymorphisms (SNPs) +45T/G and +276G/T of the adiponectin gene as predictors for the conversion from impaired glucose tolerance to type 2 diabetes in the STOP-NIDDM trial, which aimed to investigate the effect of acarbose compared with placebo on the prevention of type 2 diabetes. Compared with the TT genotype, the G-allele of SNP +45 was associated with a 1.8-fold risk for type 2 diabetes (95% CI 1.12–3.00, $P = 0.015$) in the placebo group. Subjects treated with placebo and simultaneously having the G-allele of SNP +45 and the T-allele of SNP +276 (the risk genotype combination) had a 4.5-fold (1.78–11.3, $P = 0.001$) higher risk of developing type 2 diabetes compared with subjects carrying neither of these alleles. Women carrying the risk genotype combination had an especially high risk of conversion to diabetes (odds ratio 22.2, 95% CI 2.7–183.3, $P = 0.004$). In conclusion, the G-allele of SNP +45 is a predictor for the conversion to type 2 diabetes. Furthermore, the combined effect of SNP +45 and SNP +276 on the development of type 2 diabetes was stronger than that of each SNP alone. *Diabetes* 54:893–899, 2005

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IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

Adiponectin is a specific protein having 247 amino acids and sharing structural homology with collagen VIII, X, and complement C1q (1,2). It is encoded by the most abundant gene transcript one (apM1) in adipose tissue and is expressed exclusively in differentiated adipocytes (1). Paradoxically, the levels of adiponectin are decreased in obesity, although adipose tissue mass is increased (3). Adiponectin levels are negatively correlated with serum insulin levels (4,5), insulin resistance (6), type 2 diabetes (4), and cardiovascular diseases (7), whereas weight loss (4) and the administration of insulin-sensitizing drugs such as thiazolidinediones (5) increase serum adiponectin levels. Studies on Rhesus monkeys suggest that hypoadiponectinemia is the primary defect leading to the development of obesity and related phenotypes (8). Administration of recombinant globular or full-length adiponectin to laboratory animals leads to weight loss and improvement of insulin sensitivity and glucose tolerance via stimulation of glucose utilization and free fatty acid oxidation in skeletal muscle and a decrease of hepatic glucose output and gluconeogenesis (9–14). Adiponectin accumulates in injured vessel walls (15) and inhibits tumor necrosis factor-α–induced monocyte adhesion and expression of adhesion molecules in endothelial cells (16). These findings support the notion that adiponectin is protective against obesity, type 2 diabetes, and atherosclerosis.

The adiponectin gene is located on chromosome 3q27 (17), in a region that was recently mapped as a susceptibility locus for type 2 diabetes in a genome-wide scan (18). Several studies have been performed aiming to investigate the association of genetic variations in the adiponectin gene with serum adiponectin level, obesity, insulin resistance, and type 2 diabetes (17,19–28). The most common reported variants are the T/G polymorphism of SNP +45 in exon 2 and the G/T polymorphism of SNP +276 in intron 2, which have been found to be related to obesity in German (21), Swedish (22), and Taiwanese subjects (19), insulin resistance syndrome in Italian Caucasians (24), and type 2 diabetes in French Caucasians (25) and Japanese subjects (26). Recently Fumeron et al. (28) reported that these two SNPs and SNPs in the promoter region of the

adiponectin gene were associated with the risk of hyperglycemia in normoglycemic subjects of the prospective study cohort. However, there are no previous longitudinal studies on the association of SNP +45T/G and SNP +276G/T polymorphisms of the adiponectin gene with the risk of type 2 diabetes in subjects with a high prevalence of obesity and impaired glucose tolerance (IGT). Therefore, we investigated whether SNP +45 and SNP +276 polymorphisms of the adiponectin gene and their genotype combinations predict the conversion from IGT to type 2 diabetes in the STOP-NIDDM study (29).

RESEARCH DESIGN AND METHODS

The STOP-NIDDM study was an international, longitudinal, placebo-controlled, randomized, multicenter trial conducted in Canada, Germany, Austria, Norway, Denmark, Sweden, Finland, Israel, and Spain aiming to investigate the effect of acarbose (α-glucosidase inhibitor) on the conversion from IGT to type 2 diabetes (29). Detailed description of the study design and main results have been previously published (29,30). Briefly, 1,429 subjects were enrolled to the study from a high-risk population with IGT (World Health Organization 1985 criteria [31]: fasting plasma glucose <7.8 mmol/l and 2-h glucose concentration ≥7.8 and <11.1 mmol/l) and a fasting plasma glucose value between 5.6 and 7.8 mmol/l. All participants were from 40 to 70 years of age (mean age 54.4 ± 7.9 years) with BMI between 25 and 40 kg/m^2 (mean 30.9 ± 4.2 kg/m^2). Subjects with elevated serum creatinine, triglycerides, and liver enzymes levels were excluded, as well as subjects treated with drugs that could affect intestinal motility and absorption of nutrients.

