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Human G-Protein β3 Subunit C825T Polymorphism is Associated with Serum Total Cholesterol and LDL-Cholesterol Levels in Koreans

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Abstract: Essential hypertension results from the complex interaction between genetic and environmental factors. A C825T polymorphism of the gene encoding G-protein β3 subunit (GNB3), associated with enhanced G-protein coupled signaling and increased Na^+-H^+ exchanger, has been implicated in the development of essential hypertension in several human populations, especially in Caucasian population. We examined the disease relevance of this candidate gene by performing an association study in a study group of Korean heritage. Participants comprised 109 essential hypertensives and 109 normotensives, respectively. Genotyping was performed with PCR-*Bsa*JI restriction digestion method. Observed genotype frequencies were in Hardy-Weinberg equilibrium in all groups. Genotype and allele frequencies did not differ significantly between normotensives and essential hypertensives ($P > 0.05$). However, the serum total cholesterol (TC) and LDL-cholesterol levels were significantly higher in subjects with the TT genotype compared to those with the CC or CT genotypes in normotensives of our study subjects ($P < 0.05$). Thus, these results suggest that GNB3/C825T polymorphism might be significantly associated with abnormality in serum lipid metabolism.

Key words: essential hypertension, G-protein β3 subunit, Korean population

Essential hypertension affects approximately 20 to 30% of individuals in industrialized countries (Carretero and Oparil, 2000; Gu et al., 2002) and is commonly believed to develop on the basis of both genetic and environmental factors (Beevers et al., 2001). The identification of genes susceptible to essential hypertension is hampered by the fact that blood pressure is a poorly defined phenotype that is modulated by multiple factors, such as age, gender, body mass index, physical activity, nutrition and race etc (Bots et al., 1991). Until now, one method that has been widely used in defining genes susceptible to these multifactorial diseases including essential hypertension is a candidate gene approach of case-control type (Izawa et al., 2003; Kang et al., 2000). This method is becoming increasingly popular as a result of a great number of genes and genetic polymorphisms having been identified for which some functional information is available (Yagil and Yagil, 2004). Because many biochemical and physiological systems impact blood pressure regulation and essential hypertension susceptibility, many of these identified genes and polymorphisms are candidates for population-based case-control study involving blood pressure levels or essential hypertension status (Zhu et al., 2003). Among these multiple candidate genes, G-protein β3 subunit (GNB3) gene has been considered as a candidate gene that may be implicated in the pathogenesis of essential hypertension (Stiffert, 1998).

G-proteins are a group of heterotrimeric proteins (α, β and γ) transducing signals from extracellular receptors to intracellular effectors (Rosskopf et al., 1995; Siffert and Düsing, 1995). Because of their crucial role in the function of many types of cells, genetic abnormalities in G-protein subunits have the potential to be involved in the aetiology of a wide range of clinical conditions (Dong et al., 1999; Benjafield et al., 2001; Willeit et al., 2003).

The gene coding the GNB3 has been cloned and localized in the short arm of human chromosome 12 (12p13) (Levine et al., 1990a). It spans 7,500 bp and is

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composed of 11 exons and 10 introns (Levine et al., 1990b). A C825T polymorphism located in exon 10 of this gene has been described by Stiffert et al. (1998), and C substitution by T causes alternative splicing in exon 9, resulting in the loss of 41 amino acids from the polypeptide chain (Stiffert et al., 1998). The available data account for the association of C825T polymorphism in the GNB3 gene with essential hypertension in some populations (Stiffert et al., 1998; Dong et al., 1999). The mechanism whereby the T allele may lead to essential hypertension in human remains known, but it may involve increased Na^+-H^+ exchanger activity (Stiffert et al., 1998). Increased activity of this exchanger provides several mechanisms of potential relevance to the development of essential hypertension including enhancement of renal tubular sodium reabsorption that leads to an increase in extracellular volumn (Stiffert, 1998).

Although it has been implicated that a C825T polymorphism in the GNB3 gene may predispose to essential hypertension, the results in all populations studied have been inconsistent (Brand et al., 1999; Huang et al., 2003; Shioji et al., 2004). In addition, there are few data on its association with essential hypertension in a group of Korean heritage. Therefore, we investigated the relationship between the GNB3/C825T polymorphism and essential hypertension in a group of Korean heritage, and also determined whether this polymorphism is significantly associated with conventional cardiovascular risk factors in our subjects.

