G-Protein \( \beta_3 \) Subunit Gene Variant and Left Ventricular Hypertrophy in Essential Hypertension

Esteban Poch, Daniel González, Elisenda Gómez-Angelats, Montserrat Enjuto, Joan Carles Paré, Francisca Rivera, Alejandro de la Sierra

Abstract—A functional genetic variant consisting of a C825T substitution in the \( GNB3 \) gene, encoding for the G-protein \( \beta_3 \) subunit, has been associated with enhanced G-protein activation and cell growth. The aim of the study was to investigate the association of this polymorphism with left ventricular hypertrophy (LVH) in a sample of patients with essential hypertension. Left ventricular mass was assessed by 2-mode echocardiography in 86 patients with essential hypertension, and \( GNB3 \) C825T genotype was determined by polymerase chain reaction and restriction digestion. Thirty-seven (0.43) patients were homozygous for the C allele (CC), 40 (0.47) were heterozygous (CT), and 9 (0.10) were homozygous for the T allele (TT). The genotype distribution among the patients was in Hardy-Weinberg equilibrium. Values of left ventricular end-diastolic diameter (52.0±0.7 versus 48.9±0.9 mm, \( P=0.007 \)), posterior wall thickness (11.3±0.2 versus 10.6±0.2 mm, \( P=0.042 \)), and left ventricular mass index (152.7±4.4 versus 135.2±6.4 g/m², \( P=0.023 \)) were significantly higher in patients with CT and TT genotypes considered together (CT+TT) than in CC patients. The distribution of the genotypes was significantly different when comparing patients with LVH: 20 (0.33) CC and 40 (0.67) CT+TT patients had this complication, and 17 (0.65) CC and 9 (0.35) CT+TT patients did not (\( P<0.01 \)). The frequency of the T allele was significantly different among patients with (0.40) and without (0.20) LVH (\( P<0.01 \)). A logistic regression analysis showed that the association between the T allele and LVH was independent of age, mean blood pressure, body mass index, and alcohol consumption. The relative risk of LVH in patients bearing the T allele (CT+TT group) compared with CC hypertensive patients was 3.03 (95% CI 1.14 to 8.05). The findings suggest an association between LVH and the 825T allele in hypertensive patients. (Hypertension. 2000;35[part 2]:214-218.)

Key Words: hypertrophy ⋅ G proteins ⋅ genes ⋅ hypertension, essential ⋅ polymorphism

Left ventricular hypertrophy (LVH) is a major independent risk factor for morbidity and mortality from cardiovascular disease.\(^1\) Blood pressure is an important determinant of LVH,\(^2,3\) and a substantial percentage of patients with essential hypertension develop this complication. However, the degree of such hypertrophy varies greatly from patient to patient.\(^4,5\) Moreover, epidemiological studies have demonstrated that subjects with LVH may have near-normal blood pressure,\(^4\) suggesting that other factors may be important in the development of the hypertrophy. Studies in families and twins have shown that left ventricular mass is a familial trait, indicating the influence of both genetic and environmental factors.\(^5,6\) The genes influencing the control of cardiac growth and hypertrophy are candidates for LVH.

A single base substitution (C→T) at position 825 of the \( GNB3 \) gene, which encodes the \( \beta_3 \) subunit of G proteins, is related to alternative splicing of exon 9, resulting in the loss of 41 amino acids.\(^7\) The 825T allele has been associated with enhanced stimulated binding of labeled GTP in cell lines from hypertensive patients and in transfected insect cells.\(^7\) This polymorphism was discovered after investigation of the mechanism underlying enhanced Na\(^+\)-H\(^+\) exchange activity, an intermediate phenotype displayed by 30% to 50% of patients with essential hypertension.\(^8\) Na\(^+\)-H\(^+\) exchange is involved in the control of pH and cell volume and may also participate in the initiation of cell growth and proliferation.\(^8,9\) Rosskopf et al\(^10\) have demonstrated that the abnormal kinetics of the Na\(^+\)-H\(^+\) exchanger is genetically fixed, because it persisted in immortalized lymphocytes from patients with essential hypertension. In addition, the enhanced Na\(^+\)-H\(^+\) phenotype observed in the “hypertensive” cell lines has been associated with an enhanced proliferation pattern and enhanced G-protein activation of the corresponding cell line.\(^10,11\) The possible association between the 825T allele and essential hypertension has been very recently reported in case-control studies. However, the results have been conflicting,\(^7,12–15\) and no reported data regarding its possible relation to organ damage are available. Because we and others
previously reported that Na\textsuperscript{+}-K\textsuperscript{+} exchanger activity was associated with LVH in patients with essential hypertension,\textsuperscript{16} our aim in the present study was to analyze the association between the C825T polymorphism of the G-protein β\textsubscript{3} subunit gene and LVH in a sample of patients with essential hypertension.

