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Preliminary report: genetic variation within the *GPBAR1* gene is not associated with metabolic traits in white subjects at an increased risk for type 2 diabetes mellitus

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Abstract

Bile acids are signaling molecules with important endocrine functions. Some of these, including the induction of energy expenditure in brown adipose tissue and skeletal muscle as well as the stimulation of glucagon-like peptide–1 (GLP-1) production in enteroendocrine L-cells, are mediated by the G-protein–coupled bile acid receptor 1 (GPBAR1). Therefore, we investigated in a cohort of white subjects at increased risk for type 2 diabetes mellitus whether a genetic variation within the *GPBAR1* gene contributes to prediabetic phenotypes, such as disproportionate fat distribution, insulin resistance, or β-cell dysfunction. We genotyped 1576 subjects (1043 women, 533 men) for the single nucleotide polymorphism rs3731859 in the *GPBAR1* gene. All subjects underwent an oral glucose tolerance test; a subset additionally had a hyperinsulinemic-euglycemic clamp. Regional fat distribution, ectopic hepatic and intramyocellular lipids were determined by magnetic resonance techniques. Peak aerobic capacity, a surrogate parameter for oxidative capacity of skeletal muscle, was measured by an incremental exercise test on a motorized treadmill. Total GLP-1 and gastric inhibitory peptide levels were determined by radioimmunoassay. After appropriate adjustment and Bonferroni correction for multiple comparisons, rs3731859 was not significantly associated with regional or ectopic fat distribution, peak aerobic capacity, levels of incretins, insulin sensitivity, or indices of insulin secretion. Nominal associations were found between rs3731859 and body mass index, waist circumference, fasting GLP-1 levels, and intramyocellular lipids in the soleus muscle ($P = .02$, $P = .02$, $P = .05$, and $P = .03$, respectively). Our data suggest that a common genetic variation within the *GPBAR1* gene may not play a major role in the development of prediabetic phenotypes in our white population.

1. Introduction

Bile acids play key roles in dietary lipid absorption and cholesterol homeostasis. Bile acids are signaling molecules with important endocrine functions that are mediated by activation of mitogen-activated protein kinase pathways of nuclear hormone receptors, such as farnesoid X receptor, and of the G-protein–coupled bile acid receptor 1 (GPBAR1), also known as TGR5. GBPAR1 is expressed in many tissues, including target tissues of insulin action, such as liver, skeletal muscle, and adipose tissue [1]. Some of the metabolic effects of bile acids, such as the induction of energy expenditure in brown adipose tissue and skeletal muscle [2], the stimulation of glucagon-like peptide–1 (GLP-1) production in enteroendocrine cells [3], and the antidiabetic effects of olive leaves [4], are mediated by GPBAR1. In line with these findings, in a murine *Gpbar1* knockout model, high-fat diet resulted in a significantly higher fat accumulation compared with wild types [5]. In light of these endocrine functions, *GPBAR1* appears to be an

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attractive prediabetes candidate gene. Therefore, we studied the impact of a common genetic variation in *GPBAR1* on prediabetes phenotypes including β-cell dysfunction, insulin resistance, and disproportionate body fat distribution.

2. Patients and methods

2.1. Subjects

One thousand five hundred seventy-six nondiabetic subjects at increased risk of type 2 diabetes mellitus were recruited from southern Germany and participated in an ongoing study on the pathophysiology of type 2 diabetes mellitus [6]. All subjects were metabolically characterized by an oral glucose tolerance test (OGTT). In randomly selected subgroups, a hyperinsulinemic-euglycemic clamp was performed. Total, visceral, and nonvisceral fats were determined by magnetic resonance imaging; and intramyocellular and intrahepatic lipids, by magnetic resonance spectroscopy. Peak aerobic capacity, a surrogate parameter for oxidative capacity of skeletal muscle, was measured using an incremental exercise test on a motorized treadmill (Saturn; HP-Cosmos, Traunstein, Germany); and total GLP-1 and total gastric inhibitory peptide (GIP) plasma concentrations were determined by radioimmunoassay. Participants gave informed written consent to the study. The protocol was approved by the local ethical committee.

