Association Between a Polymorphism in the G Protein β3 Subunit Gene and Lower Renin and Elevated Diastolic Blood Pressure Levels

Heribert Schunkert, Hans-Werner Hense, Angela Döring, Günter A. J. Riegger, Winfried Siffert

Abstract—Gβ proteins mediate the intracellular effects of many vasoactive and proliferative stimuli. Recently such signaling was found to be enhanced in cultured cells of some hypertensive subjects. A polymorphism at position 825 (C → T) of the G protein β3 subunit gene (GNB3) was strictly related to this phenotype. The aim of the present investigation was to test the association between this polymorphism and blood pressure and plasma renin levels in humans. A population-based sample (n = 608) was analyzed by questionnaire and characterized for blood pressure; plasma renin, prorenin, and aldosterone levels; and Gβ3 C825T allele status. In individuals without antihypertensive medication (n = 474; age range, 52 to 67 years), the polymorphism was mildly associated with diastolic blood pressure (CC: 88.6 ± 0.3 mm Hg, n = 218; versus CT: 90.1 ± 0.7 mm Hg, n = 209; versus TT: 91.8 ± 1.7 mm Hg, n = 47; P = 0.02 for trend) but not with systolic blood pressure. Furthermore, the 825T allele was also significantly associated with lower renin and prorenin levels, whereas the aldosterone to renin ratio was elevated in these subjects. Significant associations between the 825T allele and diastolic blood pressure, plasma renin, and prorenin levels (inverse), and the aldosterone to renin ratio persisted after adjustment for age, gender, body mass index, and systolic blood pressure. Finally, when the entire sample was considered and an adjustment was made for covariates, the presence of arterial hypertension and the use of antihypertensive medication were both 1.8-fold higher in the TT than in the CC genotype group (P < 0.05 and P = 0.06, respectively). This observation, if replicated in further studies, suggests a molecular mechanism that unifies a higher diastolic blood pressure, a lower renin level, and an elevated aldosterone to renin ratio, ie, a combination of features frequently found in patients with arterial hypertension. (Hypertension. 1998;32:510-513.)

Key Words: hypertension ■ G protein ■ genetics ■ genotype ■ medication ■ renin

Most transmembrane receptors rely on heterotrimeric GTP-binding proteins (G proteins) to activate or inhibit intracellular signaling cascades. Specifically, a variety of vasoactive or growth-stimulating factors communicate via G proteins in virtually all cardiovascular tissues. Despite this pivotal role in the transmembrane signaling network, only recently an active participation of G proteins was considered in the pathogenesis of hypertension.1 In particular, immortalized lymphoblasts and fibroblasts of highly selected hypertensive subjects were found to respond with enhanced G protein–mediated activation of intracellular effectors after stimulation of various receptors.2,3 These specific receptors had in common the ability to activate pertussis toxin–sensitive, Gαi-type G proteins.2,3 The finding that immortalized cells of hypertensive subjects displayed enhanced G protein–mediated signaling suggested a genetic alteration rather than a blood pressure effect as the underlying mechanism. Subsequently, the genes coding for the G protein subunits Ga12, Ga13, Gβ1, and Gβ3 were screened for polymorphisms, but were found to be without alterations in affected hypertensive subjects.4 However, molecular analysis of Gβ3 revealed a point polymorphism C825T that is associated with a novel splice variant and an in-frame deletion of 41 amino acids from the wild-type protein.4 Enhanced sensitivity to agonists that stimulate intracellular signaling via pertussis toxin–sensitive G proteins was tightly associated with this splice variant and expression of recombinant mutated Gβ3 in insect cells allowed the modified protein to be generated in vitro.4

Potential implications of the Gβ3 gene for the development of hypertension were initially suggested by an excess of the 825T polymorphism in hypertensive patients of university-based referral clinics as compared with unscreened control subjects.4 These studies encouraged us to examine the relation between the Gβ3 825T polymorphism and arterial blood pressure in a sample of the general population. Furthermore, we speculated that enhanced G protein signaling via inhibition of cAMP generation may suppress renin formation and result, thereby, in lower renin yet higher diastolic blood pressure levels in subjects carrying the Gβ3 825T genotype.
Methods
The subjects of this study had participated in the WHO-MONICA (World Health Organization—Multinational Monitoring of Trends and Determinants in Cardiovascular Disease), Augsburg, baseline survey of 1984/85 and its follow-up examinations in 1987/88 and 1994.1 In 1994, biochemical and anthropometric measurements were offered to a total of 1010 men and women, aged 52 to 67 years, of whom 646 (64%) attended. Plasma renin measurements and determinations of the Gβ3 C825T allele status were successfully performed in 608 of these subjects. All subjects responded to an extensive questionnaire. Body mass index was computed as weight in kilograms divided by height in meters squared (kg/m²). Resting blood pressure level was measured in sitting subjects after at least 5 minutes of rest using a mercury sphygmomanometer. In this single appointment, blood pressure was read 3 times at the right arm by 2 investigators. The mean of 3 measurements was used for this study. Hypertension was defined as blood pressure level ≥160 (systolic) and/or 95 mm Hg (diastolic) or intake of antihypertensive agents. Antihypertensive drugs were considered to be used for treatment of hypertension when subjects were aware of hypertension. Antihypertensive combination therapy was considered when drugs acting via different blood pressure—lowering mechanisms, eg, diuretics and ACE inhibitors, were taken either as separate pills or in a fixed combination.

