G Protein β3 Gene Variant, Vascular Function, and Insulin Sensitivity in Type 2 Diabetes

José Manuel Fernández-Real, Georgina Peñarroja, Cristóbal Richart, Antoni Castro, Joan Vendrell,Montserrat Broch, Abel López-Bermejo, Wifredo Ricart

Abstract—A common polymorphism (825 C/T) in exon 10 of the GNB3 gene, that encodes for the β-3 subunit, has been associated with different degrees of activation of heterotrimeric guanine nucleotide binding proteins (G proteins). Many hormones and neurotransmitters use specific receptors that interact noncovalently with G proteins in the transmembrane signaling process. Among them, insulin uses an inhibitory G protein–sensitive mechanism that is involved in metabolic and vascular events, leading to enhanced glucose transport and vasodilation. We hypothesized differences in peripheral and vascular insulin sensitivity according to GNB3 gene polymorphism in type 2 diabetic patients. To address this issue, we used an intervention-optimization protocol to examine whether diabetic patients with the variant show a different response in terms of insulin-sensitivity. Interindividual differences in baseline insulin sensitivity and vascular dysfunction (vasodilatory response to glyceryl trinitrate) were not attributable to this polymorphism of the GNB3 gene. However, in contrast to normal homozygotes, insulin sensitivity (Si) significantly improved (P=0.01) in carriers of the 825T variant. Parallel to these findings, stimulated C-peptide tended to decrease, and the response to glyceryl trinitrate significantly improved (P=0.004) among 825T carriers. Body mass index, systolic and diastolic blood pressure, heart rate, or serum lipid levels did not significantly change in either group. Our findings suggest an effect of GNB3 gene polymorphism on important phenotypic variations in type 2 diabetes mellitus. The GNB3 gene polymorphism might be an example of pharmacogenetics, with the underlying etiological genetic defect altering the response to treatment.

(Hypertension. 2003;41:124-129.)

Key Words: G protein • endothelium • vascular resistance • nitric oxide • polymorphism • insulin • diabetes mellitus

Many hormones and neurotransmitters use specific receptors that interact noncovalently with guanine nucleotide binding proteins (G proteins) in the transmembrane signaling process.1 G proteins comprise a family of ubiquitous signal-transducing proteins. The large G proteins are heterotrimers of alpha, beta, and gamma subunits. The β-3 subunit preferentially links to the inhibitory G protein (Gi). A common polymorphism (825 C/T) in exon 10 of the GNB3 gene, which encodes for the β-3 subunit, has been recently identified.2 The 825T allele was associated with a splice variant, which shortens the protein by 41 amino acids and one domain. In vitro studies showed that this truncated protein was associated with increased activation of heterotrimeric G proteins. The GNB3 825T homozygous genotype has been associated with increased body mass index (BMI) among different populations.3,4 One of the proposed mechanisms is increased signaling by pertussis toxin–sensitive G proteins, which have been shown to stimulate adipogenesis.5 Increased Gi activity could also attenuate Gi-mediated lipolysis, leading to impaired adrenergic-mediated lipolysis and obesity.6 In fact, primiparous homozgygous carriers of the G protein β3 825T allele were at high risk of obesity and postpregnancy weight retention if they did not exercise regularly.7

The 825T allele of GNB3 is related to increased stimulated binding of labeled GTP in cell lines from hypertensive patients,2 in accordance with enhanced G protein activation and Na+–H+ exchange activity in these patients.8 The latter is associated with insulin resistance in hypertensive individuals.9 In the recent years, several studies have demonstrated that a Gs-sensitive mechanism is involved in insulin action.10–14 Insulin can modify responsiveness to agents that operate via G proteins,10–11 and reciprocally, pertussis toxin treatment attenuates some effects of insulin.12–13 Genetically engineered mice with a defect in Gs protein clearly demonstrate resistance to insulin action.14 In humans, immunodetection of G1a,2, in the liver was decreased by 40% in diabetic patients compared with obese nondiabetic subjects.15

Insulin is a pleiotropic signal for the target tissues. Independently of its effect on intermediary metabolism, insulin may have relevant effects on vascular tone, and these effects...
seem to be mediated by nitric oxide (NO). Recent studies suggest that the insulin interaction with NO also involves a specific receptor-mediated signal transduction pathway. Insulin appears also to sensitize endothelial cells to the effects of the α2-adrenergic pathway, which is closely coupled to G proteins to transduce their signal.

