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Association of *ADIPOR2* With Liver Function Tests in Type 2 Diabetic Subjects

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Objective: Adiponectin protects against liver dysfunction in insulin-resistant states such as obesity and type 2 diabetes (T2DM), but the role of adiponectin receptors in this disorder is largely unknown. We studied whether common single-nucleotide polymorphisms (SNPs) in *ADIPOR1* and *ADIPOR2* are associated with liver function tests (LFTs) in human subjects with various degrees of insulin resistance.
Methods and Procedures: Serum alanine (ALT) and aspartate (AST) aminotransferases, homeostasis model assessment of insulin resistance (HOMA-IR), –8503 G/A (rs6666089) and +5843 C/T (rs1342387) SNPs in *ADIPOR1*, –64,241 T/G (rs1029629) and +33447 C/T (rs1044471) SNPs in *ADIPOR2* were assessed in 700 white subjects from a population-based study.
Results: In nondiabetic subjects, the at-risk alleles for the common –64,241 T/G and +33447 C/T SNPs in *ADIPOR2* were associated with increased circulating adiponectin ($P < 0.05$ to $P < 0.005$), but not with LFT. Conversely, in T2DM subjects (who are at risk for liver dysfunction), the same alleles were associated with increased serum ALT and AST ($P < 0.05$ to $P < 0.0001$), but not with circulating adiponectin. No significant associations with these parameters were evident for the common –8503 G/A and +5843 C/T SNPs in *ADIPOR1.* In a replication study, the –64,241 T/G and +33447 C/T SNPs in *ADIPOR2* were associated with ALT and AST ($P < 0.05$ to $P < 0.0001$) in pooled obese and T2DM subjects.
Discussion: Common SNPs in *ADIPOR2* are associated with LFT in T2DM subjects, which suggests a possible role of this receptor in liver dysfunction associated with insulin resistance.

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INTRODUCTION

Adiponectin is an anti-inflammatory adipocyte-derived plasma protein known to alleviate steatosis and inflammation in nonalcoholic fatty liver disease (NAFLD), a condition frequently observed in insulin-resistant states such as obesity and type 2 diabetes mellitus (T2DM) (1).

A role for adiponectin in NAFLD is supported by reports showing (i) protection from hepatic fibrosis in adiponectin null mice sequentially treated with adiponectin and carbon tetrachloride (2); (ii) decreased circulating adiponectin, independently of insulin resistance, in patients with NAFLD (3); and (iii) improved hepatic histology in NAFLD patients treated with rosiglitazone and pioglitazone, which are known to exert insulin-sensitizing actions and to upregulate adiponectin expression in adipose tissue (4,5). Hypoadiponectinemia was also closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients (6).

Two putative adiponectin receptors (AdipoR1 and AdipoR2, with 67% amino acid identity) have been recently cloned (7). In mice, AdipoR1 is ubiquitously expressed, with the most abundant expression occurring in skeletal muscle. AdipoR2 is predominantly expressed in the liver. In humans, both receptors are expressed in muscle, liver, and adipose tissue. AdipoR1 is a high-affinity receptor for globular adiponectin and a low-affinity receptor for full-length adiponectin in skeletal muscle. In contrast, AdipoR2 is an intermediate-affinity receptor for both globular and full-length adiponectin, which seems to be predominantly responsible for the effects of adiponectin on the liver (8).

A possible role for ADIPOR1 and ADIPOR2 in NAFLD has been recently investigated. Although no changes were demonstrated for ADIPOR1, conflicting data have been reported for the expression of ADIPOR2 in NAFLD, with results showing either increased or reduced expression in this disease (9,10). In the latter study, the expression of ADIPOR2, but not that of ADIPOR1,

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was negatively associated with both alanine (ALT) and aspartate (AST) aminotransferases and grade of fibrosis (10).

Common single-nucleotide polymorphisms (SNPs) have been identified in both *ADIPOR1* and *ADIPOR2* (11–15). Genetic association analyses have shown modest contributions of either gene to diabetes risk (11–15). In one of these studies, a SNP in *ADIPOR1* (−8503GA; rs6666089) was also associated with liver fat content, as measured by proton magnetic resonance spectroscopy (14). To our knowledge, the contribution of these SNPs to NAFLD has not yet been reported.