2.2. Genotyping

Using the publically available phase II data of the International HapMap Project derived from a population of Utah residents with ancestry from northern and western Europe (release #23a March 2008, http://www.hapmap.org/index.html.en), we screened in silico the complete *GPBAR1* gene spanning 2844 bases from nucleotide 218 833 983 to nucleotide 218 836 826 (2 exons, located on human chromosome 2q35) as well as 5 kilobases each of its 5′- and 3′-flanking regions. Among 7 informative single nucleotide polymorphisms (SNPs), only 1 SNP, rs3731859 (located in the 5′ untranslated region), had a minor allele frequency (MAF) of at least 5%. Genotyping for rs3731859 was performed using the TaqMan assay (Applied Biosystems, Foster City, CA). The overall genotyping success rate was 100%, and rescreening of 3% of subjects gave 100% identical results.

2.3. Statistical analyses

Log-transformation of metabolic variables was performed before simple and multivariate linear regression analyses. In multivariate linear regression models, the trait was chosen as dependent variable; and sex, age, body mass index (BMI), and genotype were tested as independent variables. To account for the number of independent traits analyzed (anthropometrics, insulin sensitivity, and insulin secretion) and the 2 genetic models performed, a Bonferroni-corrected

Table 1
Associations of *GPBAR1* SNP rs3731859 with metabolic parameters

SNP	rs3731859							
Genotype	Mean	n	*d*	AA	AG	GG	$P_{additive}$	$P_{dominant}$
BMI (kg/m^2)	28.9 ± 8.2	1570	0.16	28.2 ± 8.0	29.1 ± 8.4	29.1 ± 8.1	.07	.02
Waist (cm)	94 ± 17	1522	0.16	92 ± 17	94 ± 18	95 ± 18	.04	.02
Glucose, fasting (mmol/L)	5.11 ± 0.55	1576	0.16	5.06 ± 0.51	5.12 ± 0.56	5.14 ± 0.58	.11	.10
AUC glc (mmol/L)	14.6 ± 3.4	1566	0.16	14.3 ± 2.9	15.1 ± 3.7	14.7 ± 3.3	.2	.08
Insulin, fasting (pmol/L)	64 ± 53	1563	0.16	58 ± 45	66 ± 56	66 ± 55	.3	.12
Insulin, 30-min OGTT (pmol/L)	494 ± 393	1559	0.16	480 ± 377	501 ± 404	494 ± 387	.8	.7
1st-phase insulin secretion (nmol/L)	1.27 ± 0.84	1559	0.16	1.23 ± 0.80	1.29 ± 0.87	1.28 ± 0.84	.18	.3
C-peptide, 30-min OGTT (nmol/L)	2.05 ± 0.89	1538	0.16	2.00 ± 0.84	2.08 ± 0.92	2.05 ± 0.90	.3	.8
AUC C-pep/AUC glc ([pmol/L]/[mmol/L])	320 ± 107	1520	0.16	318 ± 103	323 ± 109	317 ± 110	.2	.3
HOMA-IR (U)	2.48 ± 2.22	1563	0.16	2.23 ± 1.84	2.58 ± 2.40	2.56 ± 2.22	.2	.8
ISI, OGTT (U)	16.3 ± 10.7	1556	0.16	17.0 ± 10.5	16.2 ± 11.1	15.6 ± 10.3	.3	.14
ISI, clamp (μM·kg^{-1}·min^{-1}·mol/L^{-1})	0.085 ± 0.055	512	0.27	0.084 ± 0.049	0.087 ± 0.062	0.082 ± 0.043	.8	.7
GLP-1, fasting (pmol/L)	17.1 ± 8.7	171	0.48	18.1 ± 6.7	17.2 ± 9.4	15.7 ± 9.2	.06	.05
GIP, fasting (pmol/L)	14.5 ± 10.2	171	0.48	14.7 ± 9.2	14.0 ± 7.4	13.0 ± 6.2	.7	.5
TAT (% body weight)	30.2 ± 8.8	335	0.34	31.0 ± 7.6	30.2 ± 9.3	29.1 ± 9.3	.5	.5
VAT (% body weight)	3.3 ± 1.7	335	0.34	3.1 ± 1.7	3.4 ± 1.7	3.4 ± 1.6	.6	.4
Hepatic lipids (%)	6.5 ± 8.0	316	0.35	5.6 ± 7.6	6.8 ± 8.4	7.1 ± 7.6	.6	.4
IMCL tibialis anterior (AU)	4.1 ± 1.9	290	0.36	3.9 ± 1.6	4.3 ± 2.1	3.7 ± 1.8	.19	1.0
IMCL soleus (AU)	15.3 ± 7.7	224	0.41	16.2 ± 8.8	15.0 ± 7.7	14.4 ± 5.6	.09	.03
$VO_{2\ peak\ TM}$ (mL·min^{-1}·kg^{-1} lbm)	27.9 ± 9.3	946	0.20	27.8 ± 8.6	28.0 ± 9.7	28.0 ± 9.4	.5	.4