Subjects with the insulin-resistance syndrome show higher plasma concentrations of NO, both in the basal state and in response to insulin stimulation, compared with an insulin-sensitive control group. In fact, NO production has been described to be actually increased in type 2 diabetes mellitus (DM-2) patients and in their first-degree relatives. In the latter, insulin resistance was associated with an impairment in the ability of NO to generate its messenger (cyclic-GMP), leading to an increase in NO2-/NO3- . This increase could represent an effort to compensate for the defect in cyclic-GMP production.

We hypothesized differences in insulin sensitivity and vascular dysfunction, defined as a response to glyceryl trinitrate (GTN), according to GNB3 gene polymorphism in type 2 diabetic patients. We also put forth the hypothesis that the diabetic state per se may mask the effects of the 825T variant on diabetes-related phenotypes. To address this issue, we used an intervention-optimization protocol to examine whether diabetic patients with the variant show a different response in terms of insulin-sensitivity and GTN-sensitivity.

Methods

Patient Inclusion and Exclusion Criteria

All patients underwent a full medical history, including age, duration of diabetes, BMI, diet, smoking habits, blood pressure, total cholesterol, and a full examination to screen for diabetic complications. The diabetic patients were prospectively recruited from diabetes outpatient clinics on the basis of the following criteria: (1) age, 40 through 70 years; (2) current BMI <40 kg/m2; (3) stable metabolic control in the previous 6 months; and (4) no history of ketoadisosis. Exclusion criteria included the following: (1) clinically significant hepatic, neurological, endocrinologic, or other major systemic disease, including malignancy; (2) history of drug or alcohol abuse, defined as alcohol consumption over 80 g a day in men and over 40 g a day in women, or serum transaminase activity over twice the upper limit of normal; (3) elevated serum creatinine concentration; (4) acute major cardiovascular event in the previous 6 months; (5) acute illnesses and current evidence of acute or chronic inflammatory or infectious diseases; (6) hormone replacement therapy in women; and (7) mental illness rendering the subjects unable to understand the nature, scope, and possible consequences of the study. Informed written consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The experimental protocol was approved by the Ethical Committee of the University of Girona General Hospital, Girona, Spain.

Definition of Chronic Diabetic Complications

The clinical diagnosis of diabetic retinopathy was based on the examination of the ocular fundus after dilatation of the pupils by experienced ophthamologists. Simplex retinopathy was defined as one or more microaneurysms or hemorrhages. Diabetic macrogliopathy complications were diagnosed according to clinical findings, doppler sonography, and angiopathy. Persistent microalbuminuria was defined as an albumin excretion rate of 30 to 300 mg/d.

Study Design

All patients had been followed at our outpatient clinic for at least 1 year before the study. They had been instructed in diabetes care (diet, blood glucose monitoring, insulin administration, and self-adjustment). All patients were taught how to modify their insulin dose or diet and instructed in self-monitoring of blood glucose 3 or more times per day during a run-in period of 1 month. All patients were instructed to record the insulin dose immediately after each administration. Insulin doses were calculated during the week before initiation. Patients were seen in the outpatient unit every 4 weeks to reinforce their program compliance and to be instructed on insulin dose modifications. Patients considered eligible to participate in the study met with the doctor 4 weeks before initiation and every 2 months during the study. After the results of the laboratory tests were checked, the following tests were programmed at baseline and 4 months thereafter.