All patients were randomly allocated to the treatment with placebo or 100 mg acarbose, taken three times daily. Participants were encouraged to exercise on a regular basis and follow weight-reducing or weight-maintaining diet.

DNA was available from 770 subjects (387 men and 383 women) aged 54.7 ± 7.9 years with a mean BMI of 30.8 ± 4.1 kg/m^2. There were no significant differences in clinical characteristics (age, sex, diastolic blood pressure, weight, BMI, and waist circumference) or laboratory parameters (fasting and 2-h glucose and insulin levels in an oral glucose tolerance test [OGTT]) in subjects whose DNA was available compared with subjects whose DNA was not available. Subjects with DNA available had higher systolic blood pressure and incidence of type 2 diabetes (40.2 vs. 30.8%, $P < 0.001$) than subjects without DNA available. Type 2 diabetes was diagnosed if the fasting plasma glucose level was ≥7.8 mmol/l or the plasma glucose concentration was ≥11.1 mmol/l at 2 h after a 75-g glucose load in the OGTT. Appropriate institutional review boards approved the protocol, and each subject gave a signed informed consent form.

Measurements. Physical examination and laboratory tests were performed at baseline and during the follow-up, as previously described (29,30). The OGTT was performed at baseline and annually. Mean follow up time was 3.3 years.

Homeostasis model assessment for insulin resistance was calculated with the formula: fasting plasma glucose (mmol/l) × fasting serum insulin (mU/l)/22.5 (32).

DNA analysis. The SNaPshot ddNTP Primer Extension Kit technique was used to genotype SNP +45T/G and SNP +276G/T of the adiponectin gene. Forward, 5′-GGCTCAGGATGCTGTTGCTGG-′3, and reverse, 5′-GCT TTG CTT TCT CCC TGT GTC T-′3, primers were used to amplify a 328-bp size DNA fragment. The following cycling conditions were used for the PCR: 94°C for 4 min; 35 cycles of 94°C, 57°C, and 72°C for 30 s each; and 72°C for 4 min. The PCR product was purified with 1 unit of shrimp alkaline phosphatase and 2 units of Exonuclease I (Exo I) incubated in 37°C for 60 min and in 75°C for 15 min.

Primers used to determine the genotypes were 5′-CTGCTATTAGCTCTGC CCGG-3′ for the SNP +45T/G polymorphism and 5′-ACCTCCTACACTGATA TAAACTAT-3′ for the SNP +276G/T polymorphism. The SNaPshot reaction was performed with mix containing 3.75 μl of TRIS-HCl, 1.25 μl of SNaPshot Multiplex Ready Reaction Mix (ABI Prism; Applied Biosystems), 0.15 μl of the primer for the SNP +45, 0.075 μl of the primer for the SNP +276, and 0.775 μl of dH_2O. The mixture was incubated for 10 s in 94°C and for 45 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. Reaction mixture was purified with 1 unit of shrimp alkaline phosphatase at 37°C for 60 min and at 75°C for 15 min. Before the loading onto the ABI Prism 3100 Genetic Analyser (Applied Biosystems), 9 μl of formamide and 0.25 μl of size standard were added to 0.5 μl of the reaction mixture, and samples were heated in 95°C for 5 min.

Statistical analysis. We used the SPSS programs for Windows for the statistical analyses (version 11.0; SPSS, Chicago, IL). The Kolmogorov-Smirnov test was used to test the normality of distribution, and not-normally-distributed variables (weight, insulin, triglycerides and cholesterol levels, and HOMA-IR) were logarithmically transformed. ANOVA was used to compare the effect of the three genotypes on continuous variables and Student's t test to compare the two groups. If log transformation did not normalize the distribution or it was inapplicable, nonparametric Kruskal-Wallis or Mann-Whitney tests were used. Categorical variables were compared using the χ^2 test. Linkage disequilibrium between two SNPs was calculated with the two-locus linkage disequilibrium calculator (available from http://web1.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBSt/software.shtml). Haplotype frequencies were estimated, and model-free analysis was performed using the EH+ software (available from. http://web1.iop.kcl.ac.uk/IoP/Departments/PsychMed/GEpiBSt/software.shtml). Logistic regression analysis was applied to evaluate whether the SNP +45T/G and SNP +276G/T polymorphisms and their genotype combinations predict the conversion from IGT to type 2 diabetes. Data are presented as the mean ± SD or percentage, unless otherwise indicated.

