G-Protein \( \beta_3 \) Subunit Gene (GNB3) 825T Allele Is Associated With Enhanced Renal Perfusion in Early Hypertension

Raoul Zeltner, Christian Delles, Markus Schneider, Winfried Siffert, Roland E. Schmieder

Abstract—The \( C825T \) polymorphism of the gene encoding the G-protein \( \beta_3 \) subunit (\( GNB3 \)) is associated with increased intracellular signal transduction and arterial hypertension. The aim of the study was to investigate the impact of this polymorphism on early adaptive processes of the left ventricle and renal hemodynamic changes in young normotensive to mildly hypertensive subjects. Ninety-five white male students with normal or mildly elevated blood pressure were genotyped for the \( GNB3 C825T \) polymorphism. In each participant, 24-hour ambulatory blood pressure, left ventricular structure and function (2D-guided M-mode echocardiography), renal plasma flow (para-aminohippurate clearance), glomerular filtration rate (inulin clearance), and 24-hour urinary sodium excretion were determined. The \( GNB3 825T \) allele was not associated with casual or ambulatory blood pressure, parameters of left ventricular structure or function, glomerular filtration, or 24-hour urinary sodium excretion. However, in \( T \)-allele carriers (\( CT + TT \)), renal plasma flow was higher than in \( CC \) subjects (\( CT/TT \): 659\( \pm \)96 versus CC: 614\( \pm \)91 mL/min, \( P = 0.019 \)). ANOVA disclosed that renal plasma flow was independently influenced by both genotype and blood pressure, with hypertensives having a higher renal plasma flow than normotensive subjects. This was the fact irrespective of the criteria used for the definition of hypertension (World Health Organization or 24-hour ambulatory blood pressure criteria). The \( GNB3 825T \) variant is associated with increased renal perfusion in this study. Because early renal hemodynamic changes play a pivotal role in the pathogenesis of essential hypertension, our data suggest a relevance of increased G-protein activation in the pathogenesis of hypertension. (Hypertension. 2001;37:882-886.)

Key Words: G proteins \( \triangleright \) polymorphism \( \triangleright \) genes \( \triangleright \) hypertension, essential \( \triangleright \) hemodynamics

The early course of essential hypertension is characterized by structural and functional changes in the systemic and renal circulation. In addition, early structural changes of the left ventricle (LV) have been reported. Increased concentric remodeling of the LV and impaired diastolic function have been recognized to reflect early changes of the myocardium in early essential hypertension. Renal hemodynamics were shown to be abnormally regulated in a prehypertensive stage, and increased renal perfusion was reported in early hypertension. It has been proposed that renal vascular changes may not simply be a complication but the cause for blood pressure elevation and thus may play a pivotal role in the pathogenesis of essential hypertension.

In the search for mechanisms by which genetic markers contribute to the development of hypertension, recent interest focused on a novel gene polymorphism closely associated with a well-established intermediate phenotype, that is, the enhanced Na'/H' exchanger activity in hypertensive subjects. Immortalized lymphoblasts of these patients have been found to respond with enhanced G-protein activation on stimulation. Subsequently, a single nucleotide polymorphism (\( C/T \) at position 825) in the gene encoding the \( \beta_3 \) subunit of heterotrimeric G proteins (\( GNB3 \)) has been identified and was demonstrated to be significantly associated with essential hypertension. The 825T allele was also associated with the expression of a novel splice variant (G\( \beta_3 \)-s) displaying a 123-bp in-frame deletion within exon 9, resulting in the loss of 41 amino acids and 1 WD repeat domain of the G\( \beta \) subunit. Increased binding of [\( ^{35} \)S]GTP\( \gamma \)S in S9 insect cells expressing G\( \beta_3 \)-s suggests that this splice variant results in the enhanced activation of pertussis toxin-sensitive G proteins.

The aim of this study was to determine in early hypertension the impact of the \( C825T \) polymorphism on early adaptive processes of the LV and hemodynamic changes in the renal circulation. Therefore, we measured LV mass as well as renal hemodynamics and glomerular filtration rate (GFR) in young normotensive and mildly hypertensive subjects.

Methods

Study Population
Young white male students were elicited by announcement for participation in the study at the campus of the University of...
Blood Pressure Measurements
Casual blood pressure was measured 4 times on 2 different occasions in our outpatient clinic (at least 2 weeks apart). The cuff size of the sphygmomanometer was adjusted according to the persons’ arm circumference, and blood pressure readings were taken with the participant seated after 5 minutes of rest. Subjects were said to be mildly hypertensive if the mean blood pressure was ≥140 mm Hg systolic or ≥90 mm Hg diastolic, according to WHO recommendations. To exclude white-coat hypertension, ambulatory 24-hour blood pressure was additionally recorded with a portable device (Space Labs 90207). Measurement intervals were every 15 minutes during the daytime (6 AM to 10 PM) and every 30 minutes during the nighttime (10 PM to 6 AM). Blood pressure was said to be hypertensive if the mean daytime blood pressure values were ≥135 mm Hg systolic and/or ≥85 mm Hg diastolic.

