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Short Communication

Association of *GNB3* C825T polymorphism with plasma electrolyte balance and susceptibility to hypertension

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Abstract

The role of G-protein activation in cardiovascular disorders is well-known. G-protein β3 subunit (*GNB3)* C825T polymorphism is associated with increased intracellular signal transduction. We investigated the role of the variant in plasma sodium and potassium concentrations and association with hypertension. 345 healthy controls and 455 patients with essential hypertension were enrolled. Plasma renin activity and aldosterone concentration were measured. The variant, typed by SNaPshot, was analyzed on an ABI Prism 3100 Genetic Analyzer and GeneScan. The TT genotype and T allele were over-represented in the patients ($p < 0.001$, $p < 0.0001$). Multiple-logistic regression disclosed that the risk of hypertension was significantly greater for TT ($p < 0.0001$, OR = 6.1, CI = 2.9-12.7). One-way ANOVA revealed that hypertensive T-allele carriers (CT+TT), compared to non-carriers (CC), had a greater body mass index (BMI), mean arterial pressure (MAP) and PAC ($p = 0.01$, $p = 0.01$, $p < 0.0001$, respectively); while the patients with 825TT risk genotype showed higher plasma sodium and lower potassium ($p < 0.0001$, each). The results strongly emphasize, not only the role of C825T polymorphism by the induction of increased G-protein activity and enhancement of Na/h exchangers, but also the association with higher plasma sodium and lower potassium levels, high BMI and susceptibility to hypertension.

Key words: GNB3 C825T polymorphism, hypertension, G protein, plasma electrolytes.

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It is widely accepted that nearly all transmembrane receptors, as well as various vasoactive/growth-stimulating factors in cardiovascular homeostasis and peripheral vascular resistance, depend on G-proteins to regulate intracellular signaling cascades (Siffert *et al.*, 1995). An increased activity of G-protein enhances the Na/h exchanger (NHE-1) in almost 50% of the patients with Essential Hypertension (EH), gives evidence of the role of these proteins in the pathogenesis of essential hypertension (EH) (Siffert and Düsing, 1995; Schunkert *et al.*, 1998). The *GNB3* C825T polymorphism results in alternative splicing of exon 9 that eliminates 41 amino acids in the protein, thereby associating with the expression of a novel splice-variant (Gβ3-s) that correlates with enhanced G-protein activation. This has been investigated, in order to establish an association with blood pressure, hypertension, body-mass index, left-ventricular hypertrophy and related phenotypes (Siffert, 1996; Schunkert *et al.*, 1998; Siffert *et al.*, 1999b; Poch *et al.*, 2000). On recognizing the significance of *GNB3* C825T polymorphism in susceptibility to hypertension, we undertook an association study with a case-control design. Since alterations in electrolyte balance can be through increased G-protein activity, the next step was to evaluate association of the variant with plasma-sodium and potassium concentrations.

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The study was approved by the ethical committee. Each of the subjects involved signed an informed consent form. Study subjects consisted of 345 consecutive unrelated sex-matched healthy controls and 455 EH patients, so diagnosed through Hypertension outpatient clinics of collaborating hospitals. Details of selection criteria have already been presented in an earlier work (Nejatizadeh *et al.*, 2008). No exercise, alcohol, caffeine or smoking was allowed, for a period of 30 min prior to blood-pressure measurement. Blood pressure (BP) was measured by conventional mercury sphygmomanometer. Measurements by two different observers were taken at the left arm in the seated position after 15 min of rest. This procedure was repeated three times, systolic BP (SBP) and diastolic BP (DBP) being defined as the mean of the three independent measurements. According to the JNC VII panel, hypertension was defined as an average SBP of ≥ 140 mm Hg, an average DBP of ≥ 90 mm Hg (or both), or as a diagnosis of hypertension in subjects receiving antihypertensive medication. Demographic data were collected by means of a detailed

