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Association of Insulin Sensitivity and Glucose Tolerance with the c.825C>T Variant of the G Protein Beta-3 Subunit Gene

Daniel Kopf, M.D.¹, Li S.-C. Cheng, M.D.², Petra Blandau¹, Willa Hsueh, M.D.³, Leslie J. Raffel, M.D.², Thomas A. Buchanan, M.D.⁴, Anny H. Xiang, Ph.D.⁵, Richard C. Davis, Ph.D.⁶, Jerome I. Rotter, M.D.⁶, and Hendrik Lehnert, M.D.^{1,7}

¹ Department of Endocrinology and Metabolism, University of Magdeburg, Germany

² Department of Pediatrics, University of California at Los Angeles, CA, USA

³ Department of Medicine, University of California at Los Angeles, CA, USA

⁴ Department of Medicine, University of Southern California, Los Angeles, CA, USA

⁵ Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA

⁶ Department of Medicine, University of Southern California, Los Angeles, CA, USA

Abstract

The risk of macrovascular complications of diabetes mellitus is greatly enhanced by the presence of high blood pressure. In addition, hypertension and diabetes share insulin resistance as a common pathophysiological mechanism. Despite evidence for a common molecular genetic background of insulin resistance, glucose intolerance and hypertension, few candidate genes have been shown to influence all of these features simultaneously. We examined the association of insulin sensitivity with the c.825C>T variant of the g-protein beta-3 subunit (GNB3), a candidate gene of hypertension, in families of Mexican American hypertensive patients.

Methods—180 subjects enrolled in a family study of Mexican American hypertensive patients were recruited from hypertension clinics in Los Angeles. Subjects underwent pre-treatment blood pressure recording, an oral glucose tolerance test, euglycemic, hyperinsulinemic clamp and anthropometric measurements. DNA from peripheral blood leukocytes was genotyped by PCR and restriction enzyme digest with BseDI (GNB). Statistical analysis was performed by transmission disequilibrium testing.

Results—In carriers of the T-allele, blood glucose was significantly lower (fasting: 96.7 ± 22.9 vs. 106.7 ± 51.7 mg/dl, $p = 0.009$, oGTT 120 min: 131.7 ± 48.7 vs. 137.8 ± 64.9 mg/dl, $p = 0.036$; mean $\pm$ SD) and insulin sensitivity significantly higher (229.0 ± 108.7 vs. 188.5 ± 94.2 mg/kg/min, $p = 0.037$) than in homozygous carriers of the C-allele. Blood pressure did not differ significantly between the phenotypes.

Conclusion—In a Mexican American hypertensive population, we found evidence for higher insulin sensitivity in carriers of the T allele of the c.825C>T variant of GNB3.

Address for correspondence: Daniel Kopf, Internal Medicine Consult Service, Central Institute of Mental Health, J 5, D-68159 Mannheim, Germany, Phone: ++49 (621) 1703-145, Fax: ++49 (621) 234 25, e-mail: E-mail: Kopf@zi-mannheim.de.

⁷Currently: Department of Medicine, University of Warwick, Coventry, UK

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Keywords

Insulin resistance; GNB3; euglycemic clamp; blood pressure; metabolic syndrome

Introduction

Insulin resistance is a characteristic feature not only of diabetes mellitus type 2, but also of hypertension (Reaven 1988). Both conditions are believed to be complex genetic disorders, with multiple genes interacting with environmental and behavioural influences to bring forth the phenotype. There is evidence that a number of genes may predispose to insulin resistance, glucose intolerance and hypertension at the same time, while other genes may affect blood pressure, but not glucose tolerance, and vice versa (Kopf & Lehnert 2001; Groop & Orho-Melander 2001). On the other hand, it is possible that a given gene may predispose to hypertension, while it exerts a protective effect on insulin sensitivity and glucose metabolism, or vice versa.

However, despite epidemiological evidence of genetic linkage, few candidate genes have been characterized concerning their effects on both insulin sensitivity and blood pressure. This is most likely due to the fact that exact measurement of insulin sensitivity by the preferred technique, the euglycemic, hyperinsulinemic clamp (Ferrannini & Mari 1998), is laborious and not part of routine clinical assessment. Therefore, current knowledge about insulin sensitivity in carriers of candidate genes of hypertension is limited.

