Associations of a Human G Protein β3 Subunit Dimorphism With Insulin Resistance and Carotid Atherosclerosis

Thomas C. Wascher, MD; Bernhard Paulweber, MD; Liliane Malaimare, MD; Andreas Stadlmayr, MD; Bernhard Iglseder, MD; Isabella Schmoelzer, MD; Wilfried Renner, PhD

Background and Purpose—The C825T dimorphism of the gene encoding the human G protein β3 subunit (GNB3) is associated with hypertension and obesity. Although these findings suggest an association with insulin resistance and atherosclerosis, this hypothesis has yet been tested only partially.

Methods—To investigate this hypothesis, the C825T dimorphism was determined in a population of 932 middle-aged white subjects of middle European (Austrian) origin. Insulin sensitivity was measured with the short insulin tolerance test; intima-media thickness of the carotid artery and morphological plaque burden were measured by ultrasound.

Results—Insulin sensitivity was found to be significantly lower in carriers of the T allele (3.55±1.27 versus 3.92±1.30%/min, P=0.012) in the group of male subjects with abdominal body fat distribution (waist-to-hip ratio >0.9). No effect was observed in women or men with a waist-to-hip ratio <0.9. Advanced carotid artery plaques were more frequent (odds ratio, 1.60; 95% confidence interval, 1.002 to 2.575; P=0.04) in carriers of the T allele regardless of sex. No effect was observed with regard to carotid artery intima-media thickness.

Conclusions—In summary, our results demonstrate that the GNB3 C825T allele is associated with reduced insulin sensitivity in men with abdominal fat distribution and with more advanced carotid atherosclerosis in middle-aged white men and women. (Stroke. 2003;34:605-609.)

Key Words: atherosclerosis ■ genetics ■ insulin ■ proteins

Heterotrimeric G proteins are ubiquitously expressed, fundamental accessories of a multitude of transmembrane receptors involved in the regulation of intracellular signaling pathways. The β3 subunit of these G proteins is encoded by the GNB3 gene located on chromosome 12p13.1 A C825T dimorphism in exon 10 of GNB3 has recently been described.2 The T allele results in the expression of a splice variant with a deletion of 41 amino acids that is functional by means of increased G protein activation.2 This dimorphism was found to be associated with increased cardiac potassium channel activity3 and increased α2-adrenoceptor–mediated vasoconstriction4 in humans. Most important, the dimorphism has been shown to be associated with hypertension5–7 and obesity.8 Both phenotypes, hypertension and obesity, are linked to the insulin resistance syndrome and atherosclerosis on one hand.9 Heterotrimeric G proteins, on the other hand, are involved in the regulation of cellular transmembrane sodium exchange10 that is found to be increased in insulin-resistant subjects.11,12 Finally, worldwide distribution8 of the C825T dimorphism of GNB3 suggests that it might fulfill the criteria of a thrifty genotype.13,14

Taken together, this evidence suggests a possible further association of the C825T dimorphism with insulin resistance, as recently suggested in a small study in patients with essential hypertension,15 and atherosclerosis. Consequently, we investigated possible associations of the GNB3 C825T dimorphism with hypertension, insulin resistance, and carotid atherosclerosis in a population-based study.

Subjects and Methods

Study Population

A total of 932 subjects were included in the present study. The cohort consisted of 508 men between 40 and 55 years of age and 424 women between 50 and 65 years of age who were screening participants of the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR),16 a population-based prospective study to investigate the role of various genetic and metabolic factors in the progression of atherosclerotic vascular disease and to initiate appropriate interventions in subjects at high cardiovascular risk. All subjects were whites of middle European (Austrian) origin. Pregnant women and subjects with established coronary artery, cerebrovascular, or peripheral arterial disease; congestive heart failure; valvular heart disease; chronic alcohol or drug abuse; and morbid obesity were excluded. At baseline, all study participants were subjected to a thorough screening program that included assessment of a detailed personal and family history, physical examination, determination of anthropometric parameters, and measurement of various biochemical parameters, along with several other more specialized procedures, including measurement of...
the intima-media thickness (IMT) of the carotid arteries by B-mode ultrasound. Subjects were classified as diabetic if they were on hypoglycemic medications or when their fasting plasma glucose concentrations were >126 mg/dL. The study was conducted according to the Austrian Gene Technology Act and was approved by the ethics committee of the medical association of Salzburg. All subjects gave written, informed consent before entering the study.