RESULTS

We genotyped 770 DNA samples available for the T/G polymorphism of SNP +45 and the G/T polymorphism of SNP +276 of the adiponectin gene. The TT genotype of SNP +45 was found in 622 (80.6%) subjects, the GT genotype in 140 (18.4%) subjects, and the GG genotype in 8 (1.0%) subjects. The GG genotype of SNP +276 was found in 380 (49.2%) subjects, the GT genotype in 321 (41.8%) subjects, and the TT genotype in 69 (9.0%) subjects. The genotype frequencies of both SNPs did not differ between the placebo and acarbose groups, and their distribution was in Hardy-Weinberg equilibrium. Because only eight subjects were homozygous for the rare G-allele of SNP +45, we combined them with the subjects having the GT genotype in all statistical analyses.

There were no significant differences in baseline clinical characteristics such as age, BMI, weight, or laboratory values such as fasting and 2-h serum glucose and insulin levels in the OGTT, HOMA-IR, and triglycerides levels with respect to either the SNP +45T/G polymorphism or the SNP +276G/T polymorphism (data not shown). However, at baseline, subjects carrying the T-allele of SNP +276 had higher levels of total cholesterol (5.8 ± 1.0 vs. 5.6 ± 1.0 mmol/l, $P = 0.030$, adjusted for sex and BMI) and LDL cholesterol (3.68 ± 0.9 vs. 3.53 ± 0.8 mmol/l, $P = 0.028$, adjusted for sex and BMI) compared with subjects with the GG genotype.

Altogether 310 subjects developed type 2 diabetes during the follow-up (190 subjects in the placebo group and 120 in the acarbose group). Because of a significant effect of acarbose on the conversion to type 2 diabetes (30) and a significant genotype–treatment group interaction ($P = 0.001$), all statistical analyses were carried out separately for the placebo and acarbose groups. Similarly, as there was a trend for a genotype-sex interaction ($P = 0.061$), we analyzed men and women separately.

Compared with subjects with the G-allele of SNP +45, subjects with the TT genotype lost more weight in both treatment groups (Fig. 1*A*). The G-allele of the SNP +45 gene was associated with a higher rate of conversion from IGT to type 2 diabetes in the placebo group, as 42.9% of subjects with the TT genotype and 58.0% of subjects with the G-allele developed type 2 diabetes ($P = 0.015$, Fig. 1*B*). Weight increased in men carrying the G-allele of SNP +45 in the placebo group (Fig. 1*C*) and in women in the acarbose group (Fig. 1*E*). Correspondingly, conversion to type 2 diabetes was significantly higher in women carrying the G-allele (41.4 vs. 64.1% for subjects with the TT

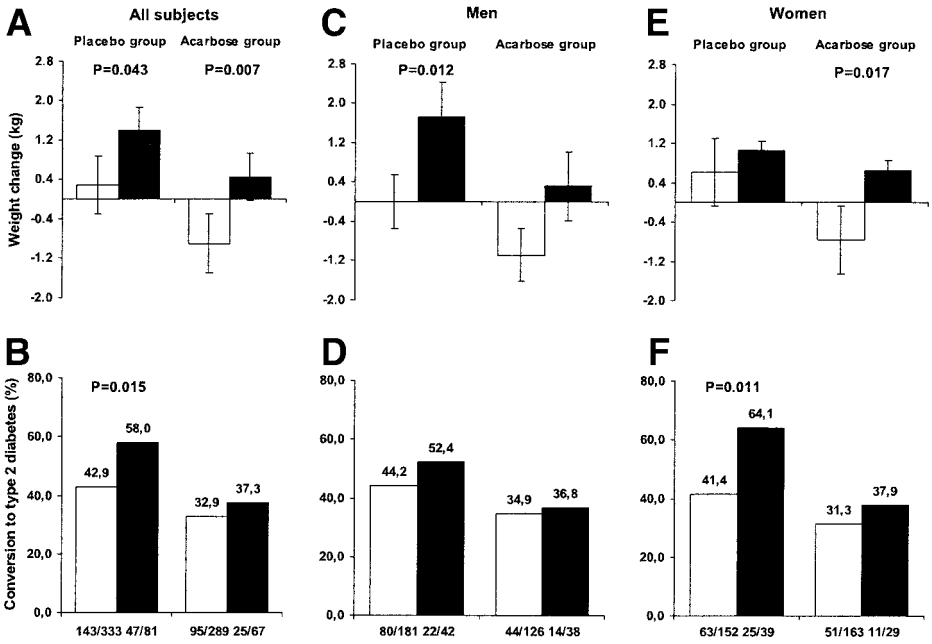

FIG. 1. Weight change in subjects treated with placebo or acarbose (*A*) (men [*C*] and women [*E*]), and the conversion to type 2 diabetes (% and number of coverters/all subjects at risk) in subjects treated with placebo or acarbose (*B*) (men [*D*] and women [*F*]) according to SNP +45T/G of the adiponectin gene. □, TT genotype; ■, G-allele.

