Signature: Med Sci Monit, 2002; 8(5): 337-340
PMID: 12011775

Received: 2001.12.07
Accepted: 2002.02.05
Published: 2002.05.15

Lack of association of G-protein subunit gene C825T polymorphism with left ventrucular hypertrophy in essential hypertension

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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Summary

Background: The aim of the present study was to determine if there is an association of G-protein β3 subunit (GNB3) gene polymorphisms and left ventricular hypertrophy (LVH) in patients with essential hypertension (EH) in a St. Petersburg population.

Material/Methods: We examined 135 patients (mean age 48±7 yrs) with mild to moderate EH recruited from the general population of an outpatient hypertension clinic. Left ventricular mass was measured by echocardiography, and the left ventricular mass index (LVMI) was calculated. The GNB3 C825T genotype was determined by polymerase chain reaction and restriction digestion.

Results: 67 patients (50%) were homozygous for the C allele (CC), 56 were heterozygous (CT) (41%) and twelve (9%) were homozygous for the T allele (TT). The distribution of genotypes among the patients was in Hardy-Weinberg equilibrium and did not differ significantly when comparing patients with or without LVH. The frequency of the T allele was only slightly higher in patients with LVH (32%) compared to those without LVH (28%), NS, and the LVMI was similar in patients with the CC, CT and TT genotypes (122.3±29.8; 118.8±29.9 and 115.2±18.3 g/m^2, respectively). No significant discrepancies were found among the various genotype groups in posterior wall thickness, interventricular septum thickness, or functional parameters such as ejection fraction, isovolumetric relaxation time and E/A ratio.

Conclusions: These observations clearly suggest the lack of association between left ventricular structure or function and the CNB3 gene variant in the studied population.

key words: **left ventricular hypertrophy • G-protein • essential hypertension • genetics**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_8/no_5/2367.pdf
File size: 83 kB
Word count: 1730
Tables: 3
Figures: –
References: 19

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BACKGROUND

Evidence is beginning to accumulate that left ventricular hypertrophy (LVH) due to pressure overload is at least partly genetically predisposed [1–4]. Numerous association studies have been undertaken to reveal the exact genes responsible for LVH development in essential hypertension [5–8]. At the same time, there is conflicting evidence for the association of several particular genetic determinants and LVH. Thus I/D polymorphism of the angiotensin-converting enzyme (ACE) gene has been tested as a potential risk factor for LVH in a large number of studies, and the proportion of negative and positive results appears to be roughly equal [9]. Recently, the genes for intracellular regulating factors have been introduced as 'candidate genes' for LVH, along with the genes for the renin-angiotensin system, membrane receptors and several growth factors. Among such intracellular messengers, a position of primary interest is occupied by the G-protein β3 subunit (GNB3) gene, which exhibits a genetic polymorphism (C825T) in exon 9, characterized by the loss of 41 aminoacid [10]. The T allele of the gene is believed to be associated with increased activity of G-protein and stimulation of cell proliferating activity [10]. Several studies have reported a possible relation between this polymorphism and essential hypertension [11,12], but this has not been confirmed by other investigators [13]. A recent pilot study by Poch et al. [14] has documented an association of GNB3 gene polymorphisms with LVH in hypertensive patients, opening a new field of scientific inquiry on this problem. It is obvious that these results should be confirmed in larger trials and other populations before a final conclusion can be drawn.

The goal of the present study, then, was to determine if there is an association between G-protein β3 subunit (GNB3) gene polymorphisms and left ventricular hypertrophy (LVH) in patients with essential hypertension (EH) from a St. Petersburg population.

MATERIAL AND METHODS

Study participants

The patients for the study were selected from the primary health care outpatient clinic in one of the municipal districts of St. Petersburg. All patients (n=156) had a previously established diagnosis of essential hypertension based on the standard WHO criteria (three consecutive blood pressure measurements on the right brachial artery in sitting position above 140 mm Hg for systolic blood pressure (BP) and/or above 90 mm Hg for diastolic BP). Possible causes for secondary hypertension were excluded. All the participants were white, ranging in age from 18 to 65, with no concomitant cardiovascular diseases and/or diabetes. Patients with a body mass index (BMI) more than 25 were considered overweight, while those with a BMI more than 30 were classified obese.

Study design

The patients were examined according to a protocol which included vital sign recordings (blood pressure and heart rate, measured in the morning without medication), and registration of weight, height, and body mass index. Blood samples were collected for DNA extraction.

The study protocol was approved by the local Ethics Committee, and informed consent was obtained from all the participants.