We have previously shown that serum adiponectin is negatively associated with various liver function tests (LFTs) in apparently healthy subjects (16), which is in accordance with the role of this protein in NAFLD. In line with our previous report (16), we now studied whether common SNPs in the adiponectin receptor 1 (*ADIPOR1* −8503 G/A (rs6666089) and +5843 C/T (rs1342387)) and in the adiponectin receptor 2 (*ADIPOR2* −64,241 T/G (rs1029629) and +33447 C/T (rs1044471)) are associated with LFT in an unselected population of white subjects with various degrees of insulin resistance.

METHODS AND PROCEDURES

Human subjects

Participants were 700 consecutive subjects enrolled in a cross-sectional, population-based study examining the prevalence of cardiovascular risk factors in Northwestern Spain (17).

All subjects were of white origin and reported that their body weight had been stable for at least three months before the study. Inclusion criteria for this group were (i) BMI <40 kg/m^2, (ii) absence of any systemic disease, (iii) alcohol intake <40 g a day in men or 20 g a day in women. Subjects underwent an oral glucose-tolerance test to diagnose T2DM, according to the criteria of the American Diabetes Association, if this disease had not been previously diagnosed according to these guidelines (18). The population-based nature of the study design allowed for newly diagnosed type 2 diabetic patients and those with previously diagnosed disease, but with stable metabolic control (i.e., with glycosylated hemoglobin values in the previous six months <9%), to be included in the study.

Both obese (n = 110) and type 2 diabetic (n = 96) subjects were characterized by being more insulin-resistant than nonobese, nondiabetic subjects (as inferred by their higher circulating insulin and homeostasis model assessment of insulin resistance (HOMA-IR) values). They were studied separately in genotype–phenotype association analyses.

Informed written consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The experimental protocol was approved by the Ethics Committee of the Hospital of Avilés (Asturias).

Measurements

Subjects were studied in the postabsorptive state. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Subjects' waists were measured with a soft tape midway between the lowest rib and the iliac crest; hip circumference was measured at the widest part of the gluteal region; and waist-to-hip ratio was accordingly calculated. Blood pressure was measured in the supine position on the right arm after a 10-min rest; a standard sphygmomanometer of appropriate cuff size was used and the first and fifth phases were recorded. Values used in the analysis are the average of three readings taken at 5-min intervals.

Insulin resistance was measured by the HOMA-IR (19). HOMA-IR correlates well with insulin sensitivity derived from the glucose-clamp technique (r = −0.82, P < 0.0001), and this correlation appears to be independent of sex, age, BMI, diabetes, and blood pressure (20).

Analytical methods

Total serum cholesterol was measured through the reaction of cholesterol estearase/cholesterol oxidase/peroxidase. High-density lipoprotein cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Low-density lipoprotein cholesterol was estimated by the Friedewald formula. Total serum triacylglycerol was measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Serum aspartate transaminase, alanine transaminase, and gamma glutamyl transpeptidase were measured by colorimetry using automated tests (Roche Diagnostics GmbH, Mannheim, Germany). Intra- and inter-assay coefficients of variation were <4% for these tests.

Serum glucose concentrations were measured in duplicate by the glucose oxidase method, using a Beckman Glucose Analyzer II (Beckman Instruments, Brea, CA). The coefficient of variation was 1.9%. Glycosylated hemoglobin was measured by high-performance liquid chromatography (Bio-Rad, Muenchen, Germany) and a Jokoh HS-10 autoanalyzer. Serum insulin concentrations were measured in duplicate by a monoclonal immunoradiometric assay (IRMA, Medgenix Diagnostics, Fleunes, Belgium). Intra- and inter-assay coefficients of variation were <7%.

