

G protein β_3 subunit gene polymorphism in Turkish hypertensives*Hipertansif Türklere G protein β_3 subunit gen polimorfizmi*

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ABSTRACT

Objective: G protein is one of the most important regulators of intracellular signaling pathways. C825T polymorphism of G protein β_3 subunit is associated with increased intracellular signal transduction. The 825T allele has been found associated with a variety of cardiovascular risk factors, including hypertension. The aim of the present study was to investigate the association between the C825T polymorphism of the G protein β_3 subunit and essential hypertension in Turkish population.

Methods: This cross-sectional, case-controlled study included 209 patients with essential hypertension (Patient group) and 82 subjects with normal blood pressure (Control group). The G protein β_3 subunit C825T gene polymorphism was determined by polymerase chain reaction. Hypertension was defined according to JNC VII criteria. Statistical analysis was performed using Chi square and unpaired t tests. Logistic regression analysis was used to study association between hypertension and genotypes.

Results: We found that the frequencies of the G protein β_3 subunit C825T polymorphism in hypertensive and control groups were 17.7%, 59.3%, 23.0% and 32.9%, 48.8%, 18.3%, (CC, CT, TT) respectively ($\chi^2=7.963$, $p=0.019$). In the multivariate logistic regression analysis CT genotype had 2.2 (OR=2.262, 95% CI 1.228-4.167, $p=0.009$), and TT genotype had 2.3 times (OR=2.335, 95% CI 1.089-5.008, $p=0.029$) greater risk of hypertension compared to CC genotype.

Conclusion: It seems that the G protein β_3 subunit C825T gene polymorphism is associated with systolic and diastolic blood pressure. Furthermore, the study indicates that the G protein β_3 subunit may be a susceptible gene to essential hypertension.

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Key words: Hypertension, G protein, gene, polymorphism, genetics, predictive models, logistic regression analysis

ÖZET

Amaç: G protein sistemi intrasellüler sinyal iletimi yollarının, en önemli düzenleyicilerinden birisidir. G protein β_3 subunitindeki C825T polimorfizmi intrasellüler sinyal transdüksiyonundaki artış ile ilişkilidir. C825T alleli ile hipertansiyonu da içeren çeşitli kardiyovasküler risk faktörleri arasındaki ilişki bilinmektedir. Bu çalışmada amacımız, Türk toplumunda C825T polimorfizmi ile hipertansiyon arasındaki ilişkiyi araştırmaktır.

Yöntemler: Vaka-kontrollü, enine-kesitsel çalışmaya esansiyel hipertansiyonu olan 209 hasta (hasta grubu) ve kan basıncı normal olan 82 birey (kontrol grubu) alınmıştır. G protein β_3 subunitindeki C825T polimorfizmi polimeraz zincir reaksiyonu ile belirlendi. Hipertansiyon tanısı JNC VII kriterlerine göre konuldu. İstatistiksel analiz Ki-kare, ve eşleştirilmemiş t testi ile yapıldı. Hipertansiyon ile genotip arasında ilişki lojistik regresyon analiz ile incelendi.

Bulgular: G protein β_3 subunitindeki C825T polimorfizmi frekansı (CC, CT, TT) hipertansif grupta ve kontrol grubunda sırası ile %17.7, %59.3, %23.0 ve %32.9, %48.8, %18.3, olarak saptandı ($\chi^2=7.963$, $p=0.019$). Lojistik regresyon analizinde CC genotipine göre CT (OR=2.262 (%95GA 1.228-4.167, $p=0.009$) ve TT (OR=2.335, %95GA 1.089-5.008, $p=0.029$) genotiplerinde hipertansiyon olma riski sırası ile 2.2 ve 2.3 kat olarak artmış olarak hesaplanmıştır.