Measurements

Each subject was studied in the research laboratory in the fasting state. The room was quiet, lights were dimmed, and temperature was controlled at 23°C. BMI was calculated as weight divided by height squared (kg/m2). Each subject’s waist was measured with a soft tape midway between the lowest rib and the iliac crest. The hip circumference was measured at the widest part of the gluteal region. The waist-to-hip ratio was accordingly calculated. Blood pressure was measured in the supine position on the right arm after a 10-minute 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 3 readings taken at 5-minute intervals. Alcohol, caffeine, and all medications, including sulfonylureas, metformin, and insulin, were withheld for 12 hours before each test.

Study of Insulin Sensitivity

After the IV injection of regular insulin, glucose levels were determined every minute for 15 minutes. Insulin sensitivity was indicated by the first-order rate constant for disappearance rate of glucose (K2/g), estimated from the slope of the regression line of the logarithm of blood glucose against time during the first 3 to 15 minutes.

Study of β-Cell Function

Plasma C-peptide was determined basally and 10 minutes after the injection of 1 mg IV of glucagon (Novo-Nordisk).

Brachial Artery Vascular Reactivity

High resolution external ultrasound (128XP/10) mainframe with a 7.5-MHz linear array transducer (Toshiba SSH-140A) was used to measure changes in brachial arterial diameter in response to reactive hyperemia (leading to flow-mediated, endothelium-dependent dilation) and in response to 400 μg of sublingual GTN, an endotheliun-undependent, direct smooth muscle dilator, as described by Celermajer et al. The lumen diameter of the artery was defined as the distance between the leading edge of the echo of the near–wall–lumen interface to the leading edge of the far wall–lumen interface echo. All scans were ECG-triggered, coinciding with the R wave–end diastolic pressure. All images were recorded with a S-VHS videotape (Panasonic MD-830AG). Endothelial-dependent vasodilation was provoked secondary to hyperemia induced by inflation of a pneumatic tourniquet placed around the forearm, distal to the scanned part of the artery, up to a pressure of 300 mm Hg for 5 minutes, followed by sudden deflation. This maneuver is recognized to raise shear-stress on the endothelial cells, which, in turn, release nitric oxide (NO) and produce vasodilatation, allowing the testing of endothelial function. Endothelial-dependent vasodilatation is expressed as the percentage of change in the arterial diameter 1 minute after hyperemia. Endothelial-independent vasodilatation is calculated after sublingual administration of a 400-μg metered dose of GTN, an exogenous NO donor (Solitinirin® spray, Almirall Prodesfarma) and is expressed as the percentage of change in the arterial diameter 3 minutes later. Reactive hyperemia is calculated as the percentage change between the maximum flow recorded in the first 15 seconds after cuff deflation and the flow during the resting scan. A first scan was recorded after 10 minutes of rest in a quiet room in the supine position. Then the tourniquet was inflated for 5 minutes. A second scan was recorded for 90 seconds, beginning 10
GTN-induced vasodilation correlated with basal artery diameter 0.39 mm. Within-subject variability in 5 consecutive days (5 coefficient of variation was 4%, with a repeatability (CI 95%) of 0.27 mm (observer A). In observer B, the correlation of fixed effects between observers A and B was 9%. The CV obtained by a same observer was 3%. The Coefficient of variation (CV) between means obtained by observers A and B was 90%. The CI 95% for the analysis was 0.27 mm (observer A). In observer B, the correlation of fixed effects between observers A and B was 9%. The CV obtained by a same observer was 3%. The Coefficient of variation (CV) between means obtained by observers A and B was 90%.