In a well established, unique family study of Mexican American hypertensive patients, extensive phenotypic characterization was carried out, including euglycemic, hyperinsulinemic clamp as well as measurements of salt sensitivity of blood pressure, intima-media thickness and various other metabolic variables and intermediate phenotypes (Xiang et al. 2001, Cheng et al. 2001). The study has yielded evidence for joint genetic control of blood pressure and insulin sensitivity.

In this population, we studied the c.825C>T polymorphism of the G-protein beta 3 subunit (GNB3) gene, which is an extensively studied candidate gene of hypertension. Its association with hypertension has first been described in a German population (Siffert et al. 1998). Further studies found association with obesity (Siffert et al. 1999; Stefan et al. 2004).

GNB3 is a member of the pertussis sensitive G protein family. The GNB3 gene is located on the short arm of chromosome 12 (12p13). The T allele of the c.825C>T polymorphism favours production of an alternatively spliced, truncated variant, which facilitates intracellular signal transduction (Siffert et al. 1998; Virchow et al. 1998). Inhibition of G protein action by pertussis toxin has been shown to impair autophosphorylation and activation of the insulin receptor (Müller-Wieland et al. 1991).

Some investigators have tested association with insulin sensitivity, yielding heterogeneous results (Stefan et al. 2004; Poch et al. 2002; Brand et al. 2003; Saller et al. 2003; Wascher et al. 2003). Most of these studies either relied on indirect estimates of insulin sensitivity (Brand et al. 2003; Wascher et al. 2003), or performed euglycemic, hyperinsulinemic clamp in very small samples (n=35, Poch et al. 2002; n=30, Saller et al. 2003).

The scope of the present study was to test the influence of this polymorphism on insulin sensitivity, assessed by euglycemic hyperinsulinemic clamp, on glucose tolerance and on other features of the metabolic syndrome. Investigations were performed within the Mexican American family study and evaluated by transmission disequilibrium testing.

Subjects and Methods

Study population

We recruited index patients of Mexican, Salvadoran, or Guatemalan ancestry, who were treated for primary hypertension according to WHO criteria at the Hypertension Clinics at Los Angeles County–University of Southern California Medical Center. In addition, their first degree relatives and spouses were examined. Age of subjects and spouses ranged from 18 to 65 years of age, while offspring had to be at least 16 years old in order to be included.

Fasting blood glucose of 140 mg/dl or higher, systolic and diastolic blood pressure above 200 or 115 mmHg, respectively, or significant cardiac disease were exclusion criteria. A total of 180 subjects out of 41 families were included into the genome scan.

All participants had given written, informed consent to the protocol, which had been approved by the Institutional Review Board.

Phenotypic characterization

After at least 5 minutes of rest, three sitting *blood pressure* readings were taken at five minute intervals using a Dinamap (Criticon, Inc.). These three measurements were averaged. In hypertensive patients, blood pressure was measured prior to initiation of antihypertensive treatment.

Following an overnight fast, an oral glucose tolerance test was performed using 75 g of glucose.

Additionally, subjects underwent *euglycemic, hyperinsulinemic clamp* with a primed infusion of human insulin (NovoNordisk) at a constant rate of 60 mU/m² body surface area per minute. Dextrose infusion was adjusted in five minute intervals to maintain blood glucose at 100 mg/dl. Potassium chloride was infused at 5 mmol/h. Insulin sensitivity was calculated as glucose infusion rate during the final 30 minutes of the clamp procedure, divided by the increment in plasma insulin over basal levels, corrected by body surface area.

Salt sensitivity of blood pressure was assessed by the Weinberger protocol (Weinberger et al. 1986), with subjects receiving an infusion of 1000 ml isotonic saline over 4 hours. They were then placed on a low sodium diet (10 mmol/d) for the next 24 h hours. On the following day, furosemide was given at a dosage of 40 mg t.i.d. The difference in blood pressure readings at the end of the saline infusion and after furosemide was then used as an estimate of salt sensitivity.

Intima-media thickness was measured at the common carotid artery using a 7.5 kHz ultrasound probe.

Laboratory assays

Blood glucose was measured by the glucose oxidase method (Beckman Glucose Analyser), insulin by radioimmunoassay (Linco Research, St. Charles, MO, USA). Cross-reactivity with human pro-insulin was less than 0.2 %. Lipoproteins were separated by lipid ultracentrifugation and phosphotungsten precipitation. Cholesterol was determined by the Chod-PAP method; triglycerides by the GPO-PAP method on automated analysers.