### Methods

#### Study Subjects

Eighty-six consecutive patients with essential hypertension (50 male and 36 female), aged 21 and 68 years (mean±SEM 51±1 years), were recruited from the Hypertension Unit, Hospital Clinic, Barcelona, Spain, on the basis of not having received prior antihypertensive therapy. These patients had an office diastolic blood pressure between 90 and 114 mm Hg in at least 3 repeated measurements. If no known cause of high blood pressure could be detected after complete clinical, biochemical, and radiological examination, a diagnosis of essential hypertension was considered. None of the patients had renal impairment (serum creatinine >132 μmol/L), cardiac failure, or evidence of coronary heart disease. Patients with any severe concomitant pathological condition or alcohol intake of >100 g of pure ethanol per day, pregnant women, or those taking contraceptive pills were excluded from the study. The protocol was approved by the Ethics Committee of the Hospital Clinic, and all patients gave their informed consent. Blood pressure was assessed by 24-hour ambulatory blood pressure monitoring with the Spacelabs 90207 monitor.

#### Evaluation of LVH

Two-dimensional-controlled M-mode echocardiograms were recorded with each patient in the partial left decubitus position after a rest of at least 10 minutes. According to the criteria of the American Society of Echocardiography,\textsuperscript{17} the following parameters relative to LVH were recorded with each patient in the partial left decubitus position after a rest of at least 10 minutes: (1) left ventricular end-diastolic diameter, (2) left ventricular end-systolic diameter, (3) left ventricular diastolic posterior wall thickness, (4) interventricular septum thickness, and ejection fraction by the Teicholz method. Left ventricular mass was determined by using the Penn convention criteria\textsuperscript{18} and divided by the body surface area to calculate left ventricular mass index (in g/m\textsuperscript{2}). LVH was diagnosed if left ventricular mass index exceeded 110 g/m\textsuperscript{2} in women and 130 g/m\textsuperscript{2} in men.\textsuperscript{19} The relative wall thickness ratio was obtained by using the standardized formula\textsuperscript{20}: 2×posterior wall thickness/left ventricular end-diastolic diameter.

#### Genotype Determination for GNB3 C825T Polymorphism

DNA was extracted from whole blood according to standard procedures. Polymerase chain reaction (PCR) was conducted in a 25-μL volume reaction containing 20 mmol/L Tris-HCl (pH 8.4), 50 mmol/L KCl, 1.1 mmol/L MgCl\textsubscript{2}, 200 μmol/L dNTPs, 0.8 μmol/L each primer, 1 U Taq polymerase (Boehringer-Mannheim), and 125 ng genomic DNA. The primer pair used for PCR amplification was 5′-TGACCACTGTCCACCCGTGC-3′ (sense) and 5′-GCAGCAGCGGTGCC-3′ (antisense), as previously reported.\textsuperscript{7} After a denaturation step at 94°C for 5 minutes, PCR was conducted for 34 cycles with denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension step at 72°C for 5 minutes. The PCR product was digested with the restriction enzyme BseDI (Fermentas), electrophoretically resolved on 2.5% agarose, and stained.

#### Statistical Analysis

Values are expressed as mean±SEM in normally distributed variables and as median (range) in variables that deviate from normal distribution. Differences among G-protein β\textsubscript{3} subunit C825T polymorphism genotypes were compared by Student t test or nonparametric Mann-Whitney test, with the CT and TT genotypes being pooled together. The association between genotype distribution and LVH was examined by contingency tables analyzed by χ\textsuperscript{2} test, in which odds ratios and 95% CIs were calculated. A logistic regression analysis was performed to test the influence of several factors in the association between the G-protein β\textsubscript{3} subunit C825T polymorphism genotype distribution and LVH. A value of P<0.05 was considered significant. All statistical analyses were performed by using the statistical software package SPSS version 6.1.3. (SPSS Advanced Statistics, 1995).