Serum adiponectin concentrations were measured in a subgroup of 197 subjects who did not differ significantly from the general cohort (see **Supplementary Table S1** online) and for whom serum samples were also available. Adiponectin was measured by validated sandwich enzyme-linked immunosorbent assay (ELISA) kits purchased from Otsuka Pharmaceuticals (Tokushima, Japan) in these subjects. Detection limit was 0.12 mg/l and intra- and inter-assay coefficients of variations were 3.3 and 7.4%, respectively.

Genetic analyses

The studied SNPs have been reported to be associated with metabolic phenotypes in previous reports (reviewed in ref. 21). The −8503 G/A SNP (rs6666089) and the intronic +5843 C/T (rs1342387) are, respectively, tagging SNPs for the promoter region and a major LD block in *ADIPOR1* (12,13,22). Similarly, the −64,241 T/G (rs1029629) and the 3′ UTR +33447 C/T (rs1044471) are, respectively, tagging SNPs for the promoter region and a major LD block in *ADIPOR2* (12,13,22).

Genomic DNA was purified from peripheral blood leukocytes using QIAGEN QIAmpBlood kits and quantified by means of a spectrophotometer (GeneQuant, GE Health Care, Piscataway NJ); 100 ng of DNA were used for PCR amplification.

All SNPs were genotyped by means of allelic discrimination assays, using an ABI Prism 7000 sequence detector and TaqMan technology (Applied Biosystems, Foster City, CA). The reaction was performed in a final volume of 25 µl. DNA was amplified after 50 cycles with an initial denaturation of 10 min at 95 °C. The cycle program consisted of 15-s denaturation at 92 °C and 1-min annealing and extension at 60 °C. Positive and negative controls, which were correctly identified, were included in all reactions. The population studied was in Hardy–Weinberg equilibrium for all the SNPs (χ^2 = 0.14, P = 0.93; χ^2 = 0.01, P = 0.99; χ^2 = 0.57, P = 0.75; and χ^2 = 0.15, P = 0.93; respectively). Successful genotyping of at least one of the studied SNPs was accomplished in more than 99% of subjects.

Replication study

The association between the studied SNP in *ADIPOR2* and LFT was also examined in 123 pooled obese and type 2 diabetic men, mean 95% confidence interval: age 53 (50–55) years, BMI 31 (31–32) kg/m^2, participants in a prospective study of cardiovascular risk factors in Northeastern Spain and previously reported by us (23).

Statistical methods

Statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL). Parameters with non-Gaussian distribution were Log10 transformed to improve symmetry for subsequent analyses. χ^2-Test was used to study differences in genotype frequencies between groups. ANOVA and general linear models (to correct for the effect of known covariates) were used to study differences in continuous variables among genotype groups. Levels of statistical significance were set at $P < 0.05$.

Table 1 Clinical and laboratory variables in the study subjects

Characteristic	Nonobese, nondiabetic subjects (*n* = 494)	Obese subjects (*n* = 110)	Type 2 diabetic subjects (*n* = 96)
Age (years)	57 (56–59)	59 (57–62)	64 (62–67)***,†
Women (*n* (%))	286 (58)	59 (54)	34 (35)*,†
BMI (kg/m^2)	26.3 (25.9–26.6)	33.7 (33.3–34.2)***	30.7 (30.0–31.4)***,†††
WHR	0.86 (0.85–0.86)	0.93 (0.91–0.94)***	0.94 (0.93–0.96)***
SBP (mm Hg)	133 (131–135)	140 (137–144)**	150 (146–154)***,††
DBP (mm Hg)	78 (77–79)	83 (81–85)***	83 (81–84)***
LDL-cholesterol (mmol/l)	3.4 (3.3–3.4)	3.6 (3.5–3.8)**	3.3 (3.1–3.5)†
HDL-cholesterol (mmol/l)	1.6 (1.5–1.6)	1.4 (1.3–1.5)***	1.3 (1.3–1.4)***
Triacylglycerol (mmol/l)	1.2 (1.1–1.2)	1.5 (1.3–1.6)***	1.7 (1.5–2.0)***
AST (U/l)	22 (21–22)	23 (22–25)	24 (23–26)***
ALT (U/l)	24 (23–26)	29 (26–33)**	33 (30–37)***,†
GGT (U/l)	24 (22–26)	29 (24–35)**	41 (33–49)***,††
Glucose (mmol/l)	5.2 (5.1–5.3)	5.2 (5.1–5.3)	7.5 (7.0–7.9)***,†††
HbA_{1c} (%)	4.6 (4.6–4.7)	4.7 (4.6–4.8)	5.9 (5.6–6.1)***,†††
Insulin (pmol/l)	56 (52–60)	79 (70–87)***	96 (83–109)***,†
HOMA-IR	1.9 (1.7–2.0)	2.6 (2.3–2.9)***	4.5 (3.8–5.1)***,†††

