

C825T Polymorphism of the G Protein β_3 Subunit Is Associated with Obesity but Not with Insulin Sensitivity

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Abstract

STEFAN, NORBERT, MICHAEL STUMVOLL, FAUSTO MACHICAO, MATTHIAS KOCH, HANS U. HÄRING, AND ANDREAS FRITSCHE. C825T polymorphism of the G protein β_3 subunit is associated with obesity but not with insulin sensitivity. *Obes Res.* 2004;12:679–683.

Objective: The common C825T polymorphism of the gene that encodes the G protein β_3 subunit has been shown to influence lipolysis in human adipocytes and to be associated with hypertension, body fat distribution, and obesity. In addition, it has been shown to be associated with insulin resistance in a small group of hypertensive subjects. We investigated whether this polymorphism contributed to the variability in obesity in our population from southern Germany and whether it was associated with insulin sensitivity of lipolysis and/or glucose disposal.

Research Methods and Procedures: We determined percentage body fat, body fat distribution, glucose tolerance [oral glucose-tolerance test (OGTT)], insulin sensitivity, and serum free fatty acids using data from OGTTs ($N = 774$) and clamp (euglycemic hyperinsulinemic clamp, $N = 216$) in normal and impaired glucose tolerant subjects who were genotyped for this polymorphism.

Results: Compared with noncarriers of the C825T mutation, subjects with the C825T variant (prevalence $\sim 32\%$) had higher percentage body fat ($p = 0.02$) and higher BMI ($p = 0.03$). No conclusive effect was seen on serum free fatty acids measured either during fasting or at the end of a 2-hour OGTT. Insulin sensitivity determined during the

OGTT and during the clamp, both adjusted for age, gender, and percentage body fat, was not different between the genotypes ($p = 0.33$ and $p = 0.48$, respectively).

Discussion: We have concluded that the C825T polymorphism in the G protein β_3 subunit played an important role in the determination of obesity in this German population. However, it probably had no direct effects on insulin sensitivity of lipolysis and glucose disposal.

Key words: type 2 diabetes, insulin resistance, lipolysis, adipose tissue

Introduction

Guanine nucleotide-binding proteins (G proteins)¹ comprise a family of ubiquitously distributed signal-transduction proteins. A large number of hormones, neurotransmitters, and chemokines exert their intracellular effects by binding to G protein-coupled receptors (1). The gene encoding the G protein β_3 subunit is located on chromosome 12p13 (2). In a Chinese population, linkage for human essential hypertension has been shown at this locus (3). It has also been linked to obesity in the Cleveland Family Study (4) and the Quebec Family Study (5). With the generation of a splice variant, a common C825T polymorphism of the gene encoding the β_3 subunit is associated with enhanced signal transduction through G proteins (2). This functionally active variant, encoded by the 825T allele, has been shown to be associated with hypertension (2,6–8). There are also well-documented data indicating that this polymorphism is associated with human obesity (9–11) and post-pregnancy weight retention (12). Furthermore, functional data have shown that possible mechanisms include a lower lipolytic response of adipose tissue to catecholamines (13,14). However, little is known about whether this variant affects insulin sensitivity in humans. This is important be-

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¹ Nonstandard abbreviations: G protein, guanine nucleotide-binding protein; TÜF, Tübingen Family; OGTT, oral glucose-tolerance test; ISI, insulin sensitivity index.

Table 1. Characteristics and genotype effects

	Genotype (<i>N</i> = 774)			<i>p</i>
	CC	CT	TT	
Sex (M/F)	227/121	222/130	45/29	
Age	37 ± 1	36 ± 1	35 ± 1	0.30
Waist-to-hip ratio	0.84 ± 0.01	0.85 ± 0.01	0.85 ± 0.01	0.88
BMI (kg/m ²)	25.3 ± 0.3	26.1 ± 0.3	26.5 ± 0.7	0.03
Fasting plasma glucose (mM)	4.93 ± 0.03	4.94 ± 0.03	4.93 ± 0.06	0.87
2-hour plasma glucose (mM)	5.44 ± 0.06	5.54 ± 0.06	5.55 ± 0.12	0.46
Fasting plasma insulin (pM)	49 ± 2	51 ± 2	49 ± 3	0.64
2-hour plasma insulin (pM)	280 ± 13	304 ± 13	307 ± 28	0.42
Fasting serum free-fatty acids (μM)*	509 ± 12	496 ± 12	462 ± 23	0.20
120-minute serum free-fatty acids (μM)*	71 ± 2	66 ± 2	77 ± 6	0.02

Data represent means ± SE. *p* values were obtained using linear regression analysis with adjustment for age, gender, and BMI (except for age and BMI).