#### Results

Among the 86 hypertensive patients studied, 37 (0.43) were homozygous for the C allele (CC), 40 (0.47) were heterozygous (CT), and 9 (0.10) were homozygous for the T allele (TT). The distribution of genotypes was in Hardy-Weinberg equilibrium.

Partly because of the low frequency of the TT genotype, patients with CT and TT genotypes were considered together for further analysis (CT+TT). This grouping was performed on 2 additional relevant grounds: (1) there were no differences in age, gender, blood pressure, and cardiac parameters between CT and TT subjects (data not shown), and (2) according to Siffert et al.,\textsuperscript{7} the associated cellular phenotype (platelet-activating factor–stimulated binding of [\textsuperscript{35}S]GTP\textsubscript{γ}S)
was not different between CT and TT genotypes. As shown in Table 1, age, gender distribution, and values of plasma renin activity, aldosterone, or atrial natriuretic peptide were not statistically different when patients with the CC genotype and patients with the CT+TT genotypes were compared. Patients with CT+TT genotypes presented slightly higher values of blood pressure, although only diastolic blood pressure with CT TT genotypes reached statistical significance (P=0.033). The relative risk of LVH in CT+TT patients compared with CC patients was 3.03 (95% CI 1.14 to 8.05). Moreover, in a stepwise logistic regression model using LVH as the dependent variable and age, body mass index, mean blood pressure, alcohol consumption, and G-protein β3 subunit C825T polymorphism genotype as independent variables, the presence of the T allele (CT+TT genotypes) was the only variable independently associated with LVH (P=0.0058).

Table 2 summarizes echocardiographic parameters of the patients classified according to the GNB3 C825T polymorphism genotype. As shown, hypertensive patients with CT+TT genotypes had greater left ventricular dimensions and thickness than did patients with the CC genotype. Values of left ventricular end-diastolic diameter (52.0±0.7 versus 48.9±0.9 mm, P=0.007), posterior wall thickness (11.3±0.2 versus 10.6±0.2 mm, P=0.042), and left ventricular mass index (152.7±4.4 versus 135.2±6.4 g/m², P=0.023) were significantly higher in CT+TT patients compared with CC patients.

The distribution of genotypes was significantly different when patients with LVH were compared with patients without this complication: 20 (0.33) CC, 32 (0.67) CT+TT patients showed echocardiographic criteria of LVH, whereas 17 (0.65) CC and 9 (0.35) CT+TT patients (P<0.01) had normal left ventricular mass index (Table 3). The frequency of the T allele was significantly higher (0.40) in patients with LVH than in those without LVH (0.20) (P<0.01). When the 3 genotypes were analyzed separately, the association between LVH and GNB3 C825T polymorphism was also significant: there were 20 (0.33) CC, 32 (0.53) CT, and 8 (0.14) TT patients with LVH, and there were 17 (0.65) CC, 8 (0.31) CT, and 1 (0.04) TT patients without LVH (P=0.019).

LVH, as defined above, was present in 40 (0.82) of CT+TT patients (0.80 in the CT group and 0.88 in the TT group), whereas only 20 (0.54) of CC hypertensive patients displayed this echocardiographic abnormality (P=0.023). The relative risk of LVH in CT+TT patients compared with CC patients was 3.03 (95% CI 1.14 to 8.05). Moreover, in a stepwise logistic regression model using LVH as the dependent variable and age, body mass index, mean blood pressure, alcohol consumption, and G-protein β3 subunit C825T polymorphism genotype as independent variables, the presence of the T allele (CT+TT genotypes) was the only variable independently associated with LVH (P=0.0058).

**Table 2. Echocardiographic Characteristics of Essential Hypertensive Patients Classified According to G-Protein β3 Subunit C825T Polymorphism Genotype**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>CC (n=37)</th>
<th>CT+TT (n=49)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD, mm</td>
<td>48.9±0.9</td>
<td>52.0±0.7</td>
<td>0.007</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>29.7±0.8</td>
<td>31.6±0.7</td>
<td>0.088</td>
</tr>
<tr>
<td>IST, mm</td>
<td>11.5±0.3</td>
<td>12.2±0.3</td>
<td>0.120</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>10.6±0.2</td>
<td>11.3±0.2</td>
<td>0.042</td>
</tr>
<tr>
<td>EF, %</td>
<td>67.5±1.3</td>
<td>67.2±1.1</td>
<td>0.829</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>135.2±6.4</td>
<td>152.7±4.4</td>
<td>0.023</td>
</tr>
<tr>
<td>WTR</td>
<td>0.44±0.01</td>
<td>0.44±0.01</td>
<td>0.918</td>
</tr>
</tbody>
</table>

Values are mean±SEM. LVEDD indicates left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IST, interventricular septum thickness; PWT, posterior wall thickness; EF, ejection fraction; LVMI, left ventricular mass index; and WTR, relative wall thickness ratio.