MATERIALS AND METHODS

Study subjects

A total of 218 individuals (109 normotensives and 109 essential hypertensives) were selected from the outpatients of Dept. of Clinical Pathology, DongIn Clinic, Seoul, Korea. Essential hypertension was diagnosed with systolic blood pressure (SBP) > 140 mmHg or diastolic blood pressure (DBP) > 90 mmHg and/or the need for antihypertensive pharmacotherapy. Subjects with secondary hypertension were excluded from this study. Clinical characteristics of each group are described in Table 1.

Table 1. Clinical characteristics of the study subjects

Variable	Mean ± SD[1](Number)		*P*
	Normotensive	Hypertensive	
Age (year)	55.3±9.2 (105)	62.2±12.7 (96)	<0.05
SBP (mmHg)[2]	118.1±9.3 (30)	152.4±21.2 (22)	<0.05
DBP (mmHg)[3]	74.5±7.2 (30)	93.9±17.9 (22)	<0.05
BMI (kg/m^2)[4]	23.4±2.5 (105)	24.1±2.7 (90)	NS[11]
TG (mg/dl)[5]	122.5±85.2 (77)	138.8±67.4 (58)	NS
TC (mg/dl)[6]	150.1±38.6 (77)	153.7±33.3 (58)	NS
LDL-chol (mg/dl)[7]	97.1±38.4 (77)	101.9±31.9 (58)	NS
HDL-chol (mg/dl)[8]	28.3±10.1 (77)	24.0±7.4 (58)	<0.05
Lp(a) (mg/dl)[9]	14.9±11.8 (89)	18.2±11.7 (49)	NS
ApoAI (mg/dl)[10]	71.0±22.2 (12)	112.7±28.8 (23)	NS
Glucose (mg/dl)	93.1±82.6 (61)	74.8±48.1 (23)	NS

[1]Standard deviation, [2]systolic blood pressure, [3]diastolic blood pressure, [4]body mass index, [5]triglyceride, [6]total cholesterol, [7]low density lipoprotein cholesterol, [8]high denstiy lipoprotein cholesterol, [9]lipoprotein(a), [10]apolipoproteinAI and [11]not significant.

Determination of clinical phenotypes

Blood samples were obtained in EDTA tubes from individuals who had been fasting for 12-16 h. The body mass index (BMI) value was calculated by the body weight (kg) divided by the square of the height (m^2). Levels of serum triglyceride (TG), total cholesterol (TC) and glucose were measured by enzymatic colorimetry methods with commercial kit (Boehringer Mannheim, Germany) and Hitachi 7150 automatic chemistry analyzer. Serum HDL-cholesterol level was determined by measuring cholesterol in the supernatant after precipitation of the plasma with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automated analyzer with reagents and calibrators from Boehringer Mannheim. Serum lipoprotein(a) (LP(a)) level was measured by the immunoprecipitation method (SPQ Test System, INCSTAR Corporation, Stillwater, Minnesota, USA), and serum apoAI concentration determined by immunoturbidimetric method (COBAS INTEGRA, ROCHE Diagnostics, USA). Serum LDL-cholesterol level was calculated by using the formular of Friedewald et al. (1972).

Genotyping

Genomic DNA was extracted from whole blood using Wizard Genomic DNA Isolation kit (Promega, Co. Ltd., USA). The C825T polymorphism of GNB3 gene was detected by PCR followed by *Bsa*JI (New England Biolabs, Inc., USA) restriction enzyme digestion. Briefly, total 50 μl of the reaction mixture contained 200 *n*g of genomic DNA, 10 *p*mol of each primer, 200 μM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for the C825T polymorphism studied were: 5'-GCT TCC TGC CGC TTG TT-3' and 5'-GAG AGT CCG AAA TGG GAG CTG A-3' (Stiffert et al., 1998). Amplification was carried out with GeneAmp 9700 thermal cycler (Perkin-Elmer, ABI, USA); one cycle at 94°C for 5 min, 35 cycles at 94°C for 45 sec, at 56°C for 45 sec and at 72°C for 45 sec with a final cycle at 72°C for 10 min. Final PCR product was digested with the restriction enzyme, *Bsa*JI, and electrophoresed in 3% agarose gel (MetaPhor agarose, FMC BioProducts, USA) and visualized by ethidium bromide staining. The size of PCR products after *Bsa*JI digestion was 268 bp for the TT genotype, and a set of 116 bp and 152 bp for the CC genotype, which were clearly