* Free fatty acids were only available in 732 subjects and were additionally adjusted for fasting insulin (fasting free fatty acids) and insulin at 120 minutes during the OGTT (120-minute free fatty acids).

cause G protein signaling has been shown to impair insulin signaling in vitro (15,16), and this may become a target for pharmacological intervention. The C825T polymorphism is one opportunity to study this question. Because the C825T mutation has been shown to alter signal transduction, through investigating associations of this variant with insulin-stimulated glucose disposal, a possible role of G proteins in human insulin sensitivity may become apparent.

Association of this polymorphism with type 2 diabetes has also been investigated. In a case-control study including subjects who have had diabetes for more than 10 years, the 825T allele is significantly associated with diabetes (17). Whether the polymorphism predisposes the subjects to obesity, which, in turn, may lead to diabetes, or whether this is independent of obesity, has not been determined.

To clarify the role of this polymorphism in metabolism, we investigated the associations of the polymorphism with obesity and insulin sensitivity of lipolysis and glucose disposal in a large, nondiabetic population of the Tübingen Family (TÜF) Study using data from oral glucose-tolerance tests (OGTTs; *N* = 774) and euglycemic hyperinsulinemic clamps (*N* = 216).

Research Methods and Procedures

Subjects

We analyzed data from 774 normal and impaired glucose tolerant subjects (18) who participated in the TÜF Study for type 2 diabetes. In this ongoing study, subjects with a family history of type 2 diabetes, from the southern part of Ger-

many, have been included. Most of them have been phenotypically and genotypically well characterized and have been included in a number of reports investigating the pathogenesis of obesity and type 2 diabetes (19–22). The participants did not take any medication known to affect glucose tolerance or insulin sensitivity. Of these, 216 underwent a euglycemic hyperinsulinemic clamp. Tests were done at 8:00 AM, after an overnight fast of 10 hours. The subjects were asked also to refrain from smoking for the same period.

Informed written consent was obtained from all participants, and the local medical ethics committee approved the protocol. The characteristics of the subjects are shown in Table 1.

Body Composition and Body Fat Distribution

Body composition was measured by bioelectrical impedance as percentage body fat. BMI was calculated as weight divided by the square of height (kilograms per meters squared). Waist and hip circumferences were measured with subjects in the supine position, and waist-to-hip ratio was calculated as an index of body fat distribution.

OGTT

All subjects underwent an OGTT. After an overnight fast, they ingested a solution containing 75 grams of dextrose, and, for determination of plasma glucose, venous blood samples were obtained at 0, 30, 60, 90, and 120 minutes. Insulin sensitivity was calculated from glucose and insulin values during the OGTT, as proposed by Matsuda and

DeFronzo (23). Serum free fatty acid concentrations were measured at 0 and 120 minutes.

Insulin Sensitivity

After a 12-hour overnight fast, an antecubital vein was cannulated for infusion of insulin and glucose at ~7:00 AM. A dorsal hand vein of the contralateral arm was cannulated and placed under a heating device to permit sampling of arterialized blood. After basal blood was drawn, subjects received a primed insulin infusion at a rate of 1.0 mU/kg per minute for 2 hours. Blood was drawn every 5 minutes for determination of blood glucose, and a glucose infusion was adjusted appropriately to maintain the fasting glucose level. An insulin sensitivity index (ISI) (in micromoles per kilogram body weight per minute per picomolar) for systemic glucose uptake was calculated as the mean infusion rate of glucose (in micromoles per kilogram per minute) necessary to maintain euglycemia during the last 60 minutes of the euglycemic hyperinsulinemic clamp divided by the steady-state serum insulin concentration.

Analytical Procedures

Blood glucose was determined using a bedside glucose analyser (glucose-oxidase method; Yellow Springs Instruments, Yellow Springs, CA). Plasma insulin was determined by microparticle enzyme immunoassay (Abbott Laboratories, Tokyo, Japan). This assay has a cross-reactivity of 0.005% with proinsulin. Serum free fatty acid concentrations were determined with an enzymatic method (WAKO Chemicals, Neuss, Germany).