genotype and the G-allele, respectively, $P = 0.011$) (Fig. 1*F*). We did not find any significant differences in the conversion to type 2 diabetes in the acarbose group or in men in the placebo group. Subjects with the TT genotype of SNP +45 had a larger decrease in their 2-h glucose level in the OGTT compared with subjects with the G-allele, but the difference was statistically significant only in the placebo group (2-h glucose change for the TT genotype 0.3 ± 2.7 and for the G-allele 0.9 ± 2.7 mmol/l, $P = 0.044$) (Table 1).

The G/T polymorphism of SNP +276 of the adiponectin gene was associated with the conversion from IGT to type 2 diabetes in subjects treated with placebo (data not shown), as 31.9% (58 of 181) subjects with the GG genotype, 31.5% (46 of 146) subjects with the GT genotype, and 57.1% (16 of 28) subjects with the TT genotype developed type 2 diabetes ($P = 0.025$). Even higher conversion rate to type 2 diabetes was observed among men treated with acarbose and who were homozygous for the T-allele (66.7% (8 of 12) compared with 35.4% (34 of 96) and 28.6% (16 of 56) in subjects with the GG and GT genotypes, respectively, $P = 0.044$). We did not find any significant differences in weight change either within the acarbose and placebo groups or in men and women according to the G/T polymorphism of SNP +276.

Neither SNP +45 genotypes nor SNP +276 genotypes influenced the response to the treatment with placebo or acarbose (data not shown). However, the conversion to type 2 diabetes was significantly lower in the acarbose group compared with the placebo group according to either SNP +45 or SNP +276 genotypes (conversion to type 2 diabetes for subjects with the TT genotype of SNP +45 was 42.9 vs. 32.9% in the placebo and acarbose groups, respectively, $P = 0.011$; and for subjects with the G-allele of SNP +45 it was 58.0 vs. 37.3% in the placebo and acarbose groups, respectively, $P = 0.012$).

TABLE 1
Changes in 2-h glucose in an OGTT according to the genotypes of SNP +45T/G and SNP +276G/T and their combinations in different treatment groups

	SNP +45T/G			SNP +276G/T			Genotype combinations of SNPs +45 and +276			
All subjects	TT	G-allele	*P**	GG	T-allele	*P**	No risk alleles	One risk allele	Both risk alleles	*P*†
Placebo	0.32 ± 2.7	0.92 ± 2.7	0.044	0.34 ± 2.7	0.51 ± 2.7	0.516	0.22 ± 2.7	0.45 ± 2.8	1.41 ± 2.4‡	0.068
Acarbose	−0.56 ± 2.6	−0.12 ± 2.5	0.214	−0.46 ± 2.6	−0.49 ± 2.6	0.912	−0.65 ± 2.6	−0.33 ± 2.6	−0.6 ± 2.3	0.533
Men										
Placebo	0.33 ± 2.9	0.88 ± 2.5	0.259	0.54 ± 2.8	0.32 ± 2.9	0.565	0.38 ± 2.8	0.46 ± 2.9	0.54 ± 2.2	0.967
Acarbose	−0.79 ± 2.8	−0.17 ± 3.0	0.244	−0.56 ± 2.9	−0.77 ± 2.8	0.648	−0.81 ± 2.8	−0.46 ± 3.0	−0.79 ± 2.6	0.745
Women										
Placebo	0.30 ± 2.5	0.97 ± 2.9	0.108	0.07 ± 2.7	0.71 ± 2.5	0.064	−0.02 ± 2.5	0.43 ± 2.6	2.35 ± 2.2§‖	0.006
Acarbose	−0.38 ± 2.4	−0.05 ± 1.7	0.368	−0.35 ± 2.3	−0.31 ± 2.4	0.919	−0.48 ± 2.5	−0.24 ± 2.3	−0.33 ± 2.1	0.814