standard questionnaire. There was also a detailed interview, physical examination and laboratory analysis. Blood samples were collected after 12 h overnight fasting. Cells were used for DNA extraction and plasma for biochemical analysis. The latter was stored at -80 °C if not analyzed immediately. *GNB3* C825T polymorphism was genotyped, and the effect of the genetic variant on clinical and biochemical parameters monitored in both study groups. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were measured by radioimmunoassay (M/s Immunotech, France). The counts were measured in duplicate on a gamma counter (Ria Calc WIZARD 1470, USA). The variant was typed by an SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, Calif), electrophoresed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), and subsequently analyzed on an ABI Prism GeneScan and Genotyper (Applied Biosystems). Sodium, calcium, potassium, total cholesterol, triglyceride, uric acid, creatinine, and glucose, were all measured on a high-throughput autoanalyzer (Elecsys 2010, Roche, Germany). Estimates were obtained in duplicate. Intra- and inter-assay coefficients of variation were less than 5%. All statistical analyses were carried out with SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA), and *EPIINFO* v. 6 softwares. Hardy-Weinberg equilibrium (HWE) with 1 *df* a goodness of fit test was calculated using the DeFinetti program. Allele and genotype frequencies were estimated by gene counting. Categorical and continuous variables were compared using χ^2-test and *unpaired* Student's t-test. Logistic regression analysis was undertaken with different models. Odds ratio (OR) was calculated through logistic regression. Where appropriate, correction for multiple testing was made by Bonferroni correction test. Power calculations were performed with the PS: Power and Sample Size Calculation program by Dupont and Plummer. A two-tailed p value of < 0.05 was considered statistically significant.

The overall percentage of successful genotyping was at least 99,4%. Genotype distribution was at Hardy-Weinberg equilibrium (p = NS). As the TT genotype was over-represented in the patients (LRT$\chi^2 = 16.2$, $df = 2$, $p < 0.001$, Table 1), consequently, the T allele was also (LRT$\chi^2 = 25.9$, $df = 1$, $p < 0.0001$). Multiple-logistic regression analysis revealed that the risk of hypertension was significantly higher for the TT genotype ($p < 0.0001$, OR = 6.1, Wald's 95% CI = 2.9-12.7) (Table 1). Significance was maintained even after adjustment by age, sex and BMI ($p < 0.001$). Significant probability values and relative risks for C825T polymorphism were obtained by implementing recessive ($p < 0.0001$, OR = 5.0, Wald's 95% CI = 2.4-10.2) and additive models ($p < 0.0001$, OR = 5.0, Wald's 95% CI = 2.9-8.4). Homozygous 825T allele carriers showed considerable relative risk, with a six-fold higher chance of developing hypertension compared to homozygous 825C allele carriers. One-way ANOVA revealed that in hypertensive T-allele carriers (CT+TT), BMI, mean arterial pressure (MAP) and PAC were higher ($p = 0.01$, $p = 0.01$, $p < 0.0001$, respectively), when compared to non-carriers (CC) (Table 1), whereas patients with the 825TT risk genotype had higher plasma sodium, serum creatinine and urea, and lower potassium levels ($p < 0.0001$, each) (Table 2). In an association of the 825T allele with MAP and PAC, increased plasma sodium and decreased potassium concentrations were observed ($p < 0.0001$, each).

Worthy of note, our study revealed for the first time an association among Asians of the 825T allele with MAP and PAC, increased plasma sodium and decreased potassium concentrations, which could reflect an enhanced activity of NHE-1 in hypertension. Furthermore, the highly significant PAC and low PRA in patients suggested of a low renin hypertension (Nejatizadeh *et al.*, 2008). Notwithstanding, studies of ethnic groups are not devoid of conflict (Beige *et al.*, 1999; Brand *et al.*, 1999; Larson *et al.*, 2000;

Table 1 - Distribution of alleles and genotype frequency of the *GNB3* C825T polymorphism between patients and controls.