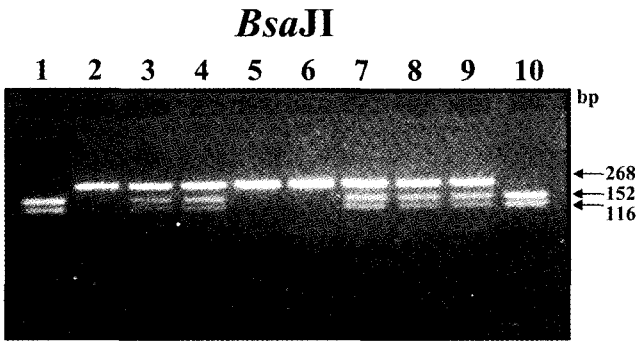

Fig. 1. A C825T polymorphism of the GNB3 gene. Lane 1 and 10, CC genotypes; 3, 4 and 7~9, CT genotypes; lane 5 and 6, TT genotypes.

resolved on 3% MetaPhor agarose gel (Fig. 1).

Statistical analysis

Allele frequencies were calculated by the gene counting method. The heterozygosity and polymorphism information content (PIC) values were calculated by the methods of Bostein et al. (1980). The significance of differences in genotype and allele frequencies between populations was estimated by χ^2-independence test. The comparisons of the variables across the clinical phenotypes were performed by using a student's t-test or one-way ANOVA test. Statistical significance was set at the P=0.05 level. All statistical analysis was performed using the computer program of SPSS for windows (version 11).

RESULTS

Genotype distribution

The objective of present study was to estimate the genotype and allele distributions of the GNB3/C825T polymorphism in Korean normotensives and essential hypertensives, respectively. Table 2 shows the genotype and allele distributions for the GNB3/C825T polymorphism in the both groups, respectively. The genotype frequencies of CC, CT and TT were 21.1, 49.5 and 29.4% in normotensives, and 22.0, 53.2 and 24.8% in essential hypertensives, respectively. Each group was consistent with Hardy-Weinberg equilibrium ($P>0.05$). Frequencies of the T allele were about 0.54 for normotensives and about 0.51 for essential hypertensives, respectively. The PIC of GNB3/C825T polymorphism indicated values of about 0.37 in the both groups, respectively. There was no statistically significant difference between normotensives and essential hypertensives in genotype and allele frequencies, respectively ($P>0.05$).

Table 2. Genotype and allele frequencies of a C825T polymorphism in the GNB3 gene between normotensives and essential hypertensives

	Genotype no. (%)			Allele no. (%)		H[1]	PIC[2]
	CC	CT	TT	C	T		
Normotensive	23 (21.1)	54 (49.5)	32 (29.4)	100 (45.9)	118 (54.1)	0.4966	0.3733
Hypertensive	24 (22.0)	58 (53.2)	27 (24.8)	106 (48.6)	112 (51.4)	0.4996	0.3748
Total	47 (21.6)	112 (51.4)	59 (27.1)	206 (47.3)	230 (52.7)	0.4985	0.3742
Chi-square	0.5879			0.2301			
Probability	0.7453			0.6315			
Odds ratio (CI[3])	0.90 (0.61-1.30)						

[1]Heterozygosity, [2]polymorphism information content and [3]confidence interval.