Sonuç: Mevcut bulgular, Türk toplumunda G protein β_3 subunitindeki C825T polimorfizmi ile sistolik ve diyastolik kan basıncı arasında bir ilişki söz konusu olduğunu göstermektedir. Ayrıca, bu çalışmada esansiyel hipertansiyona duyarlılık geni, G protein β_3 subunitindeki C825T polimorfizmi olabileceği gösterilmiştir. (*Anadolu Kardiyol Derg 2008; 8: 331-5*)

Anahtar kelimeler: Hipertansiyon, G protein, polimorfizm, genetik, prediktif modeller, lojistik regresyon analizi

Introduction

G proteins are signal transducers that communicate signals from many hormones, neurotransmitters, chemokines, and autocrine and paracrine factors (1). Polymorphism C3T at nucleotide 825 in exon 10 of the β_3 subunit of GTP binding protein (G proteins) has been identified (2). C825T polymorphism is associated with increased intracellular signal transduction (3). The C825T allele has been associated with a variety of cardiovascular risk factors, including hypertension (4, 5), obesity (6, 7), diabetes (8) or dyslipidemia (9, 10). The C825T polymorphism has been reported to induce hypertension (3). A significantly higher frequency of the T allele has been reported in subjects with essential hypertension compared with normotensive control subjects (11-13). The mechanism whereby the 825T variant may lead to hypertension remains unknown, but it may involve increased Na-H exchanger activity (13). An increase in renal sodium reabsorption through increased activity of the renal Na-H exchanger could mediate the rise in blood pressure.

The aim of the present study was to examine the C825T polymorphism of the G protein β_3 - subunit (GNB3) in relation to hypertension in Turkish population.

Methods

This cross-sectional, case-controlled study included 209 patients with essential hypertension (Patient group) and 82 subjects with normal blood pressure (Control group).

Unrelated hypertensive Caucasians of Turkish descent residing in the same geographic region that had a similar socio-economic level were included in the present study. Control subjects (n=82) blood pressures were under <120/80 mm Hg, and none were receiving antihypertensive therapy, treatment for heart disease, or hormone replacement therapy. All participants' age ranged between 30-60 years. Patients younger than 30 years and older than 60 years were excluded because of possibility of secondary hypertension. The exclusion criterion for age is needed to have a more homogenous hypertensive group. The other exclusion criteria were blood pressure more than 200/100 mm Hg or malignant hypertension, patients on oral

contraceptives, the presence of secondary hypertension, severe valvular heart disease, autoimmune disease, inflammatory arteritis, chronic or acute infectious disease, use of steroid or anti-inflammatory drugs within the last three months, renal failure and cancer.

Patients of hypertensive group were under antihypertensive treatment at the inclusion date. Informed consent was obtained from all subjects.

Blood samples were taken in the morning after an overnight fasting. The participants completed a standard questionnaire on demographic characteristics. Body mass index (BMI,) was calculated from height and weight measurements. On three consecutive visits, brachial arterial blood pressure was measured using the left arm after a 10 minutes of rest in a supine position at the first and fifth Korotkoff phases by an arm cuff of appropriate size. Hypertension was defined according to JNC VII criteria. Study group patients had stage I or above hypertension (average of three systolic blood pressure ≥ 140 mmHg or average of three diastolic blood pressure ≥ 90 mmHg on two or more separate clinic days). The serum total cholesterol, triglyceride, high-density lipoprotein cholesterol, and fasting plasma glucose were measured in all subjects.