Blood was drawn from nonfasting subjects who were in a supine resting position for at least 30 minutes. Determinations of renin were carried out in duplicate. Immunoreactive renin was measured in a 200-μL plasma sample by means of an immunoradiometric assay kit (Nichols Institute, Wycben, the Netherlands), following the methods proposed by Derkx et al.6 Prorenin was activated nonproteolytically using the renin inhibitor remikiren. The concentration of prorenin (ie, active renin) from those obtained after activation (ie, total renin).7 Aldosterone levels were determined in 100 μL serum by standard radioimmunoassays (Peninsula). The cross-reactivity of this assay with 3β,5α-tetrahydroaldosterone and 3α,5β-tetrahydroaldosterone is 4.2% and 0.3%, respectively. All other steroids cross-react to <0.1%.

After DNA purification from peripheral blood using a standard protocol, 80 ng of genomic DNA was subjected to 35 rounds of specific amplification using 5′ GGA CCC ACT TGC CAC CCG TGC 3′ (sense primer) and 5′ GCA GCA GCC AGG GCT GCC 3′ (antisense primer).1 After denaturation at 94°C, DNA was amplified using 0.5 U of Taq polymerase at 94°C for 1 minute, 61°C for 1 minute, and 72°C for 1 minute. After a final extension for 7 minutes, polymerase chain reaction products were digested with BseRI (Fermentas), separated on 2.5% agarose gels, and visualized under UV illumination. The undigested product (TT genotype) has a size of 268 bp; complete digestion (CC genotype) results in bands of 116 and 152 bp, respectively.8

Statistical Analysis
Anthropometric data of subjects were compared according to the Gβ3 C825T allele status by ANOVA for comparison of independent samples or χ² test for comparison of classified values. Regression analysis was carried out to test for trends related to genotype. Given the confounding effects of antihypertensive therapy, patients receiving such treatment were excluded from comparisons of Gβ3 C825T allele status with blood pressure, renin, and prorenin levels. A further subgroup consisted of subjects not treated for hypertension despite blood pressure levels of >95 mm Hg (diastolic) and/or 160 mm Hg (systolic; untreated hypertensives). Multivariable linear regressions, controlling for gender, age, body mass index, and systolic blood pressure level (or diastolic blood pressure), were carried out to further study the relation between diastolic blood pressure, renin, and prorenin levels and Gβ3 C825T allele status. At α=0.9, the present population offered a 70% chance to detect a 25% variation in plasma renin levels related to the Gβ3 825T allele.

Prespecified subgroups1 were used to study the relation between daily intake of antihypertensive drugs (yes versus no; and no or 1 2 or more drugs) and hypertension (antihypertensive treatment or blood pressure >95 mm Hg [diastolic] and/or 160 mm Hg [systolic]), and the Gβ3 C825T allele status was further analyzed using logistic regression, controlling for gender, age, and body mass index to estimate the adjusted relative risks of antihypertensive drug therapy in the entire sample. Values measured for plasma renin, prorenin, and aldosterone levels were nonnormally distributed and slightly skewed to the left. Thus, correlation analysis used logarithmically transformed values. Because no qualitative difference was detectable when analyses were repeated using nontransformed variables, β coefficients are reported for nontransformed renin and prorenin levels, and the aldosterone to renin ratio to allow a better estimation of the quantitative changes related to the Gβ3 C825T polymorphism. Probability values are reported for each test and statistical model, including 95% confidence intervals for the relative risk estimates.