Genotyping

DNA was extracted from peripheral blood leukocytes by phenol extraction. A specific 268 bp DNA fragment was amplified via polymerase chain reaction (PE 9600, Perkin-Elmer, Norwalk, CT, USA) at an annealing temperature of 60°C and an annealing time of 45 seconds. Custom

primers, obtained from Life Technologies Inc, Karlsruhe, Germany, had the following sequences: 5'-TGA CCC ACT TGC CAC CCG TGC-3' (sense) and 5'-GGC AGC AGC CAG GGC TGG C-3' (antisense). The C.825C>T polymorphism was typed by restriction enzyme digest with BseD1 (MBI Fermentas, Germany) at 37°C for 4 hours. Following separation on a 2 % agarose gel containing ethidium bromide, the fragments were visualized under ultraviolet light.

Statistics

Data were log-transformed when needed to obtain variables with normal distribution. For genetic markers, PedCheck 18 was used to detect inconsistencies in Mendelian segregation. The values of systolic, diastolic and mean arterial pressure were adjusted for sex, and their residual values were used in SIBPAL analysis. In order to test for quantitative traits, we examined association of variables with the GNB3 polymorphism by transmission disequilibrium testing. This method was originally designed for qualitative traits and accounts for transmission of alleles in families (Spielman et al. 1993). It utilizes all available genetic information obtained in pedigrees of variable size. We applied a modification of this technique for evaluation of quantitative traits as described by Allison (Allison 1997) and implemented in the QTDT program by Abecasis et al. (Abecasis et al. 2002).

Results

Allele frequencies

The c.825C>T polymorphism was distributed as follows: C 63,8 %; T 36,2 %. The population was in Hardy-Weinberg equilibrium.

Glucose metabolism

We found a significant association of the C.825C>T polymorphism with fasting glucose ($p = 0.009$) and 2h glucose ($p = 0.036$) during oGTT with lower values in homozygous carriers of the T allele. In addition, insulin sensitivity differed significantly between carriers of the T allele and homozygous carriers of the C allele with higher glucose infusion rate as a measure of insulin sensitivity in homozygous carriers of the T allele ($p=0.037$, Fig. 1). Plasma insulin levels were virtually identical (fasting: CC vs. CT or TT 20.2 ± 13.6 vs. 19.2 ± 16.7 ; 2 hour oGTT 99.9 ± 64.2 vs. 100.9 ± 87.7 mU/l).

Blood pressure and lipid metabolism

Our data revealed no linkage or association of systolic or diastolic blood pressure with genotype. Moreover, salt sensitivity, as assessed by the Weinberger protocol, or intima-media thickness did not differ between carriers of T or C alleles (Tab. 1). Total cholesterol, HDL, LDL cholesterol, total triglycerides and free fatty acids were unaffected by genotype.

Discussion

While our analysis of data obtained from a Mexican-American population, one of the most extensively characterized family studies of the metabolic syndrome, yielded an association of the T825 allele of the GNB3 gene with lower fasting glucose, lower glucose in oGTT and improved insulin sensitivity, we found no significant association of blood pressure with this locus.

Overall, studies examining the influence of GNB3 c.825C>T polymorphism on blood pressure are controversial, with some investigators failing to confirm association (Kato et al. 1998; Hegele et al. 1999; Brand et al. 1999; Snapir et al. 2001; Huang et al. 2003) and one study finding an association in a subgroup only (Brand et al. 2003). Ethnic differences appear to play

a role (Siffert et al. 1999). The present study, which is the first to investigate a Latin American population, also fails to support the hypothesis of a considerable role of the c.825C>T polymorphism on blood pressure regulation in this ethnic group. Interestingly, two previous studies in Spanish populations, who are closely related to Mexican Americans by migration, did not find an effect of this polymorphism on blood pressure regulation (Poch et al. 2002; Fernandez-Real et al. 2003).

In contrast, we found significant association with insulin sensitivity and glucose metabolism. An earlier study in a German population had investigated insulin sensitivity by means of euglycemic, hyperinsulinemic clamp (Stefan et al. 2004). Although in that study, the T allele was associated with increased BMI and body fat, there was no association with insulin sensitivity. Only two other clamp studies have been reported, which, however, had been performed in very small population samples (n=35 in Poch et al. 2002; n=30 in Saller et al. 2003). The paper by Poch and colleagues reported association of insulin resistance with the T allele (Poch et al. 2002), while the study performed by Saller et al. did not replicate this finding (Saller et al. 2003).