Data are means ± SD. *Comparison has been made between the two genotypes of SNP +45T/G and SNP +276G/T using Mann-Whitney test. †Comparison has been made between the three genotype combinations of SNPs +45 and +276 using Kruskal-Wallis test. ‡$P < 0.05$ when subjects with the TT genotype of SNP +45 and the GG genotype of SNP +276 were compared with subjects carrying the G-allele of SNP +45 and the T-allele of SNP +276. §$P < 0.001$ when subjects with the TT genotype of SNP +45 and the GG genotype of SNP +276 were compared with subjects carrying the G-allele of SNP +45 and the T-allele of SNP +276. ‖$P < 0.05$ when subjects with the G-allele of SNP +45 or the T-allele of SNP +276 were compared with subjects carrying the G-allele of SNP +45 and the T-allele of SNP +276.

TABLE 2
The SNP +45T/G and SNP +276G/T polymorphisms of the adiponectin gene as predictors for the development of type 2 diabetes (univariate and multivariate logistic regression analysis)

		Univariate	*P*	Multivariate*	*P*
Placebo group					
SNP +45T/G†					
All subjects	G-allele	1.84 (1.12–3.00)	0.015	1.45 (0.85–2.49)	0.173
Men	G-allele	1.39 (0.71–2.72)	0.339	0.97 (0.45–2.08)	0.939
Women	G-allele	2.52 (1.22–5.23)	0.013	2.35 (1.04–5.34)	0.041
SNP +276G/T‡					
All subjects	GT	1.37 (0.91–2.06)	0.132	1.48 (0.95–2.30)	0.087
	TT	1.32 (0.67–2.59)	0.420	1.25 (0.61–2.57)	0.541
Men	GT	1.13 (0.64–1.98)	0.679	1.11 (0.59–2.07)	0.747
	TT	1.54 (0.63–3.72)	0.341	1.56 (0.60–4.06)	0.366
Women	GT	1.69 (0.93–3.09)	0.087	1.95 (1.01–3.77)	0.047
	TT	1.06 (0.37–3.07)	0.913	0.94 (0.30–2.98)	0.916
Acarbose group					
SNP +45T/G†					
All subjects	G-allele	1.21 (0.70–2.10)	0.500	0.99 (0.54–1.80)	0.972
Men	G-allele	1.07 (0.51–2.28)	0.853	0.96 (0.42–2.21)	0.921
Women	G-allele	1.34 (0.59–3.05)	0.482	1.08 (0.43–2.69)	0.870
SNP +276G/T‡					
All subjects	GT	0.98 (0.61–1.56)	0.917	0.96 (0.58–1.58)	0.871
	TT	2.83 (1.26–6.36)	0.012	3.69 (1.45–9.35)	0.006
Men	GT	0.72 (0.35–1.47)	0.364	0.75 (0.34–1.63)	0.461
	TT	3.59 (1.01–12.8)	0.049	3.52 (0.78–15.9)	0.102
Women	GT	1.29 (0.68–2.46)	0.436	1.11 (0.56–2.21)	0.770
	TT	2.58 (0.87–7.66)	0.087	3.39 (0.99–11.5)	0.051

Data are OR (95% CI). *Adjusted for age (years), weight at baseline (kg), weight change (kg), smoking (0 = never or past smoker vs. 1 = current smoker), and the country of origin. †G-allele of SNP +45T/G was compared with the TT genotype of SNP +45T/G of the adiponectin gene. ‡GT and TT genotypes of SNP +276G/T were compared with the GG genotype of SNP +276G/T of the adiponectin gene.

Univariate logistic regression analysis showed that the G-allele of SNP +45 was associated with a 1.8-fold higher risk for type 2 diabetes compared with the TT genotype in the placebo group (odds ratio [OR] 1.84, 95% CI 1.12–3.00, $P = 0.015$) (Table 2). When SNP +276 (T-allele) was included in the model, the risk further increased (2.05, 1.23–3.41, $P = 0.006$). In women treated with placebo, the presence of the G-allele (SNP +45) was associated with a 2.5-fold (2.52, 1.22–5.23, $P = 0.013$) higher risk of developing type 2 diabetes. When SNP +276 (T-allele) was included into the model, the risk increased (3.13, 1.44–6.83, $P = 0.004$). The results for women remained significant when adjusted for age, weight at baseline, weight change, smoking, and the country of origin (Table 2).