Data are mean (95% confidence interval). *P < 0.05, **P < 0.01, and ***P < 0.001 for comparisons with nonobese, nondiabetic subjects and †P < 0.05, ††P < 0.01, and †††P < 0.001 for comparisons with obese subjects. Serum adiponectin values were, respectively, 8.8 (8.2–9.4), 7.3 (6.3–8.2), and 6.5 (5.4–7.6) mg/l in nonobese nondiabetic, in obese, and in type 2, diabetic subjects.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; DBP, diastolic blood pressure; GGT, gamma glutamyltranspeptidase; $HbA1_c$, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; SBP, systolic blood pressure; WHR, waist-to-hip ratio.

Table 2 Clinical and laboratory variables according to *ADIPOR2* –64,241 T/G (rs1029629) genotypes

	Nonobese, nondiabetic subjects				Obese subjects				Type 2 diabetic patients			
Characteristic	TT (*n* = 202)	TG (*n* = 215)	GG (*n* = 63)	*P*	TT (*n* = 40)	TG (*n* = 52)	GG (*n* = 15)	*P*	TT (*n* = 51)	TG (*n* = 31)	GG (*n* = 13)	*P*
Women (*n* (%))	122 (60)	125 (58)	31 (49)	NS	21 (53)	33 (64)	3 (20)	**0.012**	17 (33)	12 (39)	5 (39)	NS
Age (years)	57 (55–59)	58 (56–60)	56 (53–59)	NS	59 (55–63)	60 (57–63)	59 (51–66)	NS	65 (62–68)	63 (59–67)	67 (60–73)	NS
BMI (kg/m^2)	26 (25–26)	27 (26–27)	26 (25–27)	NS	34 (33–35)	34 (33–34)	34 (33–36)	NS	31 (30–32)	30 (29–32)	32 (30–34)	NS
WHR	0.86 (0.85–0.87)	0.86 (0.85–0.87)	0.85 (0.83–0.87)	NS	0.91 (0.89–0.94)	0.92 (0.90–0.94)	0.97 (0.94–1.00)	**0.019**	0.93 (0.91–0.95)	0.94 (0.92–0.97)	0.96 (0.90–1.02)	NS
ALT (U/l)	26 (23–28)	23 (22–25)	25 (21–28)	NS	25 (21–29)	29 (23–35)	39 (27–51)	**0.008**	30 (26–33)	37* (30–44)	40* (30–50)	**0.009**
AST (U/l)	22 (21–23)	21 (21–22)	21 (20–23)	NS	22 (20–24)	22 (21–24)	28 (20–36)	0.057	22 (21–24)	25 (23–27)	31** (24–38)	**<0.0001**
GGT (U/l)	24 (21–28)	25 (21–29)	21 (17–25)	NS	30 (18–42)	26 (19–33)	41 (23–60)	NS	38 (26–49)	39 (31–47)	59 (17–102)	NS
Glucose (mmol/l)	5.1 (5.0–5.2)	5.2 (5.1–5.4)	5.1 (4.9–5.3)	NS	5.2 (5.0–5.3)	5.2 (5.1–5.4)	5.4 (5.1–5.6)	NS	7.8 (7.1–8.5)	7.0 (6.3–7.6)	7.5 (6.4–8.6)	NS
Insulin (pmol/l)	55 (51–60)	59 (51–66)	48 (42–54)	NS	74 (63–84)	82 (67–97)	85 (60–109)	NS	84 (72–95)	84 (74–132)	128 (70–185)	NS
HOMA-IR	1.8 (1.6–2.0)	2.0 (1.7–2.3)	1.5 (1.3–1.7)	NS	2.4 (2.0–2.7)	2.7 (2.2–3.2)	2.9 (2.0–3.8)	NS	4.0 (3.4–4.6)	4.7 (3.1–6.2)	5.8 (3.4–8.1)	NS