Genetic analysis

Genomic DNA was isolated from whole blood using Nucleon BACC DNA extraction kit (14). The C825T polymorphism was detected by PCR followed by BseDI (MBI Fermentas) restriction-enzyme digestion as described previously (3), with minor modifications; 2 products were separated on 2% agarose gels and visualized under UV light by ethidium bromide staining (Figure 1). Genotype was confirmed by direct sequence analysis with the use of a dye terminator kit on an ABI 377 automated sequencer. To prevent observer bias, the investigator was unaware of sample origin and all gels were crosschecked by a separate individual.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (Version release 10.0, SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm standard deviation for continuous variables and as proportions for categorical variables. Kolmogorov Smirnov was used to test normality of data distribution. Among all variables included into the analysis

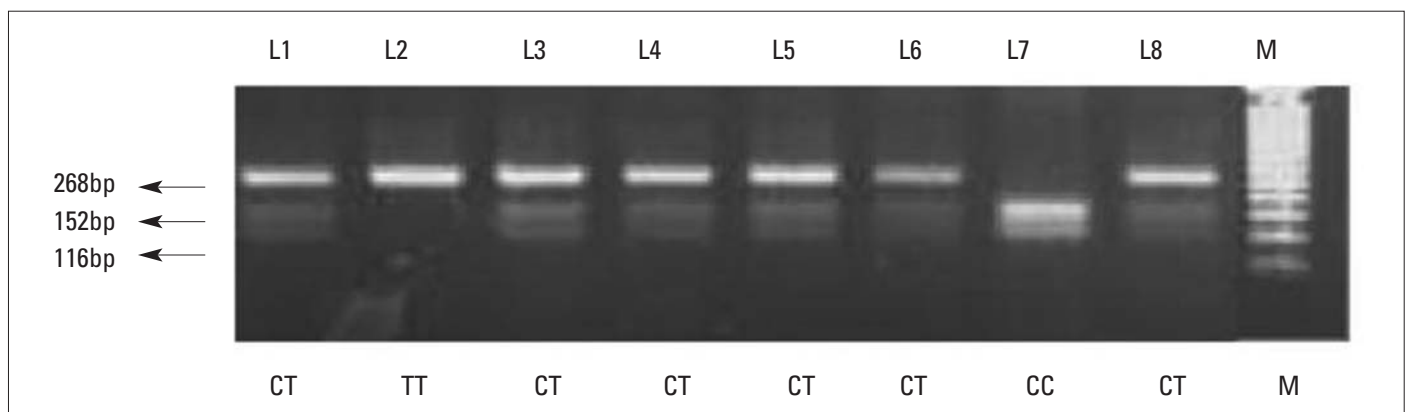


Figure 1. Polymerase chain reaction (PCR) products were detected by staining with ethidium bromide after separation on 2% agarose gel electrophoresis
CC Homozygote- 152 and 116 bp DNA fragments , CT Heterozygote- 268, 152 and 116 bp DNA fragments, TT Homozygote- 268 bp DNA fragments

only hypertension duration was abnormally distributed. Unpaired t test was used for comparison of normally distributed variables and Mann Whitney U test was used in case of abnormal distribution. Differences in C825T polymorphism for the three groups (CT, TT and CC) were tested by Bonferroni after justification by one-way analysis of variance. Association between hypertension and genotype was tested with χ^2 test. Multiple logistic regressions were used to test the effect of genotype on the likelihood of hypertension while controlling for confounding factors such as body mass index and diabetes. Dependent variable was hypertension and independent variables were C825T gene polymorphisms (CT, TT and CC) for the logistic regression analysis. We additionally assessed the association of risk of hypertension with presence or absence of T allele, where dependent variable was hypertension and independent variables were CC genotype and CT+TT genotypes (groups were composed due to presence or absence of T allele) with covariate body mass index.

A p level of less than 0.05 indicated statistical significance. The calculation of allele frequency to test for Hardy-Weinberg equilibrium was performed by use of standard methods.

Results

A total of 291 individuals were genotyped. The clinical characteristics of the hypertensive patients and controls are presented in Table 1. Hypertension duration was 6.41 ± 6.49 years (median -4.0, minimum 1.0, maximum 30.0 years) in the study group. Control and hypertensive groups were matched by their baseline characteristics except BMI and blood pressure values. Body mass index, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were significantly higher in hypertensive group than in control one ($p < 0.001$ for all).