Genotyping of G Protein β3 Subunit C825T Polymorphism

The polymorphism in exon 10 of the guanine nucleotide binding protein β polypeptide 3 (GNB3) was detected by restriction fragment length polymorphism (RFLP). DNA was extracted from cellular blood components by the salting-out method. Genomic DNA was amplified using the following primer pair: forward 5′ TGA CCC ACT TGC CAC CCG AGG GCT GCC 3′ and reverse 5′ GCC GCA GCC AGG GCT GCC 3′. The polymerase chain reaction was carried out in a final volume of 25 μL containing 2 mM of MgCl2, 0.2 mM of each dNTP (Boehringer Mannheim), 0.2 μM of each primer, and 1U of Taq DNA polymerase (Biotherm, Gene Craft). After an initial denaturation of 5 minutes at 94°C, the samples were subjected to 30 cycles at 94°C for 45 seconds, 68.1°C for 45 seconds, and 72°C for 45 seconds, 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-bp and 152-bp fragments. The 3 genotypes were scored after running on a 2.5% agarose gel with ethidium bromide 10 μg/mL.

Other Determinations

The serum glucose concentrations were measured in duplicate by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments). Glycosylated hemoglobin (HbA1c) was measured by high performance liquid chromatography using a fully automated glycosylated hemoglobin analyzer system (Hitachi L-9100). Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase, using a BM/Hitachi 747. High-density lipoprotein cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Low-density lipoprotein cholesterol was calculated using the Friedewald formula when applicable. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. Serum C-peptide concentrations were measured using a fluorimetric immunoassay (EG & G Wallac, Wallac Oy) with intra- and interassay coefficients of variation lowered to 6%.

Statistical Methods

We used a χ2 test for comparisons of proportions and either unpaired or paired t tests for comparisons of quantitative variables. A multiple linear regression analysis was used for age adjustments.

Results

Eleven subjects (2 women) were homozygous for the C allele and 18 (5 women) were carriers of the 825T allele. Tables 1 and 2 show baseline clinical characteristics of carriers and noncarriers of the variant allele, who were similar in BMI, waist-to-hip ratio (0.97±0.05 versus 0.97±0.06), and duration of diabetes. 825T carriers tended to be older (58.2±8.2 versus 52.2±7.2, P=0.06). Carriers and noncarriers did not differ significantly with regard to pharmacologic treatment and diabetic complications. Three subjects in each group were smokers. No subject had proliferative retinopathy. Baseline artery diameter and response to GTN were also not significantly different among genotypes. The program did not induce significant changes in BMI, systolic and diastolic blood pressure, heart rate, or serum lipid levels (Table 1). HbA1c decreased significantly during the optimization program, from 6.71±1.04% to 6.42±1.1% (P=0.02) when all
individuals were considered as a group. Reduction in HbA1c was not significantly different between carriers and noncarriers of the variant allele (Table 1). However, insulin sensitivity (SI) significantly improved after the optimization program in carriers of the 825T variant in contrast to normal homozygotes (Figure). In parallel with these findings, stimulated C-peptide tended to decrease among 825T carriers. The apparent decrease in insulin sensitivity (KITT) in homozygous C allele carriers was almost entirely due to one subject in which K unexpectedly decreased by 50%. After excluding this subject, the SI remained essentially unchanged in this group of subjects.

Among carriers and noncarriers of the gene variant, basal artery diameter remained unchanged throughout the study period (Table 2). However, in 6 patients, artery diameter significantly increased after the optimization program (from 4.1±0.87 mm to 4.5±0.95 mm (P=0.03), and 5 of these patients were carriers of the 825T allele.

Paralleling the insulin sensitivity increase, the response to GTN significantly improved after the study period among 825T carriers (Table 2 and Figure). In a multivariant analysis, the change in insulin sensitivity (P=0.04), but not age or HbA1c changes, independently predicted the change in the vascular response to GTN, contributing to 29.9% of its variance. Endothelium-dependent vasodilation did not change significantly in any group of subjects.

**Discussion**

Our findings suggest an effect of a genetic polymorphism on important phenotypic changes in type 2 diabetes mellitus. Interindividual differences in baseline insulin sensitivity and vascular dysfunction were not attributable to a polymorphism of the GNB3 gene. However, after an intervention-optimization program to examine whether diabetic patients with the variant show a different response in terms of peripheral insulin–sensitivity and vascular dysfunction, we found that the response to metabolic optimization differed according to this polymorphism. Carriers of the 825T allele were more likely to display improvements in insulin action than carriers of the C allele. The improvement in insulin action was demonstrated by the increase in SI. Furthermore, we found a tendency toward lower stimulated C-peptide levels after the intervention. Recent studies have found a predominance of β-cell downregulation in response to improved insulin sensitivity. Significant reductions in insulin secretion accounted for the majority of the overall increase in S1. Thus, in our patients, lower stimulated C-peptide is a probable reflection of improved β-cell sensitivity.