Echocardiography
Two-dimensionally guided M-mode echocardiography at rest (Picker-Hitachi CS 192, 2.5-MHz probe) was performed in the third to fourth intercostal space lateral to the left sternal border, with the patient lying in the partial left decubitus position. LV structure was assessed by measurement of septal wall thickness, posterior wall thickness, and LV end-diastolic diameter. Midwall fractional fiber shortening was taken as a parameter of systolic function.8 Concentric LV hypertrophy was assessed by calculation of the relative wall thickness as 2×posterior wall thickness divided by end-diastolic diameter.10 LV mass was calculated according to the American Society of Echocardiography (ASE) recommendations but was subsequently corrected by the regression LV = 0.8 (ASE cube LV mass) + 0.6 g, following the suggestions of Devereux et al.12 LV diastolic filling was determined by pulsed-wave Doppler sonography of the LV inflow, as previously described in detail.13

Measurement of Renal Hemodynamics and Urinary Sodium Excretion
GFR and renal plasma flow (RPF) were determined after 1 hour of rest in a supine position. We applied the modified clearance technique to measure RPF (para-aminohippuric clearance) and GFR (inulin clearance) without urinary sampling, as previously described in detail.14,15 Briefly, the excreted amount of para-aminohippuric acid (PAH) and inulin is equal to the infused dose of the compounds under steady-state conditions. A bolus injection was applied followed by a constant infusion for a total of 150 minutes to achieve steady state. The doses of bolus and constant infusion of PAH and inulin were adjusted to body weight. PAH and inulin were measured as outlined in detail elsewhere.16,17 This method overestimates RPF by 10% to 20%, but differences between genotypes are not affected by this potential bias. The 24-hour urinary sodium excretion was measured with participants on their usual diet. To ensure complete collection of urine, all samples containing <0.6 L and/or the expected creatinine per kilogram of body weight were excluded.

Genotyping
Genomic DNA was extracted from whole blood according to standard procedures with a QIAamp Blood Midi Kit (QIAGEN GmbH). Genotyping for the GNB3 C825T polymorphism was performed at the Department of Pharmacology at the University of Essen, as recently described in detail.8 Briefly, polymerase chain reaction (PCR) with GNB3-specific primers resulted in a 368-bp fragment. PCR products were subsequently restriction-digested with the enzyme BseD1, leading to the generation of a 116-bp and a 152-bp fragment with the C allele. The T allele is not cleaved by BseD1. Hence, CT heterozygotes exhibit all 3 fragments (368, 152, and 116 bp).

Statistics
For statistical analysis, subjects either heterozygous or homozygous for the GNB3 825T allele were taken together into one group because it has been reported that 1 T allele is sufficient for the expression of the Gβ3 splice variant and because the number of homozygous T-allele carriers was too small for separate analysis. All statistical calculations were carried out with the use of SPSS software.18 A t test and 2-way ANOVA were performed to compare CC and TC/TT genotypes. Unless otherwise specified, values are expressed as mean±SD. A 2-tailed probability value of <0.05 was considered significant.

Results
The clinical characteristics of the study group are given in Table 1. In our homogeneous study cohort of young white male students, the frequencies of the GNB3 825C and T alleles were 70% and 30%, respectively. Forty-four subjects were genotyped as homozygous for the C allele, 45 subjects were CT heterozygotes, and 6 individuals were homozygous for the T allele. There were no significant differences according to age, body mass index, body surface area, and casual and 24-hour ambulatory blood pressure between the CC and the TC/TT genotypes (Table 1).

In the whole study group, renal hemodynamic parameters were associated with the G-protein β3 subunit C825T polymorphism (Table 2). Subjects with the 825T allele (TC and TT genotypes) had a greater RPF than those homozygous for the C allele (CT/TT: 659±96 versus CC: 614±91 μL/min; P=0.019). Furthermore, T-allele carriers had a lower renal vascular resistance than subjects with the CC genotype.
whether the examined subjects were stratified for hypertensive or normotensive genotypes. This was found irrespective of the group of hypertensive subjects had an increased RPF compared with the group of normotensive subjects, irrespective of their genotypes. Hence, renal hyperperfusion in the prehypertensive stage and in borderline hypertension, whereas in sustained hypertension, the consistently observed pattern of renal hemodynamics is characterized by a decreased renal blood flow and/or plasma flow and a normal to slightly decreased GFR, resulting in an elevated renal filtration fraction. Renal hemodynamic changes have been supposed to play an important role in the development of hypertension. Evidence for this hypothesis comes from the observation of an abnormal control of renal circulation in subjects with a positive family history of hypertension in response to mental stress, and Bianchi et al found that normotensive offspring of hypertensive parents were characterized by high renal blood flow (RBF) despite normal cardiac output, suggesting a specific renal vasodilation in the prehypertensive stage. Because RBF and cardiac output (CO) are strongly correlated, the ratio of both is a reliable parameter for the assessment of renal perfusion.