In contrast to the study from Stefan et al. (Stefan et al. 2004), our data suggest that the T allele is indeed associated with improved insulin sensitivity. This finding appears robust, since fasting and oGTT 2-hour glucose were both significantly lower. Furthermore, our sample size is comparable to that of Stefan et al. and much higher than in either of the two other clamp studies (Poch et al. 2003; Saller et al. 2003). Finally, we performed transmission disequilibrium testing, a technique which utilizes information in small pedigrees, thus making it superior to mere association studies.

From an epidemiological point of view, our result is surprising: In those previous studies which found significant association with hypertension, this feature of the metabolic syndrome was coupled to the T allele. As hypertension is characterized by insulin resistance, one would have expected the T allele to be associated with insulin resistance rather than improved insulin sensitivity.

Consideration of molecular effects of the C.825C>T polymorphism provides a possible explanation: The second messenger pathway coupled to GNB3, a member of the pertussis toxin sensitive G protein family, shares important steps with insulin signalling. Inhibition of G protein action by pertussis toxin has been shown to impair intracellular insulin signalling (Luttrell et al. 1998; Müller-Wieland et al. 1991). The T allele, in contrast, enhances intracellular signal transduction of receptors coupled to pertussis toxin sensitive G proteins (Virchow et al. 1998). Thus, insulin signalling is most likely facilitated in cells carrying the T allele.

As a consequence, the 825T variant of the GNB3 appears to predispose to improved intracellular signalling both of insulin effects and of pressor hormones. The resulting phenotype – improved insulin sensitivity or hypertension – may be determined by interaction of this allele with the genetic background and behavioral characteristics of the population studied.

It is not surprising that this effect of the T allele on insulin sensitivity was first shown in the Mexican American population, in which the polymorphism obviously does not affect blood pressure and body weight. Since hypertension and obesity are known to secondarily impair insulin sensitivity, regardless of the genetic background, and carriers of the T allele in the population studied by Stefan et al. were prone to obesity (Stefan et al. 2004), this may have masked a possible direct insulin sensitizing effect of the G protein variant. To the best of our knowledge, the GNB3 c.825C>T variant is the first genetic polymorphism that may drive insulin resistance on one hand and blood pressure on the other hand in opposite directions, depending on the genetic or ethnic background of the population studied. This finding underscores the genetic complexity of the metabolic syndrome.

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Figure 1. Fasting glucose ($p=0.009$), 120 min glucose ($p=0.036$) in an oral glucose tolerance test (A) and insulin sensitivity (B; $p=0.037$) in homozygous carriers of the C allele versus carriers of the T allele.

Table 1
Age, blood pressure and metabolic parameters by genotype.

Parameter	GNB3 gene			CC vs. comb. CT and TT
	CC	CT	TT	
N	73	83	24	
Age [years]	39.5 +/-15.9	37.5 +/-14.8	38.2 +/-15.2	n.s.
Gender [males/females]	19 / 54	40 / 43	11 / 13	p=0.014 ¹
Waist-to-hip ratio	0.907 +/-0.076	0.903 +/-0.111	0.880 +/-0.121	n.s.
Fasting glucose [mg/dl]	106.7 +/-51.7	97.3 +/-25.5	94.8 +/-11.3	p=0.009
Insulin sensitivity [mg*min ⁻¹ *m ⁻²]	188.5 +/-94.2	231.0 +/-106.1	222.6 +/-119.0	p=0.037
Systolic blood pressure [mmHg]	131.1 +/-20.6	126.5 +/-27.0	129.2 +/-21.6	n.s.
Diastolic blood pressure [mmHg]	76.5 +/-9.7	75.4 +/-13.9	78.7 +/-11.7	n.s.
Salt sensitivity of mean arterial pressure [mmHg]	7.7 +/-5.6	6.9 +/-6.8	7.5 +/-3.2	n.s.
Intima media thickness [mm]	0.62 +/-0.15	0.63 +/-0.21	0.61 +/-0.14	n.s.
Total cholesterol [mg/dl]	186.6 +/-33.9	184.9 +/-38.1	179.6 +/-29.9	n.s.
HDL cholesterol [mg/dl]	49.8 +/-13.8	46.2 +/-13.4	45.9 +/-13.2	n.s.
LDL cholesterol [mg/dl]	113.9 +/-28.9	114.5 +/-33.6	108.5 +/-27.8	n.s.
Total triglycerides [mg/dl]	123.3 +/-133.1	122.4 +/-91.2	126.1 +/-70.5	n.s.

¹ Since gender was unevenly distributed between groups, the remaining parameters were calculated after adjustment for sex