Echocardiography

Echocardiography was performed in M- and 2D-mode with a Vingmed-CFM-800 machine (GE, USA) with a 3.25 MHz transducer. Echocardiographic studies of good quality were performed in the morning with the subject in supine left lateral decubitus, after 30 min of rest, by one trained observer. Interventricular septum and left ventricular posterior wall were chosen in the parasternal long axis view. All measurements were made according to the Penn convention. The left ventricular mass was calculated by the Devereux and Reicheck formula [15]:

$$LVM = 1.04\ [(IVSd + PWLVd + LVIDD)^3 - LVIDD^3] - 13.6\ g,$$

where LVM is the left ventricular mass (g), IVSd is the diastolic interventricular septum thickness (cm), PWLVd is the diastolic left ventricular posterior wall thickness (cm), and LVIDD is the left ventricular internal dimension at the end of diastole (cm).

The left ventricular mass index (LVMI) was obtained by dividing LVM on body surface area, calculated by the formula of Dubois and Dubois [16]. Left ventricular hypertrophy was diagnosed when the LVMI was greater than 134 g/m^2 in men and 110 g/m^2 in women.

The relative wall thickness (RWT) was measured as IVSd + PWLVd/LVIDD.

Patients with regional wall movement abnormalities and depressed systolic function (EF less than 50%) were excluded from the study. Patients with asymmetric LVH (IVSd/PWLVd>1.5) were also not included.

Doppler measurements

Mitral inflow velocity was recorded from the apical four-chamber view by pulsed Doppler. Diastolic filling indexes included the following: peak early velocity (E), peak atrial velocity (A), and isovolumetric relaxation time (IVRT), i.e. the time interval from aortic valve closure to mitral valve opening. The latter was measured by pulsed and continuous-wave Doppler interrogation of left ventricular outflow tract velocity.

Genotyping

Genomic DNA was isolated and purified from peripheral leukocytes in a whole blood sample (EDTA, phenol chloroform). DNA was amplified by polymerase chain reaction (PCR) in 30 μL volume reaction containing 10

mmol/l Tris-HCl, 50 mmol/l KCl, 1.5 mmol/l $MgCl_2$, 250 µmol/L dNTPs, 1.8 µL genomic DNA, 15 ng of each primer and 1 U Tag DNA polymerase. PCR was performed using specific primers described by Siffert [10]. The PCR samples were electrophoresed in 2% agarose gels, and the DNA was visualized by ethidium bromide. One investigator performed all genotype determinations in blinded mode.

Statistical analysis

The data were analyzed using the Statistica 6.0 program. Chi-squared statistics were calculated between the genotype distribution (and allele frequencies) and LVH status. The means of the different genotype groups were compared in one-way analysis of variance. The results are presented as means ± SD. All tests were two-sided.

RESULTS

135 patients (94 female and 7741 male) with echocardiographic images of reliable quality were analyzed. The mean age was 49±8 years, disease duration 12±2 years. 19 patients had normal body weight, 63 were overweight and 53 were obese. The mean body mass index (BMI) was 32±7 g/m^2. The vital signs obtained clinically were 166±17/98±12 mmHg for blood pressure (BP) and 66±7 beat/min for heart rate (HR). The echocardiographic parameters in the study group are summarized in Table 1. 68 (51%) patients were diagnosed to have LVH, and 25 of 77 patients (32%) had diastolic dysfunction.

Genotype distribution

67 patients (50%) were homozygous for the C allele (CC), 56 were heterozygous (CT) (41%) and twelve (9%) were homozygous for T allele (TT) (table 2). The distribution of genotypes among the patients was in Hardy-Weinberg equilibrium and did not differ significantly when comparing patients with or without LVH. The frequency of the T allele was only slightly higher in patients with LVH (32%) compared to those without LVH (28%). The TT and CT genotypes were more frequent in the LVH group, but again these discrepancies were not statistically significant.

The echocardiographic parameters in the various genotype groups are presented in table 3. As one can see from the data presented, the LVMI was similar in patients with CC, CT and TT genotypes. No significant discrepancies were found between the particular genotype group in terms of posterior wall thickness, interventricular septum thickness, or functional parameters, such as ejection fraction, isovolumetric relaxation time, and E/A ratio.

DISCUSSION

The present study has demonstrated a lack of association between CNG3 gene C825T polymorphism and LVH, as well as diastolic dysfunction, in hypertensive patients. It is worth mentioning that investigations of the role of G-protein genotype in the genesis of hypertensive LVH were started relatively recently and relatively little data is available. Theoretically, taking into account the positive association of CNG3 gene polymorphism and cell proliferation [10], together with evidence of the contribution of this genetic determinant to the

Table 1. Echocardiographic characteristics of the patients.