The TT genotype of SNP +276 was associated with a higher risk of type 2 diabetes than the GG genotype in all subjects treated with acarbose (OR 2.83, 95% CI 1.26–6.36, $P = 0.012$) and in men treated with acarbose (3.59, 1.01–12.8, $P = 0.049$). The inclusion of SNP +45 (G-allele) in the model further increased the risk in all subjects (3.05, 1.34–6.96, $P = 0.008$) and in men (3.80, 1.05–13.8, $P = 0.042$). After adjustment for age, weight at baseline, weight change, smoking, and the country of origin, all subjects treated with acarbose still had a significantly higher risk of developing type 2 diabetes (Table 2). The T-allele of SNP +276 was not associated with the risk of type 2 diabetes in all subjects or in men treated with acarbose.

Standardized linkage disequilibrium (D′) between two SNPs was −0.999, similar to that reported previously (25). For further statistical analyses, we formed a combination of the SNP +45 and SNP +276 genotypes. As subjects with the TT genotype of SNP +45 and with the GG genotype of SNP +276 had the lowest conversion to type 2 diabetes, they were pooled together as subjects having none of the risk alleles (the protective genotype combination). Subjects simultaneously having the G-allele of SNP +45 and the T-allele of SNP +276 were considered as subjects having both risk alleles (the risk genotype combination). Subjects not fitting to any groups above were classified as subjects carrying one risk allele (either the G-allele of SNP +45 or the T-allele of SNP +276). During follow-up, subjects with none of the risk alleles lost more weight ($P = 0.039$) compared with subjects with one or both risk alleles in the placebo group (Fig. 2*A*). The conversion to type 2 diabetes was 38.9, 46.9, and 74.1% in subjects treated with placebo and having neither, one, or both risk alleles, respectively ($P = 0.003$) (Fig. 2*B*). There were no significant differences in weight change according to the genotype combinations of the adiponectin gene in women treated with placebo (Fig. 2*E*), but only 35.1% of women without risk alleles converted to type 2 diabetes compared with 46.3 or 92.3% with one or both risk alleles, respectively ($P = 0.001$) (Fig. 2*F*). We also calculated the frequencies of haplotypes of SNP +45 and SNP +276 among converters and nonconverters to diabetes and found that they differed significantly ($P = 0.007$) (SNP +45T and SNP +276G: 0.548 vs. 0.635, SNP +45T and SNP +276 T: 0.331 vs. 0.277, amd SNP +45G and SNP +276G: 0.121 vs. 0.088).

Women carrying both risk alleles in the placebo group had a significant increase in their 2-h glucose in the OGTT compared with women having none or one risk allele of both SNPs (Table 1). Women carrying none of the risk alleles had a lower conversion to type 2 diabetes compared with men (29.8 vs. 38.0%), and women treated with place-

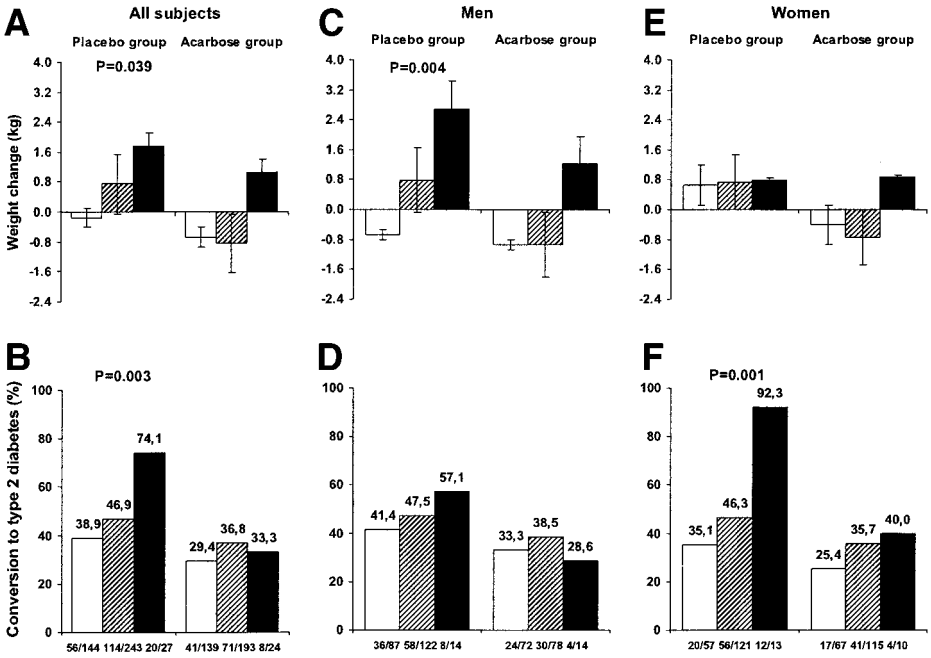

FIG. 2. Weight change in subjects treated with placebo or acarbose (*A*) (men [*C*] and women [*E*]), and the conversion to type 2 diabetes (% and number of coverters/all subjects at risk) in subjects treated with placebo or acarbose (*B*) (men [*D*] and women [*F*]) according to the combined genotypes of SNP +45T/G and SNP +276G/T of the adiponectin gene. □, TT genotype of SNP +45 and the GG genotype of SNP +276; ▨, G-allele of SNP +45 or the T-allele of SNP +276; ■, G-allele of SNP +45 and the T-allele of SNP +276.