**Laboratory Analyses**

Venous blood was collected from subjects after an overnight fast. Total serum cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, lipoprotein(a), and apolipoprotein AI and B were determined with commercially available kits (Hoffmann-LaRoche GmbH). Fasting insulin was measured with the IMX insulin kit (Abbott). Plasma glucose was measured with the glucose oxidase method. Insulin sensitivity (KITT) was estimated with the short insulin tolerance test. Briefly, after intravenous injection of 0.1 IU insulin (Actrapid, NovoNordisk GmbH) per 1 kg body weight, the constant rate of glucose disappearance was calculated. Additionally, an insulin resistance score was formed on phenotypical presentations of the insulin resistance syndrome from the following parameters: body mass index (BMI) >30 kg/m², waist-to-hip ratio > 0.90, triglycerides >150 mg/dL, HDL cholesterol <40 mg/dL, fasting blood glucose >110 mg/dL, and presence of hypertension (blood pressure >140/90 mm Hg or antihypertensive medication).

**Twenty-Four–Hour Ambulatory Blood Pressure Measurement**

The 24-hour ambulatory blood pressure measurement was performed in the SAPHIR population with the TM 2430 PC monitoring system from Boso (Bosch+Sohn). Subjects were classified as hypertensive if either antihypertensive medication was present or mean daytime blood pressure was >140/90 mm Hg.

**Carotid Artery IMT**

IMT of the carotid arteries was measured by high-resolution B-mode ultrasound (HDI 3000 CV, ATL) according to the protocol published by the Asymptomatic Carotid Artery Plaque Study investigators. Thus, average and maximal IMT relate to all 4 vessels (internal and common carotid arteries of the left and right sides). Additionally, a B score describing morphological alterations of the carotid arteries was formed as follows: 0=no alteration, 1=wall thickness >1 mm, 2=plaque <2 mm, 3=plaque 2 to 3 mm, 4=plaque >3 mm, and 5=total closure of the lumen.

**Genetic Analysis**

Venous blood was collected in 5-mL EDTA tubes; genomic DNA was isolated with the Nucleospin Blood kit (Macherey-Nagel) and stored at 4°C. For genetic analyses, a 268-bp segment containing exon 10 was amplified by polymerase chain reaction with 5′-TGA CCC ACT TGC CAC CCG TGC-3′ (forward primer) and 5′-GCA GCA GCC AGG GCT GGC-3′ (reverse primer). The reaction was performed in a 25-μL volume containing 1 U Biotherm Polymerase (Genevacraft), 110 ng TaqStart Antibody (Clontech), 15 pmol of each primer, and 5 μL genomic DNA in a GeneAmp 9700 Thermocycler (Applied Biosystems). Cycling conditions were 35 cycles with 20 seconds at 94°C, 20 seconds at 60°C, and 20 seconds at 72°C. The whole polymerase chain reaction mixture was subsequently digested overnight at 60°C with 1 U of the restriction enzyme BsaI (New England Biosystems). BsaI is an isoschizomer of restriction enzyme Bsal, which was used in the original protocol.2 Fragments were separated on 2% agarose gels and visualized by use of ethidium bromide. The C allele was cut into 2 fragments of 114 and 154 bp, whereas the T allele remained uncut.

**Statistical Analysis**

Statistic analysis was done with SPSS 10.0 for Windows. Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Numeric values were analyzed by Student’s t test; data showing unequal variance or no normal distribution were analyzed by the Mann-Whitney rank-sum test. Multiple group comparisons were made by analysis of variance. If adjustment for other parameters (as indicated) was necessary, appropriate general linear or logistic models were used. The proportions of 2 groups were compared by χ² test. The criterion for statistical significance was P<0.05.

**Results**

**Genotype Distribution and Population Characteristics**

A total of 932 subjects were genotyped for the C825T dimorphism of GNB3. Distribution of genotypes is shown in Table 1. Distribution was found to be in Hardy-Weinberg equilibrium, and no difference was observed between male and female study participants.

Because the expression of the more active splice variant of GNB3 depends on the presence of at least 1 T allele,2 CT and TT genotypes were grouped together for subsequent analyses. Characteristics of the study population according to genotype are shown in Table 2. Groups were comparable with regard to sex, age, and lipids. Additionally, BMI was comparable between groups, and prevalence of obesity, defined as BMI >27 kg/m², was not different (43.5% of CC genotypes versus 45.9% of CT/TT, P=NS). Absolute blood pressure was not statistically different between groups, but borderline significantly more subjects with CT/TT than CC genotype were...

### Table 1. Allelic Frequencies

<table>
<thead>
<tr>
<th>Genotype CC/CT/TT</th>
<th>Allelic Frequencies C/T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n=932)</td>
<td>436/418/78</td>
<td>0.692/0.308 ns</td>
</tr>
<tr>
<td>Male (n=508)</td>
<td>234/237/37</td>
<td>0.694/0.306 ns</td>
</tr>
<tr>
<td>Female (n=424)</td>
<td>202/181/41</td>
<td>0.690/0.310 ns</td>
</tr>
</tbody>
</table>

Genotype distribution and allelic frequencies of the C825T dimorphism of GNB3 in the SAPHIR population. In our study, the frequency of the T allele is in good agreement with that published for other middle European Caucasian populations,6 (P for Hardy-Weinberg equilibrium.)