Genotypes	Patients (n = 449)	Controls (n = 345)	OR†	95%CI‡	χ^2	p*
CC	185 (41%)	192 (56%)	-			
CT	211 (47%)	144 (42%)	1.5	(1.1-2.0)	7.91	0.005
TT	53 (12%)	09 (02%)	6.1	(2.9-12.7)	28.4	0.0000
C	581 (65%)	528 (77%)				
T	317 (35%)	162 (23%)	1.8	(1.4-2.2)	25.9	0.0000
HWE	$\chi^2 = 0.05$, p = 0.97	$\chi^2 = 2.95$, p = 0.22				
Recessive model			5.0	(2.4-10.2)	22.9	0.0000
Dominant model			5.0	(0.4-0.7)	16.3	0.0000
Additive model			5.0	(2.9-8.4)	43.0	0.0000

†OR (CI %95), indicates crude odds ratio and 95% confidence interval. ‡OR denotes adjusted odds ratio by age, sex and BM I. *p values are calculated by logistic regression. Number of the individuals (%) Dominant model compares a combination of heterozygous and homozygous genotypes of the least frequent allele to homozygotes of the most frequent. Recessive model compares a combination of heterozygous and homozygous genotypes of the most frequent allele to the variant allele homozygous genotype. Additive model compares a combination of the two genotypes with weight 2 and 1 respectively to the homozygote of the most frequent allele.

Table 2 - Anthropometric and demographic data in hypertensive patients according to 825C/T polymorphism of the *GNB*3 gene.

Parameter	CC (n = 179)	CT (n = 220)	TT (n = 50)	p*	CT+TT (n = 270)	p†
Age, yr	50.1 ± 11.6	52.6 ± 12	51.8 ± 9.1	0.1	52.2 ± 10.5	0.05
Gender, %	58.1	58.6	62	0.83	60.3	0.77
BMI, kg/m^2	23.8 ± 3.7	24.5 ± 4.4	24.8 ± 2.8	0.13	24.65 ± 3.6	0.01
SBP, mmHg	168 ± 18.2	169 ± 18	171. 3 ± 17	0.51	170 ± 34.5	0.45
DBP, mmHg	99.4 ± 8.4	100 ± 10	102 ± 8	0.21	101 ± 9	0.04
MAP, mmHg	121 ± 15.3	123 ± 10	125 ± 9	0.08	124 ± 9.5	0.01
PP, mmHg	67.6 ± 16	69.2 ± 18	69 ± 16	0.63	69.1 ± 17	0.35
PAC, Pmole/L	155 ± 41	220 ± 62	255 ± 6	< 0.0001	237.5 ± 65	< 0.0001
Serum Na^+, mmol/L	135 ± 7.8	137 ± 6.6	142 ± 7.2	< 0.0001	139.5 ± 6.9	< 0.0001
Serum K^+, mmol/L	4.5 ± 0.8	4.6 ± 0.85	5.0 ± 0.54	0.0005	4.8 ± 0.69	0.00008
Serum creatinine, mg/dL	1.1 ± 0.48	1.32 ± 0.6	1.41 ± 0.5	0.000002	1.36 ± 0.55	< 0.0001
Serum urea, mg/dL	32 ± 8	35 ± 8.5	36.4 ± 7.2	0.0001	35.7 ± 7.85	0.000005
Total cholesterol, mg/dL	110 ± 40	107 ± 39	108 ± 32	0.78	107.5 ± 35	0.85
Triglyceride, mg/dL	100 ± 30	105 ± 35	90 ± 25		0.1996 ± 29	0.46
Uric acid, mg/dL	5.2 ± 1.6	5.3 ± 1.5	5.5 ± 1.7		0.47 ± 1.6	0.19

Values are mean ± STD. BMI indicates body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; Na^+, sodium; K^+, potassium; Ca^+, calcium. CC, CT, TT, and CT +TT refer to genotype groups described in this study. *Statistical analysis was performed by analysis of variance among the 3 genotypes. †Statistical analysis was performed by Student's t-test for CT +TT *vs.* CC genotypes.