Two-way ANOVA disclosed that genotype and blood pressure independently influenced RPF (Table 3). Thus, first the group of hypertensive subjects had an increased RPF compared with the group of normotensive subjects, irrespective of their genotypes. This was found irrespective of whether the examined subjects were stratified for hypertension according to the WHO (hypertensives: 611 ± 99 versus normotensives: 611 ± 84 mL/min, P = 0.003) or ambulatory (hypertensives: 665 ± 96 versus normotensives: 618 ± 90 mL/min, P = 0.011) blood pressure criteria. And, second most important, T-allele carriers had a greater RPF than individuals with the CC genotype (659 ± 96 versus 614 ± 91 mL/min, P = 0.005). Hypertensive T-allele carriers had the greatest RPF, followed by normotensive individuals with the CT/TT genotype or hypertensive individuals homozygous for the C allele with intermediate values, and finally normotensive homozygous C allele carriers having the lowest values for RPF (Table 3).

**Discussion**

In this study, we show that the 825T allele of the G-protein β-3 subunit is associated with increased RPF in young male normotensive to mildly hypertensive subjects. Previous studies have reported normal to enhanced renal blood flow in the prehypertensive stage and in borderline hypertension, whereas in sustained hypertension, the observed pattern of renal hemodynamics is characterized by a decreased renal blood and/or plasma flow and a normal to slightly decreased GFR, resulting in an elevated renal filtration fraction. Renal hemodynamic changes have been supposed to play an important role in the development of hypertension. Evidence for this hypothesis comes from the observation of an abnormal control of renal circulation in subjects with a positive family history of hypertension in response to mental stress. Bianchi et al. found that normotensive offspring of hypertensive parents were characterized by high renal blood flow (RBF) despite normal cardiac output, suggesting a specific renal vasodilation in the prehypertensive stage. Because RBF and cardiac output (CO) are strongly correlated, the ratio of both is a reliable parameter for the assessment of renal perfusion.

In this study, CO was not measured. However, potential confounding factors such as blood pressure or the size and structure of the heart, which reflect profound alterations in systemic hemodynamics, were similar between the different genotypes in our study population. Hence, renal hyperperfusion observed in our study is most likely due to a selective renal vasodilation rather than to increased CO. Consequently, the GNB3 polymorphism per se might account for the

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**TABLE 2. Echocardiographic and Renal Hemodynamic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>CC (n=44)</th>
<th>TC/TT (n=51)</th>
<th>P</th>
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<tbody>
<tr>
<td><strong>Echocardiographic data</strong></td>
<td></td>
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<tr>
<td>Posterior wall thickness, mm</td>
<td>9.9±1.4</td>
<td>9.9±1.3</td>
<td>NS</td>
</tr>
<tr>
<td>Septal wall thickness, mm</td>
<td>10.5±1.6</td>
<td>10.4±1.4</td>
<td>NS</td>
</tr>
<tr>
<td>End-diastolic diameter, mm</td>
<td>50.7±3.5</td>
<td>51.5±3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Relative wall thickness (−)</td>
<td>0.39±0.07</td>
<td>0.38±0.06</td>
<td>NS</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>243±50</td>
<td>245±46</td>
<td>NS</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>65.9±6.2</td>
<td>65.3±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>Midwall fractional fiber shortening, %</td>
<td>17.1±2.4</td>
<td>17.2±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Vmax A/E (−)</td>
<td>0.57±0.2</td>
<td>0.59±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>VTI A/E (−)</td>
<td>0.29±0.2</td>
<td>0.31±0.1</td>
<td>NS</td>
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</tbody>
</table>

**Renal parameters**

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>GFR, mL/min</td>
<td>119±16</td>
<td>118±14</td>
<td>NS</td>
</tr>
<tr>
<td>RPF, mL/min</td>
<td>614±91</td>
<td>659±96</td>
<td>0.019</td>
</tr>
<tr>
<td>RBF, mL/min</td>
<td>1107±175</td>
<td>1176±184</td>
<td>0.065</td>
</tr>
<tr>
<td>Renal vascular resistance, mm Hg/[mL/min]</td>
<td>91±14</td>
<td>83±16</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Vmax A/E indicates ratio of peak late to peak early diastolic influx velocities; VTI A/E, ratio A/E of velocity-time integrals.

(CT/TT: 83±16 versus CC: 91±14 mm Hg/[mL/min], P = 0.021).