### Table 2. Study Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT/TT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>436</td>
<td>496</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male, %</td>
<td>53.7</td>
<td>55.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age, y</td>
<td>52.8±5.7</td>
<td>52.7±5.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26±4.2</td>
<td>26.9±4.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.90±0.08</td>
<td>0.89±0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>2.3</td>
<td>2.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Blood pressure systolic, mm Hg</td>
<td>133±14</td>
<td>134±14</td>
<td>n.s.</td>
</tr>
<tr>
<td>Blood pressure diastolic, mm Hg</td>
<td>82±8</td>
<td>83±8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>36.5</td>
<td>42.5</td>
<td>n.s. (0.061)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.06±1.06</td>
<td>6.01±1.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.58±0.44</td>
<td>1.56±0.40</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.81±0.96</td>
<td>3.80±0.98</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.48±1.06</td>
<td>1.46±1.01</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Characteristics of the study population according to the GNB3 genotype (presence of the T allele). With the exception of the presence of hypertension (borderline significance), no influence of the genotype on any parameter was observed. All data are either mean±SD or absolute numbers or percentage.
Insulin Sensitivity

A linear-regression model with stepwise inclusion identified fasting insulin ($r = -0.322, P < 0.001$), insulin resistance score ($r = -1.99, P < 0.001$), age ($r = -0.99, P = 0.002$), and BMI ($r = -0.94, P = 0.024$) as independent predictors of insulin sensitivity. In the entire population, CT/TT genotypes had significantly higher fasting blood glucose (5.24 ± 1.05 versus 5.16 ± 1.06 mmol/L, $P = 0.045$) and fasting insulin (8.24 ± 5.31 versus 7.63 ± 4.74 mU/L, $P = 0.043$) compared with subjects with CC genotype. Insulin sensitivity ($K_{ITT}$) derived from the short insulin tolerance test was lower in CT/TT subjects (4.02 ± 1.27%/min) than in CC subjects (4.19 ± 1.31%/min) although formally not reaching statistical significance ($P = 0.052$). Subsequent analysis revealed that the effect of the $GNB3$ genotype on insulin sensitivity was seen only in male subjects (4.13 ± 1.37 versus 3.87 ± 1.35, $P = 0.036$). In-depth analysis revealed that it was restricted to those with a waist-to-hip ratio above the median of 0.9 (Table 3). No effect was observed in men with a waist-to-hip ratio <0.9 or in women regardless of the waist-to-hip ratio.

Carotid Atherosclerosis

In a stepwise-inclusion, linear-regression model, age ($r = 0.362, P < 0.001$), systolic blood pressure ($r = 0.167, P < 0.001$), apolipoprotein B ($r = 0.151, P < 0.001$), waist-to-hip ratio ($r = 0.094, P = 0.013$), and HDL cholesterol ($r = -0.078, P = 0.044$) were found to be significant predictors of IMT in the SAPHIR population. In the entire population, neither average IMT nor maximal carotid artery IMT was different between CC and CT/TT genotypes (Table 4). In contrast to the results regarding insulin sensitivity, no influence of sex or waist-to-hip ratio was observed with regard to a possible influence of the $GNB3$ genotype on carotid IMT (data not shown). In addition, no influence of the $GNB3$ genotype was seen on the mean B score. However, the proportion of subjects with a single-vessel B score >2, indicating at least 1 plaque ≥2 mm thickness, was higher in CT/TT subjects (11.3%) than in CC subjects (7.3%, $P = 0.040$). In a logistic-regression model including the above-mentioned predictors of IMT, $GNB3$ CT/TT genotype remained significantly predictive for a single-vessel B score >2 ($r = 0.474, P = 0.04$). This translates into an odds ratio of 1.606 (95% confidence interval, 1.002 to 2.575) for carriers of a T allele to have at least 1 carotid artery affected by a plaque ≥2 mm thick. Similar results were found in male (odds ratio, 1.483) and female (odds ratio, 1.737) study participants and those with and without hypertension (data not shown), although statistical significance was not reached because of the size of the groups.

Discussion

The major results of our population-based study in middle European whites are the associations of the T allele of the C825T dimorphism of $GNB3$ with reduced insulin sensitivity in men with abdominal fat distribution and with an increased risk of advanced carotid atherosclerosis in the entire population.

Allelic frequencies in our study were almost identical to those in other investigations in white populations of middle European origin. The T allele was found with a frequency of 0.308; consequently, 53% of the entire population investigated had a CT or TT genotype. This high prevalence of the variant allele highlights the importance of our findings on a population base.