Genotyping

The polymorphism in exon 10 of the G protein β_3 polypeptide 3 was detected by restriction fragment-length polymorphism. DNA was extracted from cellular blood components by the salting-out method. Genomic DNA was amplified using the following primer pair: forward, 5'-TGACCCACTTGCCACCCGTGC-3'; reverse, 5'-GCAG-CAGCCAGGGCTGGC-3'. The polymerase chain reaction was carried out in a final volume of 25 μ L containing 2 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate (Boehringer Mannheim, Mannheim, Germany), 0.2 mM each primer, and 1 U of Taq DNA polymerase (Biotherm; Gene Craft, Münster, Germany). After an initial denaturation of 5 minutes at 94 °C, the samples were subjected to 35 cycles at 94 °C for 1 minute, 69.3 °C for 45 seconds, and 72 °C for 1 minute, with a final extension of 5 minutes at 72 °C. The 268-bp product was restricted with BseD1 (Fermentas). The unrestricted 268-bp product represents the *T* allele, whereas a *C* allele was cut into 116- and 152-bp fragments. The three genotypes were scored after running on a 2.5% agarose gel with 10 μ g/mL ethidium bromide.

Statistical Analyses

Unless otherwise stated, data are given as mean \pm SE. Subjects with normal and impaired glucose tolerance were

analyzed together to prevent groups from becoming too small to be informative. Statistical comparison between genotype groups was performed by ANOVA, using logarithmically transformed data (for non-normally distributed parameters). To adjust the effects of relevant covariates (age, sex, BMI, plasma insulin), multivariate linear regression analyses were performed. The statistical software package JMP (SAS Institute, Cary, NC) or SPSS version 10.0 software (SPSS, Chicago, IL) was used.

Results

The frequency of the rare allele (*T*) was ~0.32 in our population. The genotype distribution was in Hardy-Weinberg equilibrium ($p = 0.4$, χ^2 test).

As reported in Table 1 and Figure 1, carriers of the 825*T* allele had higher BMI and percentage body fat compared with noncarriers of this allele. There was no association between genotype and body fat distribution and fasting and 2-hour glycemia and insulinemia before and after adjustment for age, gender, and BMI. There was also no association between genotype and fasting serum free fatty acids, which were additionally adjusted for fasting insulin. However, serum free fatty acids at 120 minutes during the OGTT were lower in the heterozygotes but higher in the subjects who were homozygous for the 825*T* allele compared with subjects who were homozygous for the 825*C* allele. This was the case before and after adjustment for age, gender, BMI, and insulin at 120 minutes. ISI determined during the OGTT (CC, 21.0 ± 0.6 ; CT, 19.5 ± 0.6 ; TT, 20.0 ± 1.3 arbitrary units), and during the euglycemic hyperinsulinemic clamp (CC, 0.11 ± 0.006 ; CT, 0.11 ± 0.007 ; TT, 0.10 ± 0.02 μ mol/kg/min per picomolar) were not statistically different between the genotypes (Figure 1).

Discussion

The aim of this study was to investigate whether the C825T polymorphism of the G protein β_3 subunit contributes to the variability in obesity and serum free fatty acids, an indirect measurement of lipolysis, in a population from southern Germany. In addition, we assessed whether this variant was associated with insulin sensitivity of glucose disposal in our large cohort of nondiabetic subjects.

The polymorphism was clearly associated with obesity. However, the association with body fat distribution that has been reported by others (11) was not apparent in our study. We also did not find an association of the polymorphism with fasting serum free fatty acids. There was a significant association of the polymorphism with free fatty acids at 120 minutes during the OGTT. However, subjects with CT had lower, whereas subjects with TT had higher, serum concentrations compared with those with CC. We cannot explain this finding entirely; however, it is possibly a chance finding, because of the low number of subjects in the group with