We found that the frequencies of the G protein β3- subunit C825T polymorphism in hypertensive and control groups were 17.7%, 59.3%, 23.0% and 32.9%, 48.8%, 18.3%, (CC, CT, TT) respectively ($\chi^2 = 7.963$, $p = 0.019$) (Table 2).

In the multivariate logistic regression analysis, CT genotype had 2.2 (OR= 2.262; 95% CI: 1.228-4.167, $p = 0.009$), and TT genotype had 2.3 times (OR= 2.335; 95% CI 1.089-5.008, $p = 0.029$) greater hypertension risk compared to CC genotype (Table 3). Odds ratio was 1.815 (95% CI 1.565-2.106, $p = 0.0001$) when the body mass index was selected as covariate in the multivariate logistic regression analysis.

When the patients divided into two groups according to presence of T allele, patients with T allele had 2.7 times greater risk of hypertension (OR=2.786, 95% CI 1.114-6.967, $p = 0.028$). This association was still significant after adjustment for BMI (OR=1.790, 95% CI 1.546-2.074, $p = 0.0001$).

Total number of diabetic patients were 49. Nine patients (11%) in the control group and 40 patients (19.1%) in the hypertension group were diabetic (Table 1). Fifteen of them had CC genotype, 25 of them - CT genotype and 9 of them - TT genotype. Of 242 nondiabetic patients CC genotype was found in 49 patients, CT genotype - 139 patients and TT genotype - 54 patients. No statistically significant difference in genotype distribution was found according to diabetes mellitus prevalence.

Genotype distribution of GNB3 in the Patients group was not consistent with Hardy-Weinberg equilibrium ($p < 0.05$) because of an observed increase in heterozygotes (124 vs. 104 expected), but was included in the analysis because other quality-control checks (including genotyping of samples with known genotypes) suggested this deviation was not caused by genotyping error. Deviations from expected values may be due to a variety of causes. If an excess of heterozygotes is observed this may indicate the presence of over dominant selection or the occurrence of out breeding.

Discussion

In present study, we investigated the association between a GNB3 825T/C polymorphism and essential hypertension in a Turkish population. We found that the GNB3 825T/C polymorphism is associated with essential hypertension.

Table 1. Demographic characteristics

Parameters	Control group (n=82)	Patient group (n=209)	*p
Male, n (%)	30 (36.6)	74 (35.4)	ns
Age, years	43.9±8.6	41.6±8.3	ns
BMI, kg/m ²	23.5±2.1	30.3±5.3	<0.001
Systolic BP, mmHg	113.0±6.5	156.4±11.3	<0.001
Diastolic BP, mmHg	72.8±6.8	87.0±6.4	<0.001
Hyperlipidemia, n (%)	24 (29.3)	82 (39.2)	ns
Familial CAD history, n (%)	16 (12.4)	26 (19.5)	ns
Cigarette smoking, n (%)	13 (15.9)	56 (26.8)	ns
Diabetes mellitus, n (%)	9 (11.0)	40 (19.1)	ns
Hypertension duration, years	-	6.41±6.49	

Data are expressed as mean±SD and proportions/percentages *unpaired Student's t test for comparison of continuous variables and Chi-square test for comparison of categorical variables BMI- body mass index, BP- blood pressure, CAD- coronary artery disease, ns- non-significant

Table 2. Allele and genotype frequencies of the GNB3 gene

GNB3 genotypes	Control groups (n=82)	Hypertension group (n=209)	*p
CC, n (%)	27 (32.9)	37 (17.7)	$p = 0.019$ $\chi^2 = 7.963$
CT, n (%)	40 (48.8)	124 (59.3)	
TT, n (%)	15 (18.3)	48 (23.0)	
Allele frequencies C/T, n (%)	0.573/0.427	0.474/0.526	

Data are expressed as proportions/percentages
* Chi-square test

Table 3. Logistic regression analysis of association between G-protein polymorphism and hypertension