**TABLE 3. Renal Plasma Flow Stratified According to C825T Polymorphism of G-Protein β-3 Subunit Gene and Blood Pressure**

<table>
<thead>
<tr>
<th></th>
<th>CC (n=24)</th>
<th>CT/TT (n=20)</th>
<th>CC+CT/TT (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>According to WHO criteria</strong></td>
<td></td>
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</tr>
<tr>
<td>Normotensives</td>
<td>587±73 (n=16)</td>
<td>625±88 (n=27)</td>
<td>611±84 (n=43)</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>629±97 (n=28)</td>
<td>698±91 (n=24)</td>
<td>661±99 (n=52)</td>
</tr>
<tr>
<td>All participants</td>
<td>614±91 (n=44)</td>
<td>659±96 (n=51)</td>
<td>638±96 (n=95)</td>
</tr>
<tr>
<td><strong>According to 24-h ambulatory blood pressure criteria†</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensives</td>
<td>589±86 (n=24)</td>
<td>643±87 (n=32)</td>
<td>618±90 (n=56)</td>
</tr>
<tr>
<td>Hypertensives</td>
<td>643±89 (n=20)</td>
<td>687±106 (n=19)</td>
<td>665±96 (n=39)</td>
</tr>
<tr>
<td>All participants</td>
<td>614±91 (n=44)</td>
<td>659±96 (n=51)</td>
<td>638±96 (n=95)</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*Genotype CC vs CT/TT: P = 0.005; normotensives vs hypertensives: P = 0.003; interaction: P = NS.
†Genotype CC vs CT/TT: P = 0.012; normotensives vs hypertensives: P = 0.011; interaction: P = NS.
Genetic polymorphism or renal perfusion (GNB3 825T) is associated with structural and functional changes in hypertension. There is a significant variance in renal perfusion and therefore might be of significance in the early stages of the development of arterial hypertension. The pathogenetic relevance of the C825T polymorphism relies on the fact that the 825T allele of GNB3 is related to enhanced stimulated G-protein activation in cell lines from hypertensive patients. There is substantial evidence that the enhanced Na+/H+ exchange activity observed in 30% to 50% of patients with essential hypertension is mediated by this genetically fixed G-protein activation. Na+/H+ exchange is involved in the regulation of pH, and contributes to sodium reabsorption in the kidney. Thus, a faster tubular sodium reabsorption may lead to an increase in RPF by means of the macula densa feedback mechanism and inhibition of renin secretion in subjects with the GNB3 825T allele. However, the fact that we have not found any change in GFR is in conflict with this hypothesis. Alternatively, one may speculate that a faster Na+/H+ exchange could be involved in selective renal vasodilation by a direct effect at the vascular smooth muscle or endothelial cell levels.

An additional finding of our study is that RPF was higher in hypertensive than in normotensive subjects regardless of the definition used for arterial hypertension. This observation is in line with the above-mentioned studies that found enhanced renal perfusion in the prehypertensive and in very early stages of hypertension. Because we examined a young homogeneous study cohort, including only mildly hypertensive subjects without signs of advanced target organ damage, it can be presumed that our hypertensive individuals had early essential hypertension.

Besides renal perfusion, we also determined GFR and cardiac structural adaptation to assess abnormalities occurring with early essential hypertension. Thus, an increase in LV mass and impaired LV systolic function has been demonstrated in young patients with borderline to mild hypertension. Furthermore, glomerular hyperfiltration has been related to incipient LV hypertrophy in mild hypertension, indicating early target organ damage. In this study, GFR and functional or structural parameters of the heart were not linked to the G-protein β1 subunit gene polymorphism. Poch et al recently reported an association between the GNB3 825T allele and LV hypertrophy in hypertensive patients. In a study including patients with mild to moderate hypertension, we found that the C/T genotype was associated with impaired LV diastolic filling, which is an early sign of hypertensive heart disease. Because we have investigated young volunteers with normal or mildly elevated blood pressure, it is not surprising that we did not find such associations in the present study.

**Study Limitations**

There are several limitations of our study that must be emphasized. We studied young white subjects and therefore we do not know whether or not our results can be extended to older individuals, to subjects with more advanced stages of essential hypertension, or to other populations. Furthermore, because we only included men in our study, we cannot extrapolate the results to women. However, we believe that the use of a homogeneous study population is also advanta-

**Conclusions**

We have demonstrated that the 825T allele of the β1 subunit of heterotrimeric G proteins is associated with increased renal perfusion in early hypertension and thus may be of relevance in the pathogenesis of essential hypertension. However, we currently can only speculate on how the GNB3 825T allele leads to increased RPF. We anticipate that this is due to functional changes because we have not found any association of the G-protein β1 subunit gene variant with structural alterations. Subsequent studies are needed to further clarify the role of the GNB3 825T allele in the regulation of renal hemodynamics.

**References**


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