Unadjusted data are shown and given as mean ± SD. For statistical analysis, data were log-transformed; BMI and waist circumference were adjusted for age and sex; first-phase insulin secretion, C-peptide (30-minute OGTT), and AUC C-peptide/AUC glucose were adjusted for sex, age, BMI, and insulin sensitivity (OGTT); all other data were adjusted for sex, age, and BMI. Power calculation was performed in the dominant inheritance model ($1 - \beta > 0.8$, α level $P < .05$, 2-tailed *t* test) using G*power software available at http://www.psycho.uni-duesseldorf.de/aap/projects/gpower. AUC indicates area under the curve; C-pep, C-peptide; *d*, effect size *d*; glc, glucose; HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; lbm, lean body mass; TAT, total adipose tissue; TM, treadmill; U, units; VAT, visceral adipose tissue; $VO_{2\ peak}$, peak aerobic capacity.

α level of $P < .008$ was considered statistically significant. The statistical software package JMP 7.0 (SAS Institute, Cary, NC) was used. Hardy-Weinberg equilibrium was tested using the χ^2 test. Power calculation was performed in the dominant model ($1 - \beta > 0.8$; α level of $P < .05$) by 2-tailed tests using G*power software available at http://www.psycho.uni-duesseldorf.de/aap/projects/gpower.

3. Results

3.1. Characterization and genotyping of the study population

The characteristics of the 1576 nondiabetic subjects (1043 women, 533 men) are shown in Table 1.

rs3731859 was in Hardy-Weinberg equilibrium ($P = .2$). The observed MAF and the MAF published by HapMap were 0.458 and 0.400, respectively.

3.2. Associations between GPBAR1 SNP rs3731859 and metabolic traits

After appropriate adjustment and Bonferroni correction for multiple comparisons, rs3731859 was not significantly associated with regional or ectopic fat distribution, peak aerobic capacity, levels of incretins, insulin sensitivity, or indices of insulin secretion (all Ps $\geq .02$). Nominal associations were found between rs3731859 and BMI, waist circumference, fasting GLP-1 levels, and soleus muscle intramyocellular lipids (IMCL) ($P = .02$, $P = .02$, $P = .05$, and $P = .03$, respectively; Table 1).

4. Discussion

Genotyping of a metabolically well-characterized population for rs3731859 revealed no reliable association of the *GPBAR1* gene with regional fat distribution, aerobic physical fitness, insulin secretion, insulin sensitivity, or incretin levels. However, for some traits, for example, incretin levels, our study was insufficiently powered to detect small or moderate effect sizes. Furthermore, we cannot exclude that rare variants in the *GPBAR1* locus that have not been investigated in the present study may be associated with prediabetic phenotypes. Interesting trends for associations with whole-body adiposity, GLP-1 levels, and IMCL in the soleus muscle were found, the clinical relevance of which has to be investigated in larger studies.

It is worth noting that rs3731859 lies within a high-linkage disequilibrium block ($r^2 > 0.8$) that spans 195.7 kilobases and comprises 91 SNPs with MAFs of more than 5% located in 8 distinct genes. None of the recently reported diabetes-risk SNPs [7-10] lies within this high-linkage disequilibrium block.

In conclusion, our data suggest that common genetic variation within the *GPBAR1* gene may not play a major role in the development of prediabetic phenotypes in our white population at an increased risk for type 2 diabetes mellitus.

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