Table 3 summarizes C825T genotypes and allele frequencies in hypertensive patients with and without LVH.

**Table 3. C825T Genotypes and Allele Frequencies in Hypertensive Patients With and Without LVH**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Total Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>CT+TT</td>
</tr>
<tr>
<td>Patients with LVH</td>
<td>40 (0.67)</td>
</tr>
<tr>
<td>Patients without LVH</td>
<td>9 (0.35)</td>
</tr>
</tbody>
</table>

χ² tests were performed for patients with LVH vs patients without LVH for analyses of genotype distributions and allele frequencies.

*P<0.02 and †P<0.01.
these processes. Most of the DNA markers studied are not related with functional changes in the gene of interest. Therefore, positive associations are usually attributed to linkage disequilibrium with putative functional changes close to the genetic locus, which may vary depending on the population studied. It is relevant to test the G-protein β3 subunit C825T polymorphism because it is related to functional changes that have been previously linked to essential hypertension. A recent case-control study in Germany initially showed that the T allele was significantly associated with essential hypertension. A subsequent population-based study confirmed the association of the T allele with elevated diastolic blood pressure and low renin. However, studies performed in populations of different ethnicity failed to show association of the T allele with blood pressure or hypertension. Moreover, a recent report failed to show association between the GNJB3 polymorphism and the severity of hypertension assessed by ambulatory blood pressure monitoring. Of note, in the study performed in Japan, the frequency of the T allele was remarkably higher (49%) than that reported for the white population (≈30%), and this difference could have accounted for the discrepancy in the result. In the present study, the frequency of genotypes among the subjects was similar to that reported by Schunkert et al in a white population and was in Hardy-Weinberg equilibrium. In contrast to the study of Beige et al, in our sample of patients, the T allele was associated with slightly higher 24-hour ambulatory diastolic blood pressure values.

LVH is a major independent risk factor for morbidity and mortality from cardiovascular disease. Although blood pressure, stroke volume, and decreases in contractile efficiency are important determinants of LVH, this complication is not present in all patients with essential hypertension and can develop before the establishment of hypertension in some individuals. Thus, the pathogenic mechanism of LVH may be multifactorial, involving both hemodynamic and nonhemodynamic factors, such as the sympathetic nervous system and the renin-angiotensin system. In addition, epidemiological studies have demonstrated that subjects with LVH may have near-normal blood pressure, indicating that additional factors besides blood pressure may be important in the development of the hypertrophy. On the other hand, studies in families and twins have shown that left ventricular mass is a familial trait, indicating the influence of both genetic and environmental factors. Recent investigation into the genetic factors for LVH have focused mainly on the renin-angiotensin system, with focus on the insertion/deletion polymorphism of the angiotensin-converting enzyme gene associated with LVH, but results have been conflicting.

G-protein β3 subunit gene polymorphism may constitute a genetic basis for the enhanced growth phenomena observed in essential hypertension, such as LVH and media hypertrophy of the vessel wall, because it is directly associated with a functional change and may not merely represent a DNA marker. G proteins mediate part of the actions of vasoactive hormones, such as angiotensin II and norepinephrine. Angiotensin II exerts trophic effects on cardiomyocytes in culture and is known to stimulate growth in other cell systems. On the other hand, growth factors, such as platelet-derived growth factor, can act in part through pertussis-sensitive G proteins in skin fibroblasts, vascular smooth muscle cells, and cardiac fibroblasts and may participate in the pathogenesis of LVH. To our knowledge, no previous study has investigated the possible association between the G-protein β3 subunit and LVH. In our sample of hypertensive patients, the T allele was associated with LVH. Although patients with the T allele had slightly higher diastolic blood pressure levels, a logistic regression analysis showed that the association of the T allele with LVH was independent of age, blood pressure, body mass index, and alcohol consumption.

In summary, we have shown that the 825T allele in the G-protein β3 subunit gene is associated with higher diastolic blood pressure, left ventricular mass, and LVH in patients with essential hypertension. Additional studies in other populations will be needed to confirm this association.

Acknowledgments

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