bo having both risk alleles significantly more often developed type 2 diabetes compared with men (92.3 vs. 57.1%, $P = 0.037$).

In univariate logistic regression analysis (Table 3), subjects in the placebo group and simultaneously carrying the G-allele of SNP +45 and the T-allele of SNP +276 had a 4.49-fold higher risk of developing type 2 diabetes than subjects having neither of these risk alleles (95% CI 1.78–11.3, $P = 0.001$). Moreover, these two alleles were related to even higher conversion rate to diabetes in women treated with placebo (OR 22.2, 95% CI 2.7–183.3, $P = 0.004$). These associations remained unchanged, even after adjustment for age, weight at baseline, weight change, smoking, and the country of origin (Table 3). None of genotype combinations predicted the conversion to type 2 diabetes in the acarbose group.

DISCUSSION

The novel finding of our study was that the adiponectin gene is a susceptibility gene for type 2 diabetes in subjects with IGT having a high risk of developing type 2 diabetes. We demonstrated that the G-allele of SNP +45 was associated with a 1.8-fold risk for the development of type 2

TABLE 3
Combinations of the risk alleles (G-allele of SNP +45, T-allele of SNP +276) of the adiponectin gene as predictors for the development of type 2 diabetes in univariate and multivariate logistic regression analyses. Subjects having one or both risk alleles were compared with subjects having none of the risk alleles

	Univariate	*P*	Multivariate*	*P*
Placebo group				
All subjects				
One risk allele ($n = 243$)	1.39 (0.91–2.11)	0.125	1.30 (0.81–2.09)	0.281
Both risk alleles ($n = 27$)	4.49 (1.78–11.3)	0.001	4.20 (1.31–13.5)	0.016
Men				
One risk allele ($n = 122$)	1.28 (0.74–2.24)	0.378	1.16 (0.61–2.22)	0.644
Both risk alleles ($n = 14$)	1.89 (0.60–5.91)	0.275	1.60 (0.34–7.59)	0.556
Women				
One risk allele ($n = 121$)	1.59 (0.83–3.06)	0.160	1.49 (0.71–3.13)	0.288
Both risk alleles ($n = 13$)	22.2 (2.7–183.3)	0.004	22.5 (2.2–233.3)	0.009
Acarbose group				
All subjects				
One risk allele ($n = 193$)	1.38 (0.86–2.20)	0.180	1.42 (0.84–2.41)	0.197
Both risk alleles ($n = 24$)	1.18 (0.47–2.98)	0.722	0.78 (0.22–2.73)	0.695
Men				
One risk allele ($n = 78$)	1.22 (0.63–2.39)	0.555	1.36 (0.59–3.10)	0.469
Both risk alleles ($n = 14$)	0.78 (0.22–2.76)	0.704	0.39 (0.63–2.47)	0.320
Women				
One risk allele ($n = 115$)	1.63 (0.83–3.18)	0.153	1.52 (0.73–3.18)	0.262
Both risk alleles ($n = 10$)	1.96 (0.49–7.79)	0.339	0.96 (0.14–6.59)	0.967

Data are OR (95% CI). *Adjusted for the G-allele of SNP +45G/T of the adiponectin gene, age (years), weight (kg) at baseline, weight change (kg), smoking (0 = never or past smoker vs. 1 = current smoker), and the country of origin.

diabetes ($P = 0.015$) in the placebo group and that subjects treated with placebo having both the SNP +45 (G-allele) and SNP +276 (T-allele) risk alleles had an even higher risk of developing type 2 diabetes than subjects carrying neither of these risk alleles (OR 4.49, $P = 0.001$).