Data are mean (95% confidence interval). *P* values shown on the right are from linear trend one-way ANOVA. *Post hoc* comparisons are from general linear models, adjusted for age, sex, BMI, WHR, HOMA-IR, and medication use (AST and ALT). *P < 0.05 and **P< 0.005, compared to TT homozygous subjects. Boldface *P* values indicate significant comparisons.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltranspeptidase; HOMA-IR, homeostasis model assessment of insulin resistance; NS, nonsignificant; WHR, waist-to-hip ratio.

Within the number of patients included, the study had a 90% power to detect a difference of at least 1 standard deviation in LFT among the study subgroups.

RESULTS

Clinical and biochemical variables of the study subjects are summarized in **Table 1**. Both obese and type 2 diabetic subjects exhibited higher circulating insulin and HOMA-IR values and higher LFT than nonobese, nondiabetic subjects.

The –64,241 T/G and +33447 C/T SNPs in ADIPOR2 are associated with LFT

None of the studied SNPs in either *ADIPOR1* or *ADIPOR2* was significantly associated with LFT in the study subjects, considered as a group. However, further analysis in obese and type 2 diabetic subjects—who are known to be at risk for developing NAFLD—documented a significant association between both the –64,241 T/G and +33447 C/T SNPs in *ADIPOR2* and LFT.

The at-risk allele for the –64,241 T/G SNP in *ADIPOR2* was associated with increased ALT in both obese ($P = 0.008$) and type 2 diabetic ($P = 0.009$). In the latter, the same SNP was also associated with increased AST ($P < 0.0001$). These associations proved to be independent of known covariates in type 2 diabetic subjects (**Table 2**).

The at-risk allele for the +33,446 C/T in *ADIPOR2* was associated with increased concentrations of both ALT ($P = 0.005$) and AST ($P = 0.026$) in type 2 diabetic subjects, and the former association proved to be independent of known covariates (**Table 3**).

The –64,241 T/G and +33447 C/T SNPs in ADIPOR2 are associated with circulating adiponectin

In a sample of 197 subjects who did not differ significantly from the general cohort (see **Supplementary Table S1** online), circulating adiponectin was also measured. In these subjects, the at-risk allele for the –64,241 T/G SNP in *ADIPOR2* was associated with increased circulating adiponectin ($P = 0.017$). This finding, however, was limited to nonobese, nondiabetic subjects, mean, 95% confidence interval for circulating adiponectin: 7.4 (6.5–8.4) mg/l vs. 8.8 (8.0–9.6) mg/l, for TT homozygotes and G carriers subjects, respectively; $P < 0.005$, adjusted for sex, age, BMI, waist-to-hip ratio, HOMA-IR, and medication use. No such association was evident in obese or in type 2 diabetic subjects (not shown).

Similarly, the at-risk allele for the +33,446 C/T in *ADIPOR2* was significantly associated with increased adiponectin in nondiabetic subjects, mean, 95% confidence interval for circulating adiponectin: 8.3 (7.7–8.9) mg/l vs. 7.2 (6.1–8.3) mg/l, for C carriers and TT homozygotes, respectively; $P < 0.05$, adjusted for sex, age, BMI, waist-to-hip ratio, HOMA-IR, and medication use. No such association was evident in type 2 diabetic subjects (not shown).