Staessen *et al.*, 2003). Our findings are in accordance with those of Siffert (2000). It is well-established that obesity, salt retention and low plasma renin activity are associated with NHE-1 hyperactivity (Delva *et al.*, 1993; Diez *et al.*, 1995). As enhanced NHE-1 activity is subject to G-protein hyperactivation in the presence of the 825T allele, a consequential increase of these phenotypes in 825T allele carriers is to be expected. Furthermore, the association of this allele with obesity could not be overlooked, since BMI in T-allele carriers (CT+TT) higher than in non-carriers (CC), thus in agreement with an earlier report (Siffert *et al.*, 1999b). Although, G proteins are known to play a key role in adipogenesis and 825T alleles predict enhanced G protein activation, the association between 825T allele and obesity cannot attenuate the influence of non-genetic factors, such as life-style, for a susceptibility gene to present its effect on obesity. Predictably, the 825T allele increases the risk of obesity, which, over the years, leads to hypertension.

Moreover, given the causative nature of the C825T variant, it is likely that the vasoconstrictors, norepinephrine and angiotensin II, also transmit their effect to pertussis toxin-sensitive G proteins, which, in turn, enhance vascular resistance in individuals carrying mutated GNB3 proteins. It is known that angiotensin II, as a main stimulator of aldosterone, can act via Gi proteins, thereby enhancing phospholipase C activity, cytosolic calcium liberation, and *CYP11B*2 gene expression (Lu *et al.*, 1996). Therefore, in brief, in hypertensive TT carriers, lower angiotensin II levels are regulated by increased postreceptor signaling and subsequent high aldosterone and suppressed renin levels, thus emphasizing 825T allele efficacy for enhancing intracellular signal transduction in humans. In conclusion, our results strongly suggest the contribution of *GNB3* C825T polymorphism to the risk of essential hypertension and high BMI, and also highlight the role of increased Na^+-H^+ exchanger activity. Of course, it is likely that this association could have arisen from a direct physiological effect of the genetic variation or linkage disequilibrium, with an additional functional alteration at the *GNB3* locus. Nonetheless, validation of the functionality of the 825T allele, nonetheless, is desirable, as a means of establishing its causative role. Moreover, the role of other variants in or near *GNB3* cannot be overlooked. The identification and functional significance of novel polymorphisms, such as A (-350) G in the promoter region and A C1429T in the 3'-untranslated region of the *GNB3*, having occurred with variable frequency in several ethnic groups, including black Africans, Chinese, and Germans, have also been investigated (Rosskopf *et al.*, 2000). Another assumption is that *GNB3* 825T, possibly in concert with these and other variants, influences variations in blood pressure and bodyweight. Additional work will be needed to elucidate the associations between these polymorphisms and G-protein functions.

Interaction analysis of the *GNB3* gene with other genes involved in G protein-mediated pathways may be helpful in developing an understanding of the association between G protein-mediated pathways and cardiovascular diseases. Despite universal recognition that blood pressure

is influenced by interactions between the effects of several genetic and environmental factors, the possibility of detecting statistically significant interactions between genetic effects measured by gene polymorphisms, and effects of other genetic and environmental factors, have received wide attention. Such interactions may give rise to the different relationships between genotypic and phenotypic variations in diverse environments. Genotyping of these polymorphisms, along with a positive family history of cardiovascular disease, could help in identifying individuals with high susceptibility to EH.

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Internet Resources

DeFinetti program, http://ihg.gsf.de/cgi-bin/hw/hwa1.pl.

PS: Power and Sample Size Calculation program, by William D. Dupont and Walton D. Plummer, Jr., http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

Note that the information previously found at http://www.mc.vanderbilt.edu/prevmed/ps/index.htm has been moved to http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize.

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