Table 3. The comparison of the clinical characteristics according to a C825T polymorphism of the GNB3 gene in normotensives

Variable	Mean±SD[1] (Number)		
	CC (No.)	CT (No.)	TT (No.)
Age (year)	55.9±10.3 (22)	56.6±8.5 (51)	52.9±9.2 (32)
SBP (mmHg)[2]	118.1±11.9 (7)	118.5±8.0 (14)	117.4±10.0 (9)
DBP (mmHg)[3]	72.9±7.2 (7)	75.4±5.4 (14)	74.2±9.9 (9)
BMI (kg/m^2)[4]	22.9±2.3 (22)	23.6±3.0 (51)	23.4±1.8 (32)
TG (mg/dl)[5]	150.8±140.8 (15)	112.6±71.5 (40)	121.0±53.2 (22)
TC (mg/dl)[6*]	130.3±35.0 (15)	149.2±37.9 (40)	165.2±37.1 (22)
LDL-chol (mg/dl)[7*]	71.9±45.7 (15)	99.0±32.3 (40)	110.8±36.7 (22)
HDL-chol (mg/dl)[8]	26.9±8.7 (15)	27.7±11.0 (40)	30.2±8.8 (22)
Lp(a) (mg/dl)[9]	16.8±17.0 (19)	13.6±8.7 (43)	15.8±10.6 (27)
ApoAI (mg/dl)[10]	70.9±7.0 (4)	74.1±14.0 (3)	69.2±34.7 (5)
Glucose (mg/dl)	97.6±75.8 (11)	102.0±100.2 (32)	74.3±43.0 (18)

[1]Standard deviation, [2]systolic blood pressure, [3]diastolic blood pressure, [4]body mass index, [5]triglyceride, [6]total cholesterol, [7]low density lipoprotein cholesterol, [8]high denstiy lipoprotein cholesterol, [9]lipoprotein(a) and [10]apolipoproteinAI. *There are significant differences in serum TC and LDL-cholesterol levels among the gnotypes (For serum TC level, one-way ANOVA test, $P = 0.0230$. For serum LDL-cholesterol level, one-way ANOVA test, $P = 0.0080$).

Association with clinical phenotypes

Table 3 displays the comparison of clinical phenotypes across the GNB3/C825T polymorphism in normotensives. This polymorphism was significantly associated with serum TC and LDL-cholesterol levels, respectively (one-way ANOVA test, $P<0.05$). Especially, the subjects with TT genotype indicated significantly higher levels of serum TC and LDL-cholesterol than those with CC or CT genotype. In the case of essential hypertensives, there were no statistically significant differences in any clinical phenotypes across the genotypes (one-way ANOVA test, $P>0.05$) (Table 4).

Table 4. The comparison of the clinical characteristics according to a C825T polymorphism of the GNB3 gene in essential hypertensives

Variable	Mean±SD[1] (Number)		
	CC (No.)	CT (No.)	TT (No.)
Age (year)	61.8±12.0 (22)	63.3±12.7 (51)	60.0±13.6 (23)
SBP (mmHg)[2]	152.7±11.2 (6)	154.0±24.4 (10)	149.3±26.0 (6)
DBP (mmHg)[3]	91.7±5.6 (6)	98.5±24.6 (10)	88.5±11.8 (6)
BMI (kg/m^2)[4]	24.2±2.2 (22)	23.8±2.9 (50)	24.8±2.6 (18)
TG (mg/dl)[5]	138.3±37.3 (8)	137.5±73.8 (38)	143.4±65.6 (12)
TC (mg/dl)[6]	136.3±33.4 (8)	153.9±29.3 (38)	164.5±42.2 (12)
LDL-chol (mg/dl)[7]	85.5±40.0 (8)	103.3±24.9 (38)	108.3±43.9 (12)
HDL-chol (mg/dl)[8]	23.1±7.7 (8)	23.1±7.6 (38)	27.5±6.2 (12)
Lp(a) (mg/dl)[9]	21.5±13.2 (11)	16.7±12.7 (28)	18.7±5.9 (10)
ApoAI (mg/dl)[10]	111.3±28.0 (9)	109.7±29.6 (7)	117.5±33.0 (7)
Glucose (mg/dl)	63.8±48.8 (6)	82.6±49.7 (12)	69.2±50.4 (5)

[1]Standard deviation, [2]systolic blood pressure, [3]diastolic blood pressure, [4]body mass index, [5]triglyceride, [6]total cholesterol, [7]low density lipoprotein cholesterol, [8]high denstiy lipoprotein cholesterol, [9]lipoprotein(a) and [10]apolipoproteinAI.