Results
In the entire cohort and the subgroups presented below, the genotype distribution of the Gβ3 allele was in Hardy-Weinberg equilibrium. Anthropometric and biochemical data for individuals without antihypertensive medication are listed in Table 1. The T allele was found to be significantly related to higher diastolic blood pressure and, in an opposite fashion, to renin and prorenin levels. Because aldosterone levels were not significantly affected by the Gβ3 allele status, the poly-

<table>
<thead>
<tr>
<th>Variable</th>
<th>CC Genotype</th>
<th>CT Genotype</th>
<th>TT Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>218</td>
<td>209</td>
<td>47</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.4±0.2</td>
<td>57.2±0.2</td>
<td>57.7±0.5</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>47.5</td>
<td>43.4</td>
<td>59.9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±0.2</td>
<td>27.0±0.2</td>
<td>26.1±0.3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>70±0.8</td>
<td>71±0.9</td>
<td>69±1.5</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>143±1.2</td>
<td>145±1.3</td>
<td>142±3.0</td>
</tr>
<tr>
<td>Diastolic DBP, mm Hg</td>
<td>88±0.3</td>
<td>90±0.7</td>
<td>91±1.7§</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>1.9</td>
<td>2.2</td>
<td>0</td>
</tr>
<tr>
<td>Myocardial infarction, %</td>
<td>1.4</td>
<td>0.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Estrogen replacement, %</td>
<td>38.4</td>
<td>26.7</td>
<td>27.8</td>
</tr>
<tr>
<td>Renin, mU/L</td>
<td>17.3±0.8</td>
<td>15.7±0.7</td>
<td>13.2±1.0§</td>
</tr>
<tr>
<td>Prorenin, mU/L</td>
<td>226±11</td>
<td>190±6</td>
<td>189±13‡</td>
</tr>
<tr>
<td>Aldosterone, pmol/L</td>
<td>128±6</td>
<td>127±6</td>
<td>115±10</td>
</tr>
<tr>
<td>Aldosterone/renin ratio</td>
<td>9.7±0.6</td>
<td>10.6±0.6</td>
<td>12.3±1.3*</td>
</tr>
<tr>
<td>All participants (including treated hypertensives)</td>
<td>274</td>
<td>266</td>
<td>68</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>46</td>
<td>51</td>
<td>63§</td>
</tr>
<tr>
<td>Antihypertensive med, %</td>
<td>22.3</td>
<td>22.6</td>
<td>32.4</td>
</tr>
<tr>
<td>2+ Antihypertensive med, %</td>
<td>8.4</td>
<td>9.9</td>
<td>19.7§</td>
</tr>
</tbody>
</table>

CC indicates homozygosity for the Gβ3 C825T genotype; CT, heterozygotes for the Gβ3 C825T genotype; TT, homozygosity for the Gβ3 825T genotype; BMI, body mass index; SBP and DBP, systolic and diastolic blood pressure, respectively; antihypertensive med, percent subjects on antihypertensive medication; 2+ antihypertensive med, percent subjects taking 2 or more antihypertensive drugs. Values are expressed as mean±SEM and percentages. *P for trend <0.05. †P for trend <0.01.
morphism was also related to an increased aldosterone to renin ratio. Other parameters (including age, gender, heart rate, body mass index, and systolic blood pressure level) displayed no significant differences between the CC, CT, and TT genotype groups (Table 1).

Because previous studies have suggested that renin levels may be weakly related to blood pressure levels in an inverse fashion, we analyzed whether this presumed downregulation of renin levels is affected by the Gβ3 allele status. Interestingly, untreated hypertensive subjects carrying the TT genotype presented with markedly depressed renin levels (n=22, 11.8±1.1 mU/L) compared with untreated hypertensive subjects with the CC (n=67, 16.8±1.6; P<0.05) or CT (n=78, 15.3±1.1; P<0.05) genotype and compared with normotensive individuals (all genotypes: n=306, 16.7±0.6 mU/L; P<0.05; CC: n=150, 17.6±0.9 mU/L; CT: n=131, 16.0±1.0 mU/L; TT: n=25, 14.5±1.5 mU/L).

Significant associations between the TT genotype and diastolic blood pressure, plasma renin, and prorenin levels (data not shown), and the aldosterone to renin ratio persisted after adjustment for age, gender, body mass index, and systolic blood pressure (Figure). Furthermore, significant associations between the Gβ3 allele status and renin and the aldosterone to renin ratio were replicated when diastolic blood pressure was replaced by systolic blood pressure in the multivariate model (data not shown).

When all individuals including those taking antihypertensive drugs were analyzed, the T allele was significantly related to the prevalence of hypertension and the intake of antihypertensive combination therapy (Table 1), a finding that persisted after adjustment for age, gender, and body mass index (Table 2).

Discussion
In the present study, carriers of a frequent polymorphism at position 825 of the Gβ3 subunit gene were found to have higher diastolic blood pressure levels, lower renin levels, and a higher aldosterone to renin ratio. Interestingly, these features are similar to some of the alterations found in patients with low renin hypertension.