The findings described here mirrored what has been found in primiparous homozygous carriers of the G protein β3 825T allele who were at high risk of postpregnancy weight retention if they did not exercise regularly. These subjects specifically benefit from physical activity, because, otherwise, postpregnancy weight retention may result in increased BMI associated with higher risk for diabetes and cardiovascular disorders. In a similar way, we can hypothesize that DM-2 patients with the 825T allele are those who specifically benefit from an optimization program.

Increased Gs activity could tonically attenuate Gi-mediated lipolysis in 825T carriers, and this effect would be observable only after improvements in insulin bioavailability. In fact, impaired G, function, as found in pseudohypoparathyroidism,
is characterized by impaired adrenergic-mediated lipolysis and obesity. The differences in improvement of insulin sensitivity among carriers and noncarriers of the 825T variant were observed despite a similar decrease in glycosylated hemoglobin in both groups. Thus, subjects with 825C allele seem somewhat resistant to the beneficial effects of treatment of insulin sensitivity.

Baseline lipid levels did not further improve after the optimization program, although a tendency toward reduction in serum triglycerides was observed among normal homozygotes. The concomitant treatment with statins in some patients (in 2 C/C homozygotes and in 3 T allele carriers) precludes, however, a correct interpretation of the lipid response to therapy in these patients. Statins were maintained during the trial. The influence of such treatment probably masks any effect of this polymorphism on lipids.

The arterial smooth muscle response to GTN has been consistently demonstrated to be impaired in humans with risk factors for atherosclerosis and in diabetic patients. In fact, primary nitrate tolerance has been recognized to occur in diabetes mellitus. Extensive evidence is available to show that the chemical nature, mechanism of action, and biologic actions of NO and the organic nitrates parallel one another. This was specific for GTN vascular response, because endothelium-dependent vasodilation remained essentially unchanged in both groups of subjects. Insulin appears to sensitize endothelial cells to the effects of the α2-adrenergic pathway, which is closely coupled to G proteins to transduce their signal. Previous studies had suggested that insulin stimulates NO production and release from the endothelium. However, those conclusions were derived from the use of pharmacological insulin levels. The employment of more physiological insulin concentrations has shown that this sensitizing effect is accounted for by levels of the hormone that have no direct effect on the release of NO, and that cause vasorelaxation. Lembo et al have proposed a specific cross-talk between insulin and the α2-adrenergic pathways at the endothelial level, and both pathways seem to be closely coupled through G proteins. Insulin significantly blunts the sympathetic vasoconstriction, and this is in line with the higher change in artery diameter after optimization in some 825T carriers. Furthermore, the improved response to GTN might be secondary to improved insulin sensitivity itself: the improved vascular insulin action might lead to enhanced GTN-sensitivity through the α2-adrenergic pathway.

In recent years pharmacogenetics is increasingly reinforced in clinical research and clinical medicine. The possibility that disease could be treated according to genetic and specific individual markers may improve outcomes by predicting individual response to drugs. The GNB3 gene polymorphism might be an example of pharmacogenetics, with the underlying etiological genetic defect altering the response to treatment.

**Perspectives**

Many hormones and neurotransmitters use specific receptors that interact noncovalently with G proteins in the transmembrane signaling process. The large G proteins are heterotrimers of alpha, beta, and gamma subunits. The β-3 subunit preferentially links to the inhibitory G protein (G). Insulin is a pleiotropic signal for the target tissues, and, independently of its effect on intermediary metabolism, may have relevant effects on vascular tone. Insulin appears to sensitize endothelial cells to α2-adrenergic pathways via G proteins, which transduce their signal.