DISCUSSION

In the present study, we examined the relationship between the GNB3/C825T polymorphism and essential hypertension in a group of Korean heritage. In the case-control sample of a total of 218 subjects, we found that the GNB3/C825T polymorphism was not significantly associated with essential hypertension. Thus, our results suggest that it is likely that the GNB3/C825T polymorphism contributes in any important way to the risk of essential hypertension in Koreans. Stiffert et al. (1998) first reported that this polymorphism was significantly associated with essential hypertension in Caucasian population, but subsequent studies have produced contradictory results (Brand et al., 1999; Huang et al., 2003; Shioji et al., 2004). Difference in genetic background among racial groups may be a major factor for inconsistant results in association studies. The T allele frequency of GNB3/C825T polymorphism ranges from 21~56% in Caucasians to 65~91% in black Africans, with intermediate values of 43~52% in Asians (Stiffert et al., 1999). The T allele frequency (about 0.53) in our study group is similar to those reported in other Asians. It seems to be important for carefully designed studies to minimize the ethnic heterogeneity of the case and control populations (Pollak et al., 2000).

One of the most valuable finding of our study reported here was the observation of a significant association between the GNB3/C825T polymorphism and serum lipid concentrations such as TC and LDL-cholesterol in normotensives. Analysis of variance revealed that subjects with TT genotype indicated higher serum TC and LDL-cholesterol levels than those with CC or CT genotype in our subjects. It is probable that the T allele of this polymorphism might be significantly associated with abnormality in serum lipid metabolism. Until now, the published data showed inconsistent correlations between the GNB3/C825T polymorphism and these biochemical parameters. Ishikawa et al. (2000) reported that the subjects with T allele (CT+TT) had higher serum TC level than those with CC genotypes, consistent with the results of our study. In contrast, Dzida et al. (2002) proposed that the subjects with CC genotype had a higher serum TC level than those with other genotypes (CT+TT) in Caucasian diabetic patients. The reasons for these discrepant results are far from clear, but disease status and/or ethnic background may explain some of the variances. The study by Dzida et al. (2002) was performed with diabetic patients, and therefore, it is likely that genetic and/or environmental factors associated with the development of diabetes modify the effect of the GNB3/C825T polymorphism on serum lipid metabolism. The mechanism on the association between GNB3/C825T polymorphism and serum lipd level remains to be clarified. Further studies are needed to precisely define the biochemical mechanism by which G-protein signal transduction pathway may contribute to the lipid metabolism.

Unlike the normotensives, this polymorphism was not significantly associated with serum TC and LDL-cholesterol levels in essential hypertensives of our study subjects. Since essential hypertension is known to be caused by both genetic and environmental factors and its clinical condition also influences the serum lipid and lipoprotein components of each individual, the significant association between GNB3/C825T polymorphism and serum lipd level may be masked by uncontrolled gene-gene and/or gene-environment interaction in this group (Fuh et al., 1987).

There are several limitations of our study that must be emphasized. An important consideration is the statistical power when small differences in allele frequencies do not achieve statistical significance. Essential hypertension is a polygenic disorder (Bae et al., 2001), and many genes with minor effect are likely to be involved in its development (Izawa et al., 2003; Zhu et al., 2003). Thus, large samples are required for detection of an association. The present study involves a modest sample size of 109 normotensives and 109 essential hypertensives, and thus, has relatively low statistical power in detecting statistical significance. Secondly, we did not measure any intermediate phenotypes such as plasma renin and aldosterone levels as well as salt sensitivity in this study. Although our data do not suppose a probable role of GNB3/C825T polymorphism in the development of essential hypertension, we cannot exclude the possibility that there are significant association between this genetic polymorphism and some subgroup of hypertensive patients. Actually, some studies reported that the T allele of this polymorphism was associated with low-

renin hypertension (Schunkert et al., 1998). In the case of other candidate genes for essential hypertension, the significant association with salt-sensitive hypertension has also been documented in many ethnic groups (Cusi et al., 1997).

In conclusion, we could not find any significant association between the GNB3/C825T polymorphism and essential hypertension in a group of Korean heritage. However, significant associations between the T allele of this polymorphism and the elevation of serum TC and LDL-cholesterol levels was detected in normotensives of our study subjects. Further studies are needed to define the role of the GNB3/C825T polymorphism in the control of serum lipid metabolism.

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