Given the nature of the underlying polymorphism, namely an in-frame deletion of the Gβ3 protein associated with enhanced signaling via pertussis toxin–sensitive G proteins, it is tempting to speculate that this single molecular alteration may contribute to the distinct levels of diastolic blood pressure and biochemical findings. First, some vasoactive factors (including norepinephrine via α2-adrenoceptors, angiotensin II, and thrombin) are known to transmit some of their effects via pertussis toxin–sensitive G proteins. Thus, these vasoconstrictors, if activated, may cause enhanced vascular resistance in individuals carrying the mutated Gβ3 protein. Alternatively, the higher diastolic blood pressure levels in these subjects might be explained by vascular hypertrophy. In particular, growth factors of vascular smooth muscle cells, like platelet-derived growth factor, are known to activate G protein–dependent pathways.

Second, renin synthesis (reflected by total renin or prorenin plus renin levels) and renin release are strongly augmented by cAMP, i.e., the intracellular messenger that is under negative control by G, proteins. Thus, enhanced G, protein–related signaling is likely to suppress plasma renin levels.

Third, despite genotype-related differences in renin and presumably angiotensin II levels, no differences were found with respect to aldosterone levels. It may be interesting to note, therefore, that some stimulatory factors of aldosterone release (eg, angiotensin II) can act via G, proteins in a stimulatory fashion, enhancing phospholipase C activity.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>1.84 (1.03–3.3)</td>
</tr>
<tr>
<td>Antihypertensive med</td>
<td>1.80 (0.96–3.38)</td>
</tr>
<tr>
<td>2 + Antihypertensive med</td>
<td>3.60 (1.61–8.18)</td>
</tr>
</tbody>
</table>

Values are odds ratios (95% confidence intervals) for the prevalence of hypertension, antihypertensive drug use, and use of 2 or more antihypertensive drugs in carriers of the TT vs CC genotype. The ratios are adjusted for gender, body mass index, and age.
cytosolic calcium liberation, and the expression of the aldosterone synthase gene.\textsuperscript{14} Thus, it may be speculated that in individuals with the TT genotype, lower angiotensin II levels can be compensated for by enhanced postreceptor signaling. Thereby, aldosterone levels may be inappropriately high given the suppressed renin levels. Taken together, the contention that the $G_\beta_3$ polymorphism may contribute to these features seems to be well supported by recent experimental evidence. However, further studies are necessary to confirm the present observations and to precisely elucidate the mechanisms that may explain the associations between the $G_\beta_3$ 825T allele with higher diastolic blood pressure level, lower renin level, and an elevated aldosterone to renin ratio. Furthermore, these effects of the $G_\beta_3$ polymorphism should be reassessed with regard to enzymatically measured plasma renin activity because this method may offer enhanced sensitivity in the lower range of renin levels.\textsuperscript{15}

The $G_\beta_3$ 825T allele has been overrepresented in prespecified groups\textsuperscript{2} characterized by arterial hypertension or the use of antihypertensive agents. With regard to blood pressure regulation, these findings may underscore the pathophysiological relevance of the polymorphism. Moreover, these data corroborate a recent case-control study on selected individuals that initially suggested an association of the polymorphism with hypertension.\textsuperscript{3} Combined, these studies on 2 distinct MONICA cohorts of the Augsburg study region in Bavaria involve more than 1000 subjects and accumulate evidence that a polymorphism in the $G_\beta_3$ subunit gene may be related to essential hypertension in white populations. Given the recent experience that association studies on polygenic disorders may have discrepant results, a word of caution has to accompany the present findings.\textsuperscript{16} In particular, polygenic disorders may have discrepant results, a word of caution has to accompany the present findings.\textsuperscript{16} In particular, polygenic disorders may have discrepant results, and thus, the alterations in intracellular signaling may allow investigators to discriminate candidate genes and thereby to discover the polymorphism in the $G_\beta_3$ subunit gene that is related to in-frame deletion of the wild-type protein.\textsuperscript{3,4} In turn, the discovery of the molecular basis of the cellular phenotype made it possible to revisit subjects with hypertension and to test the implications using population genetic methodologies. Thus, the alterations in transmembrane signaling caused by the polymorphism had been established before the $G_\beta_3$ 825T genotype was assessed by the present association study, thus giving strength to the reported findings.

In summary, this study provides evidence that low renin and elevated diastolic blood pressure levels may be related to a splice variant of the $G_\beta_3$ subunit gene that had been shown to enhance $G$ protein-related transmembrane signaling.

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References

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