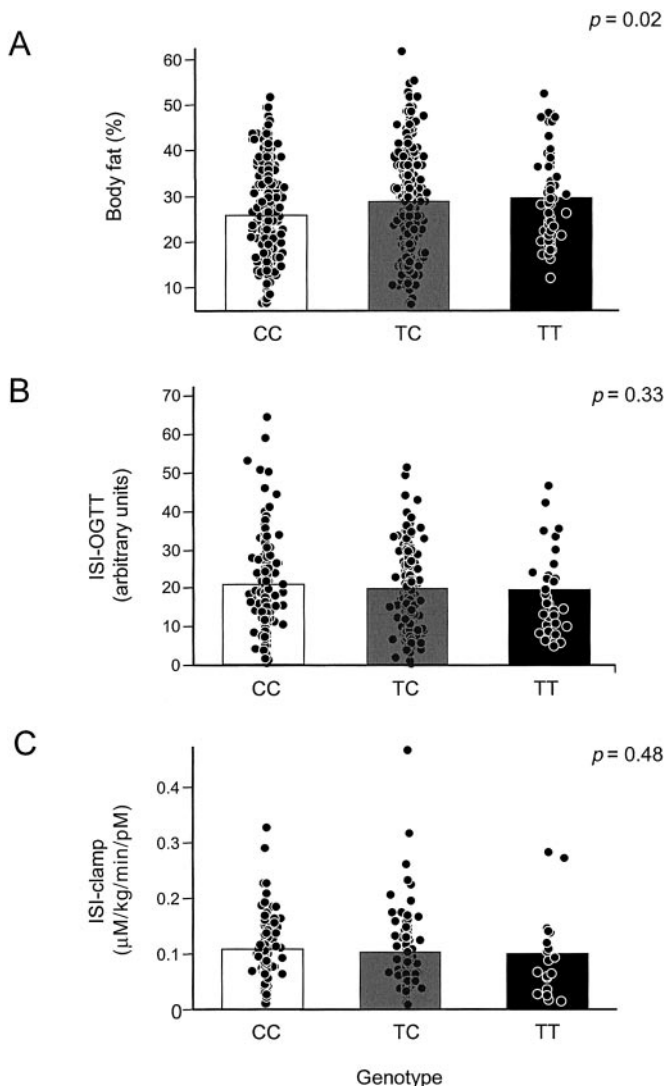


Figure 1: Associations between genotype according to the C825T polymorphism in the G protein β_3 subunit and percentage body fat (A), ISI determined during the OGTT (B), and ISI determined during an euglycemic hyperinsulinemic clamp (C).

TT. Thus, based on our data, this polymorphism does not seem to influence lipolysis *in vivo*. Nevertheless, this lack of an association between the polymorphism and measurements of lipolysis may well be different under conditions of catecholamine stimulation.

We did not find an association of the polymorphism with insulin sensitivity of glucose disposal in the cohort of subjects who underwent measurement of insulin sensitivity with the gold standard method (i.e., the euglycemic hyperinsulinemic clamp), nor did we find such an association in an even larger group, in whom we determined insulin sensitivity during the OGTT. To date, one study has found a lower insulin sensitivity, measured during an euglycemic hyperinsulinemic clamp, in carriers of the 825T allele

among a small ($N = 35$) group of hypertensive subjects. This association has been shown independent of obesity (24). Another study has found no association of the polymorphism with insulin sensitivity measured during an insulin tolerance test in patients with type 2 diabetes at baseline. After a standardized intervention program, however, carriers of the 825T allele become significantly more insulin sensitive than noncarriers (25).

In our large cohort of phenotypically well-characterized nondiabetic subjects, we were able to investigate the role of the C825T polymorphism in insulin sensitivity in humans. A priori power calculations showed that the design and population used in this study allowed detection of a 10% difference in insulin sensitivity, with a power of 99% (OGTT group) and 75% (clamp group), respectively ($\alpha = 0.05$). Therefore, it seems unlikely that we missed a strong or moderate effect of this polymorphism on insulin sensitivity. However, the analysis is only cross-sectional, and longitudinal data would be clearly superior, because subtle effects of this polymorphism on metabolism may become apparent when changes in age, adiposity, and insulin sensitivity are taken into account. Moreover, these types of association studies need to be interpreted with caution because, generally, the polymorphism under investigation need not be the causative one but may be in linkage disequilibrium with such a mutation.

In conclusion, the C825T polymorphism of the G protein β_3 subunit explains some of the variability in obesity seen in our population in southern Germany. However, the polymorphism is not associated, independent of obesity, with insulin sensitivity of lipolysis and glucose disposal.

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