With regard to insulin sensitivity, our results confirm and extend those of Poch and coworkers who found an association between the T allele and reduced insulin sensitivity in a group of 35 subjects with essential hypertension using the euglycemic clamp technique. In our study, insulin sensitivity was measured with the short insulin tolerance test. This method has very good correlation with the euglycemic clamp technique, the gold standard for measuring insulin sensitivity, but unlike the euglycemic clamp technique, it is suitable for large studies. Although a reduction in insulin sensitivity in our study population was observed in the entire group of male subjects, it was finally found restricted to those with abdominal fat distribution. Therefore, our findings strongly suggest a sex- and body fat distribution–specific association of the T allele with insulin resistance. This, in turn, might favor...
phenotypical manifestations of the metabolic syndrome such as hypertension. It is of interest that the association of the GNB3 T allele with obesity was reported only in male populations and that the largest study reporting associations with hypertension in a white population shows stronger associations for men than for women. In addition, the association with type 2 diabetes was observed until now only in a male population. All these studies, however, did not further take into account body fat distribution. Because reduced insulin sensitivity was observed only in men with abdominal body fat distribution, we suggest that sex-specific differences in fat distribution (visceral versus subcutaneous) or absolute visceral fat mass could contribute to this effect. Such an assumption is supported by the notion that men, for any given total body fat, have more visceral than subcutaneous fat, resulting in metabolic alterations such as increased postprandial lipemia or disturbed glucose homeostasis and dyslipidemia. In our study, because of the inclusion criteria, female participants were 10 years older than male subjects. Because stepwise-inclusion regression analysis did identify age as a determinant of insulin sensitivity, we cannot completely exclude an observation bias in the female population based on a possible diminution of the contribution of the T allele to insulin sensitivity with age. This, however, seems unlikely because such a phenomenon was not observed in the male population.

Compared with other studies, the association with hypertension that we observed was less pronounced, and no differences between men and women were observed. On the other hand, a significant T allele–specific trend for elevated diastolic blood pressure was also found in our subjects comparable to that observed in an earlier study. The reason for this weaker association with hypertension might be the use of the mean daytime blood pressure derived from 24-hour ambulatory blood pressure monitoring in our study. Because all other investigations used office readings, the unavoidable inclusion of subjects with white coat hypertension in these studies might be responsible for the differences observed.

We did not find any association of the C825T dimorphism with obesity in male or female study participants. This contrasts the findings of several large investigations in populations of different ethnic origins. It has to be taken into account that these associations were reported only in young male subjects with a mean age well below 30 years. Another large study did not find an association with obesity in a mixed (male and female) population from Germany with a mean age of 55 years, an age very comparable to that of our population, as did several other studies.

Both carotid artery IMT and quantification of plaque burden are suitable vascular parameters for the identification of subjects at elevated cardiovascular risk. In our study, the GNB3 dimorphism was not associated with increased IMT (average or maximum) or overall plaque burden, expressed as the mean B score. Carriers of the T allele of GNB3 at position 825, however, had a significantly increased risk (1.6-fold) of advanced carotid plaques (thickness ≥2 mm) in single vessels. This is an important finding because it has recently been shown that subjects with at least moderately sized carotid plaques are at a substantially higher risk for subsequent cardiovascular events than those without this size of plaque. With regard to IMT, it has to be noted that we observed increased IMT in subjects with carotid plaque compared with those without, supporting previous studies that suggested IMT as a predictor for carotid plaque. Carotid plaque, however, seems to be influenced by vascular risk factors other than IMT and to be more specific for coronary artery disease. In addition, IMT in our study was measured strictly outside plaque areas, which also might contribute to the effects observed in our population. Finally, there is inconsistency in the studies published as to whether plaque is only detected or also quantified. Thus, we suggest that the T allele of GNB3 might be associated with progression rather than the presence of carotid atherosclerosis.

Because G proteins are involved in a multitude of regulatory processes, it can currently not even be speculated why associations with metabolic parameters seem to be restricted to men, whereas the association with advanced carotid atherosclerosis seems not to be restricted.

Finally, a very recent publication suggests that several additional polymorphisms in the GNB3 gene are in strong linkage disequilibrium with the C825T dimorphism, giving rise to 2 major haplotypes in whites. Therefore, the C825T dimorphism itself might only be a marker of other as-yet-unidentified genetic variants involved in the metabolic and vascular alterations observed.

In summary, our results demonstrate that the GNB3 825T allele is associated with reduced insulin sensitivity in men with abdominal fat distribution and with more advanced carotid atherosclerosis in middle-aged white men and women. Because of its high prevalence, that dimorphism can be expected to be associated with a high population-attributable risk and morbidity.

Acknowledgments
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