Parameters	Means±SD
LVPWd [mm]	10.1±1.7
IVSd [mm]	10.9±1.8
LVDDd [mm]	49.7±4.3
RWT	0.42±0.07
LVMI [g/m^2]	120±29
IVRT [ms]	95±20
E/A	1.01±0.29
EF [%]	67.2±4

LVMI – left ventricular mass index, PWLVd – posterior wall diastolic thickness, IVSd – interventricular diastolic wall thickness, LVIDD – left ventricular internal diastolic dimension, RWT – relative wall thickness, EF – ejection fraction, E/A – early to atrial peak velocity ratio, IVRT – isovolumetric relaxation time

Table 2. Relative frequencies of CNG3 genotypes and alleles in patients with and without LVH.

	Relative frequencies				
	genotype			allele	
	CC	CT	TT	C	T
With LVH	31 (0.463)	29 (0.433)	7 (0.104)	0.68	0.32
Without LVH	35 (0.522)	27 (0.403)	5 (0.075)	0.72	0.28

Odds ratio for having LVH

TT and CT vs. CC – 1.12 (CI 95%, p=0.54)

Table 3. Characteristics of the patients according to C825T polymorphism of the CNB3 gene.

CNB3 genotype	CC	CT	TT
n	67	56	12
Age [years]	48±5	49±7	47±8
Duration of hypertension [years]	10±3	12±3	12±3
Male/female	21/46	16/40	4/8
Blood pressure, [mm Hg]	167±22/ 98±11	170±23/ 99±9	167±24/ 98±10
LVDd [mm]	49.6±4.3	48.8±4.5	50.1±4.0
LVPWd [mm]	10.1±1.7	10.0±1.8	9.9±1.3
IVSd [mm]	10.9±2.0	11.1±1.7	10.9±1.5
RWT [mm]	0.43±0.08	0.43±0.07	0.42±0.04
EF [%]	66.7±7.1	67.2±7.2	67.5±8.0
LVMI [g/m^2]	122.3±29.8	118.8±29.9	115.2±18.3
IVRT [ms]	96±17	94±16	101±18
E/A	1.04±0.26	0.98±0.22	1.01±0.18

LVMI – left ventricular mass index, PWLVd – posterior wall diastolic thickness, IVSd – interventricular diastolic wall thickness, LVIDD – left ventricular internal diastolic dimension, RWT – relative wall thickness, EF – ejection fraction, E/A – early to atrial peak velocity ratio, IVRT – isovolumetric relaxation time

development of hypertension [11,12], the T allele of the gene might be involved in the genetic predisposition to LVH. This possible association appears to be reasonable, as all the major hemodynamic and humoral stimuli of LVH are at least partly mediated by G-protein-coupled receptors. Such a link has been recently documented in a study by Poch and colleagues [14]. Besides, the CNB3 gene polymorphism is also associated with increased activity of the Na^+-H^+ exchanger, which in turn is known to be a hypertension-enhancing factor [17–18]. This circumstance has also been assumed to be one of the explanations of the results reported by Poch et al. The major aims and design of the present study, which was conducted almost simultaneously, seem to be similar to those of the research by Poch et al. In both case-control studies, patients with similar age, sex distribution, and hypertension severity were included. The only discrepancy was the population selected, and the fact that the group size was 2 times larger in the present investigation. At the same time, while in the Spanish population from the above-mentioned study the T allele frequency was two times higher in patients with LVH, and the LVMI index was significantly increased in TT+CT patient group, we failed to find any significant relation of the CNB3 gene genotype either with LVMI level, or with LVH. The explanation of these conflicting results may lie in the genetic heterogeneity of the populations selected. Similar conflicting results were obtained in studies performed with populations of different ethnicity concerning the association of the C825T GNB3 gene polymorphism with essential hypertension. In this case, however, the differences of genotype distribution in the populations studied could serve as a reasonable explanation for the contradictions. Thus in a study on a Japanese population [13], the frequency of the T allele was significantly higher than in Germany [11] or Canada [19]. Notably, the genotype and allele distribution in our study was comparable to the reference investigation from Spain [14]. It is well known that an association study should be always interpreted with caution, since LVH, as well as hypertension as a whole, is a pathology with a genetic predisposition, but it is not a monogenic disease. A variety of genes with complex gene-gene interactions contribute to LVH development and progression together with various environmental factors. The discrepancies between the allele distribution of the other involved genes and their interactions could be responsible for the negative results in our study. Alternatively, not only genetic, but also lifestyle discrepancies between populations may explain the observed differences. Taken together, neither LVH severity nor its occurrence was related to the CNB3 gene polymorphism in the group under investigation.

CONCLUSIONS

No relation of this genetic determinant to left ventricular diastolic function has been found. Additional studies in other larger populations will be needed to establish the real role of this genetic marker as a determinant of LVH.

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