A common polymorphism (825 C/T) in exon 10 of the GNB3 gene, which encodes for the β-3 subunit, is frequently observed in the general population. The 825T allele is associated with a splice variant, which shortens the protein by 41 amino acids and 1 domain. This truncated protein was associated with increased activation of heterotrimeric G proteins in in vitro studies. This variant has also been previously found to be associated with several metabolic abnormalities, such as weight gain. In this article, the 825T allele was not related to baseline vascular dysfunction or insulin action in type 2 diabetic patients. However, following an optimization program that led to decreased integrated glucose levels, 825T allele carriers showed a significant improvement in vascular dysfunction. These findings are probably linked to the concomitant improvement in insulin sensitivity, given the known effect of insulin on sympathetic vasoconstriction. The GNB3 gene polymorphism could be considered an example of pharmacogenetics, with the underlying etiological genetic defect altering the response to treatment.

**Acknowledgments**

This work was partially supported by grant 00/0024-01 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain. We thank Drs Dolores Cabrero and Nuria Aleixandre for technical assistance.

**References**


G Protein β3 Gene Variant, Vascular Function, and Insulin Sensitivity in Type 2 Diabetes
José Manuel Fernández-Real, Georgina Peñarroja, Cristóbal Richart, Antoni Castro, Joan Vendrell, Montserrat Broch, Abel López-Bermejo and Wifredo Ricart

Hypertension. 2003;41:124-129; originally published online November 11, 2002;
doi: 10.1161/01.HYP.0000042428.24031.73

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/41/1/124

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2003/01/02/41.1.124.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
EXPANDED MATERIALS AND METHODS

Brachial artery vascular reactivity

High resolution external ultrasound (128XP/10 mainframe with a 7.5-MHz linear array transducer, (Toshiba SSH-140A, Japan) was used to measure changes in brachial artery diameter in response to 400 µg of sublingual nitroglycerin (NTG), an endothelium-independent, direct smooth muscle dilator, as described by Celermajer et al. (22).

The lumen diameter of the artery was defined as the distance between the leading edge of the echo of the near wall-lumen interface to the leading edge of the far wall-lumen interface echo. All scans were taken ECG-triggered coincident with the R wave –end diastolic-. All images were recorded with a S-VHS videotape (Panasonic MD-830AG). Endothelial-independent vasodilatation is induced after sublingual administration of a 400-µg metered dose of glyceryltrinitrate (GTN), an exogenous NO donor (Solinitrina spray®, Almirall Prodesfarma, Barcelona, Spain) and expressed as the percentage of change in the arterial diameter 3 minutes later. A first scan was recorded after 10 minutes of resting in a quiet room in the supine position. A last scan was recorded starting 2 minutes after GTN administration for 70 seconds. All images registered on super-VHS tape were subsequently analyzed by two independent observers blinded to the randomization of the subject and the stage of the experiment. Each observer analyzed the arterial diameter during four cardiac cycles for each condition, and these measurements were averaged.

Prior to the initiation of the study in diabetic subjects, validation of this technique was performed through the evaluation of inter- and intra-observer reproducibility in twenty-two healthy subjects (12 men and 10 women, mean age 30.1 years (95% confidence interval (CI) 27.1, 33.2), body mass index 22.6 Kg/m² (CI 21.3, 23.8). Measurements were performed by two observers (A and B). Intraclass coefficient of correlation of fixed effects between observers A and B was 0.90. Coefficient of variation (CV) between means obtained by observers A y B was 9%. The CV obtained by a same observer was 3%. The reproducibility (CI 95%) was 0.27 mm (observer A). In observer B the coefficient of variation was 4%, with a reproducibility (CI 95%)
of 0.39 mm. The study of the variability of the means by the same observer in 5 consecutive
days showed a CV of 6% (observer A) and 2% (observer B). The GTN-induced vasodilation
correlated with basal artery diameter ($r = -0.67; \ p = 0.025$).