Multivariate analyses

Using stepwise multivariate regression analyses, the at-risk alleles for the common –64,241 T/G and +33,446 C/T SNPs in

Table 3 Clinical and laboratory variables according to *ADIPOR2* +33,446 C/T (rs1044471) genotypes

	Nonobese, nondiabetic subjects				Obese subjects				Type 2 diabetic patients			
Characteristic	CC (*n* = 159)	CT (*n* = 233)	TT (*n* =91)	*P*	CC (*n* = 34)	CT (*n* = 53)	TT (*n* = 21)	*P*	CC (*n* = 30)	CT (*n* = 43)	TT (*n* = 22)	*P*
Women (*n* (%))	81 (51)	146 (63)	52 (57)	NS	14 (41)	34 (64)	9 (43)	NS	10 (33)	15 (35)	9 (41)	NS
Age (years)	56 (54–58)	58 (56–60)	58 (55–61)	NS	60 (56–64)	60 (56–63)	58 (53–64)	NS	65 (62–69)	64 (61–68)	63 (58–69)	NS
BMI (kg/m^2)	26 (26–27)	27 (26–27)	25 (25–26)	NS	34 (33–35)	34 (33–34)	34 (33–35)	NS	31 (30–32)	31 (30–32)	31 (29–32)	NS
WHR	0.86 (0.84–0.87)	0.86 (0.85–0.87)	0.86 (0.84–0.87)	NS	0.94 (0.92–0.97)	0.92 (0.90–0.94)	0.92 (0.88–0.96)	NS	0.95 (0.92–0.98)	0.94 (0.92–0.96)	0.92 (0.89–0.94)	NS
ALT (U/l)	25 (23–27)	24 (22–25)	25 (22–29)	NS	31 (25–37)	28 (23–34)	28 (24–33)	NS	40* (32–48)	32 (28–35)	28 (22–33)	**0.005**
AST (U/l)	22 (21–23)	21 (21–22)	23 (21–24)	NS	24 (20–28)	23 (21–25)	23 (20–25)	NS	27 (24–30)	24 (22–26)	23 (19–26)	**0.026**
GGT (U/l)	23 (19–27)	23 (20–26)	29 (22–35)	NS	39 (24–55)	24 (20–27)	30 (15–44)	NS	43 (28–57)	43 (28–59)	35 (24–46)	NS
Glucose (mmol/l)	5.2 (5.0–5.24)	5.1 (5.0–5.2)	5.2 (5.0–5.4)	NS	5.3 (5.1–5.5)	5.2 (5.0–5.3)	5.3 (5.1–5.5)	NS	7.0 (6.3–7.6)	7.8 (7.0–8.6)	7.5 (6.7–8.3)	NS
Insulin (pmol/l)	55 (48–62)	55 (49–61)	59 (50–67)	NS	79 (60–97)	85 (72–97)	67 (53–81)	NS	104 (78–123)	97 (74–119)	85 (67–103)	NS
HOMA–IR	1.8 (1.5–2.1)	1.8 (1.6–2.1)	2.0 (1.6–2.5)	NS	2.7 (2.0–3.3)	2.8 (2.3–3.2)	2.2 (1.7–2.7)	NS	4.5 (3.4–5.6)	4.7 (3.5–5.8)	4.0 (2.9–5.1)	NS

Data are mean (95% confidence interval). *P* values shown on the right are from linear trend one-way ANOVA. *Post hoc* comparisons are from general linear models, adjusted for age, sex, BMI, WHR, HOMA-IR, and medication use (AST and ALT). *$P < 0.05$, compared to TT homozygous subjects. Boldface *P* values indicate significant comparisons.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltranspeptidase; HOMA-IR, homeostasis model assessment of insulin resistance; NS, nonsignificant; WHR, waist-to-hip ratio.

ADIPOR2 explained, respectively, 6 and 9% of the variance of ALT in type 2 diabetic subjects.

Replication study

The association between the common −64,241 T/G and +33,446 C/T SNPs in *ADIPOR2* and LFT was also examined in a different population of pooled obese and type 2 diabetic men (n = 123) previously reported by us (23).

In this population, the at-risk allele for the −64,241 T/G SNP in *ADIPOR2* was associated with increased AST ($P < 0.0001$) and ALT ($P = 0.005$), explaining 13 and 7% of AST ($\beta = 0.37$, $P < 0.0001$) and ALT ($\beta = 0.27$, $P = 0.002$) variance, respectively.