The G-allele of SNP +45 was associated with the risk for type 2 diabetes and weight gain in individuals from a high-risk population. We also found that the risk genotype combination of the two SNPs (SNP +45 [G-allele] and SNP +276 [T-allele]) had a greater influence compared with that of each risk allele alone on the development of type 2 diabetes in IGT subjects participating in the STOP-NIDDM trial. Although both the G-allele of SNP +45 and the risk genotype combination were associated with weight gain, multivariate logistic regression analyses showed that changes in weight played an important, but not a crucial, role in increasing the risk of developing type 2 diabetes. This could be due to the effect of adiponectin on both glucose and lipid metabolism (33). Therefore, these SNPs could modulate the risk of type 2 diabetes via both weight-dependent and -independent pathways. Only one prospective study concerning the influence of the adiponectin gene on the risk of hyperglycemia and weight characteristics has been published. Fumeron et al. (28) found that the GG genotype of SNP +45 was associated with the risk of hyperglycemia in the study cohort. However, only a marginal significance for the influence of SNP +45 and SNP +276 genotype combinations on the risk of hyperglycemia was found ($0.05 < P < 0.1$).

Cross-sectional studies have yielded controversial findings concerning the association of SNP +45 and haplotypes of the adiponectin gene with obesity and type 2 diabetes. The G-allele of SNP +45 has been reported to be associated with an increased risk of obesity in German subjects (21) and with increased risk of type 2 diabetes in Japanese subjects (26). However, other studies have reported that the T-allele is a risk allele for obesity in Taiwanese (19) and Swedish subjects (22) and for the insulin resistance syndrome and type 2 diabetes in Italian (24) and French subjects (25). Conflicting findings between these studies could be due to true differences in allelic association with the disease phenotype in different populations. In agreement with this notion are differences in allele frequencies of SNPs in the adiponectin gene in various populations (17,19,21,22,24–26). However, because the STOP-NIDDM trial included subjects from several populations, it provides evidence that the G-allele of SNP +45 and the risk genotype combination of SNPs +45 and +276 are likely to contribute to the risk of type 2 diabetes.

We found that women treated with placebo had a 2.5-fold increased risk for developing type 2 diabetes associated with the G-allele of SNP +45 ($P = 0.013$). Furthermore, women carrying the risk genotype combination of both SNPs had an especially high risk for the conversion to type 2 diabetes (OR 22.2, $P = 0.004$). When all women without the stratification for treatment group were analyzed, carriers of the risk genotype combination had an almost 6.5-fold ($P < 0.001$) higher risk of type 2 diabetes compared with women having the protective genotype combination. The mechanisms leading to high risk of diabetes, particularly among women, remain to be elucidated. Other large studies are needed to confirm the sex difference in the risk. With respect to ORs found in our study, it should be noted that because ~40% of all subjects converted to diabetes, it is possible that ORs from logistic regression models may overestimate actual risks.

Mechanisms explaining the effect of the G-allele of SNP +45 and the T-allele of SNP +276 on the risk of type 2 diabetes are unknown. The T/G polymorphism of SNP +45 is located in exon 2 of the adiponectin gene and does not cause an amino acid change (GGT→GGG, Gly15Gly) (17). The G-allele has been reported to have higher transcriptional activity than the T-allele because it alters mRNA splicing or stability (19). The G-allele has been associated with low adiponectin concentration in one study (17), whereas two other studies have not found any effect of SNP +45 on adiponectin level (26,34). The G/T polymorphism of SNP +276 is located in intron 2 and could also influence adiponectin expression (24,26). Two recent studies have shown that carriers of the TT genotype of SNP +276 have higher adiponectin levels than noncarriers (26,34), but Vasseur et al. (25) reported that SNP +45 and +276 had only a marginal influence on adiponectin levels. Adiponectin levels were not estimated in this study.

Because the association of adiponectin gene variants with adiponectin levels remains unclear, other mechanisms must be considered to explain our findings. One possibility is that SNP +45 and SNP +276 are in linkage disequilibrium with other still undiscovered SNP(s) of the adiponectin gene or other genes having an effect on adiponectin expression, secretion, structure, or action. Another possibility is that SNP +45 and SNP +276 coordinately have an effect on the susceptibility to type 2 diabetes, although this hypothesis remains to be proven.

Our study indicates that the effects of SNP +45 and SNP +276 of the adiponectin gene on the conversion to type 2 diabetes were statistically significant only in IGT subjects treated with placebo. Therefore, the acarbose treatment was able to "neutralize" the effect of risk genotypes of the adiponectin gene on the risk of type 2 diabetes. The mechanisms of this effect remain to be determined.

In conclusion, the G-allele of SNP +45 is a predictor for type 2 diabetes in the STOP-NIDDM trial. Furthermore, the risk genotype combination of SNP +45 (G-allele) and SNP +276 (T-allele) had an even stronger influence on the conversion from IGT to type 2 diabetes compared with each of these SNPs alone.

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