Genotype	β	OR	95%CI	p
CT genotype	0.816	2.262	1.228-4.167	0.009
TT genotype	0.848	2.335	1.089-5.008	0.029
T allele	1.025	2.786	1.114-6.967	0.028

β - unstandardized regression coefficient, CI - confidence interval, OR - odds ratio

Both genetic and environmental factors contribute to the pathogenesis of essential hypertension. Hypertension is about twice as common in subjects who have one or two hypertensive parents, and many epidemiological studies suggest that genetic factors account for approximately 30% of the variation in blood pressure in various populations (14). Polymorphism in several genes has been associated with blood pressure levels (15). One of these genes is G protein. Results of examining the association between G protein β_3 subunit C825T gene polymorphism and blood pressure regulation supported the observation that the G protein β_3 subunit gene variant affects renal function. 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 (16, 17). Siffert et al. (3) have shown the significant association of the T allele with essential hypertension in Germans. After this report, many studies have investigated the association between the C825T polymorphism and hypertension (7-15). Particularly in studies with white population a positive association between the 825T allele and the increased risk for hypertension had been shown (18).

In the patient-control sample of 291 subjects, we found a significant association between C825T allele of the G protein β_3 subunit gene and essential hypertension. In our study, the frequency of C825T allele was 0.43 in normotensives and 0.52 in hypertensives. The frequency showed in the present study is lower than in black Africans. However, the frequency of the C825T allele in our study is similar to Chinese, Brazilian and Japanese populations (6, 19). Further studies from different parts of the world investigated the relation between C825T allele with essential hypertension. Brand et al. (20) investigated 681 hypertensive subjects from Northern Ireland and France. They did not find an association between hypertension and GNB3 825T/C polymorphism. However, his result was attributed to the misclassification of normotensive controls, which included subjects with borderline hypertension. In another study including hypertensive white men from Finland, no relation was found between this polymorphism and blood pressure (21). No relation was found in populations from Asia (9) and in blacks in the United States (22). There are a number of possible explanations for this inconsistency. These differences probably reflect the role of ethnicity. Ethnicity influences susceptibility to a wide range of diseases and impacts upon disease progression. The frequency of the C825T allele of the G protein β_3 subunit gene varies in different populations (5).

Another possibility is population differences in environmental factors such as diet, behavior, and lifestyle (23). Furthermore, in these studies definition of hypertension and risk factors were different.

In the present study, we have demonstrated the association between polymorphism of the G protein β_3 subunit gene and hypertension in Turkish population. A higher prevalence of hypertension was seen among the carriers of the T variant (both as heterozygotes and homozygotes) compared with the CC genotype. It seems that the 825T polymorphism was associated with systolic and diastolic blood pressure.

Limitations of the study

The first limitation of our study was the absence of data of plasma renin levels, salt intake or salt sensitivity to explain the physiological role of the G protein polymorphism. The second, we used fixed arm for the measurement of blood pressure which might cause underestimation of blood pressure.

Our study is a cross-sectional, case-control study. Essential hypertension is defined as a weak phenotype which can result from multiple hormonal and cellular alterations so that, some researchers think that genetic case-control studies are not very much informative in hypertensive subjects. Nevertheless, this study has demonstrated the relation of hypertension with 825T polymorphism.

Conclusion

We found a significant association between the C825T gene polymorphism of the G protein and hypertension in Turkish population. This is the first report examining the relationship between 825T allele of the G protein gene polymorphism and hypertension in a sample from a Turkish population. Results of some previous studies are in contradiction with our study. Despite the presence of significant number of investigations about the role of C825T gene polymorphism of G protein in the pathogenesis of hypertension, the functional significance of these polymorphisms has been still unclear. These polymorphisms may explain ethnic differences of C825T polymorphism. Further studies will be needed to clarify the relationship between G protein and other polymorphisms, which also contribute to the hypertension.

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