Similarly, the at-risk allele for the +33,446 C/T in *ADIPOR2* was associated with increased ALT ($P < 0.05$), explaining 7% of its variance ($\beta = 0.29$, $P < 0.01$) in these subjects.

DISCUSSION

Our observations suggest that common SNPs in *ADIPOR2* are differentially associated with liver function and serum adiponectin in an unselected white population. The association with liver function is replicated in a separate, geographically distinct white population. We are unaware of previous studies reporting these findings.

The beneficial role of adiponectin in liver function has been extensively studied (for review, see refs. 24,25), but data concerning the adiponectin receptors in liver physiology or disease are still scarce. It has been reported that AdipoR2 mediates most of the metabolic effects of adiponectin in the liver (7,8). The receptor is involved in peroxisome proliferator–activated receptor-α signaling and inhibition of inflammation and oxidative stress in the liver (26). It is also upregulated by rosiglitazone (27), a well-known insulin sensitizer that improves NAFLD and upregulates adiponectin secretion in the adipose tissue (4). Expression of AdipoR2 was inversely correlated with LFT in patients with NAFLD (10). However, inconclusive data exist regarding the expression of adiponectin receptors in this disease (9,10). It also remains to be established whether genetic manipulations of either of the adiponectin receptors in experimental models of liver dysfunction lead to improvements in the course of the disease, as shown for their ligand (1). Finally, it is yet to be shown whether *ADIPOR2* variants influence the phenotype of NAFLD.

The functional consequences of the common polymorphisms in *ADIPOR1* and *ADIPOR2* are unknown. Previous reports have shown that the at-risk alleles for the—64,241T/G and +33,446 C/T SNPs in *ADIPOR2* are associated with quantitative components of the metabolic syndrome (13,15; reviewed in ref. 21), which is in line with our data showing that the same alleles are associated with risk for liver damage. Altogether, these observations suggest that the presence of at-risk alleles for the common—64,241T/G and +33,446 C/T SNPs in *ADIPOR2* may result in decreased expression of the receptor and, consequently, in decreased adiponectin signaling and risk for insulin resistance and liver dysfunction. This is further supported by the fact that metabolic syndrome, a condition frequently associated with NAFLD, is also characterized by adiponectin insensitivity (28–30).

Another relevant observation in our study is the association of the *ADIPOR2* gene variants with circulating adiponectin in nondiabetic subjects, but not in type 2 diabetic subjects. These findings may indicate that healthier subjects are able to compensate for the deleterious effect of the at-risk alleles for the −64,241T/G and +33,446 C/T SNPs in *ADIPOR2* by increasing circulating adiponectin. With deterioration of insulin sensitivity, this compensatory mechanism is lost, and the negative effects of these alleles are manifested. In line with this, a negative feedback regulation of circulating adiponectin by AdipoR2 has been proposed, which appears to depend on the nutritional status and may partly account for the improved insulin sensitivity and increased circulating adiponectin observed in high fat-fed *AdipoR2* null mice (31,32). This feedback regulation may also explain, at least in part, the inconsistent associations between common SNPs in *ADIPOR2* and insulin sensitivity or glucose tolerance parameters in previous studies that did not account for the effect of changing serum adiponectin concentrations (11–13,15,33).

More difficult to reconcile with our data is a previous report showing that the common −8503 G/A SNP in *ADIPOR1* (rs6666089) was associated with liver fat content in a large sample of nondiabetic men and women (14). Genetic and clinical differences may help explain the disparity with the results of the present study. Finally, minor effects of the studied SNPs in *ADIPOR1* on LFT may have also gone undetected in our current study due to low power to detect such associations.

In summary, common SNPs in *ADIPOR2* are associated with circulating adiponectin and plasma concentrations of various LFT in type 2 diabetic subjects, suggesting a possible role of this receptor in liver dysfunction associated with insulin resistance.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

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DISCLOSURE

The authors declared no conflict of interest.

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