Original article

Genetic susceptibility of angiotensin-I converting enzyme and G-protein β3-subunit gene polymorphisms to essential hypertension

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Essential hypertension (EH) accounts for 90-95% of hypertension cases. EH, as a progressive cardiovascular syndrome arising from complex and interrelated etiologies, results from combined and interactive effects of genetic and environmental factors. The objective was to investigate the ACE I/D and GNB3 C825T polymorphisms and both genotypes combined with seeking gene-gene interactions to further clarify the role of these genes in the pathogenesis of EH. Eight hundred and ten consecutive ethnic-matched unrelated north Indian subjects, 360 healthy controls from the general population and 450 patients, were enrolled. Plasma renin activity and plasma aldosterone concentration were measured. The variant GNB3 C825T was typed by SNaPshot and analyzed on Genetic Analyzer and GeneScan. Genotypes-combinations and gene-gene interactions were also performed. We found that the plasma ACE levels were higher in hypertensive patients than healthy controls. The ACE DD genotype was associated with the highest circulating ACE levels, ID heterozygotes were associated with intermediate and II heterozygotes with the lowest ACE levels in either patients or controls. Our data suggested a significant interaction between the GNB3 825T allele and the ACE D allele in CVD and likely EH. The patients bearing (DD + CT or ID + TT) and (DD + TT) combinations, respectively, showed a significant association with EH. Logistic regression revealed a 2.7-fold greater risk of hypertension associated with the (DD + CT or ID + TT) combination, and likewise, a 6.4-fold greater chance of hypertension was associated with the (DD + TT) combination. GNB3 C825T and ACE I/D gene-gene interaction in our study revealed that (DD + CT or ID + TT) and (DD + TT) combinations were significantly associated with EH and higher risk of hypertension, respectively. In a synergistic gene-to-gene interaction among the three polymorphisms, genotypic combinations containing three and/or four unfavorable alleles had a significantly increased chance of EH. These results strengthen the hypothesis that genotypic combinations are more important than a single gene polymorphism.

Keywords: Hypertension; Polymorphism, Genetic; Renin; Aldosterone

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Introduction

Blood pressure (BP) is a quantitative multifactorial trait influenced by environmental and genetic determinants. In the general population, BP follows a normal distribution. The results of the BRIGHT and Family Blood Pressure Program (FBPP) studies have revealed that 40% of BP variability is thought to be genetically determined (1). Essential Hypertension (EH) is a common, polygenic, complex syndrome resulting from of multiple genetic determinants interacting with each other and with environmental factors (2). Of many human candidate genes and genetic loci, those encoding components of the RAS are considered to be among the most plausible candidate genes underlying hypertension genetics. RAS genes have been most extensively studied as hypertension candidate genes, including angiotensin-converting enzyme (ACE) gene. The ACE gene maps to chromosome 17q23, spans 21 kb, and comprises 26 exons and 25 introns. The absence of the segment constitutes a deletion (D) and its presence, an insertion (I) (3). Plasma ACE concentration is stable in a given individual, but varies among different subjects (4). A polymorphism of the ACE gene involves the insertion/deletion of a 287-bp Alu repetitive sequence. Approximately 50% of total phenotypic variance in ACE levels corresponds to this polymorphism, the concentration of ACE levels is lowest in homozygotes with the longer allele (II) and highest in homozygotes with the shorter allele (DD); ID heterozygotes show intermediate levels and the polymorphism is itself a neutral marker for a yet-to-be- identified closely linked variant for the level of ACE activity (5). Deletion polymorphism has been found to be a potent risk factor for myocardial infarction and coronary artery disease in diverse populations (6,7). Therefore, multiple systems intervene in the control of BP in such a way that there are a lot of genes and their interactions is responsible for genetic susceptibility to EH, making it is a complex polygenic disease.

Regarding the mechanisms by which genetic markers contribute to the development of hypertension, Siffert et al. recently reported an association between the increased activity of the trans-membrane ion transport Na+/H+exchanger (NHE-1) and hyperactivity of the protein G in hypertensive patients (8). Heterotrimeric G proteins are crucial elements of transmembrane receptor–mediated intracellular signaling cascades that are involved in a range of physiological functions such as cardiovascular homeostasis and peripheral vascular resistance. A common polymorphism of the beta-3 subunit of the protein G (GNB3) gene, namely C825T, results in alternative splicing of exon 10, is associated with an expression of a novel splice variant (Gβ3-s) and is correlated with the enhancement of G protein activation (8). Although the GNB3 C825T polymorphism has been the subject of extensive investigations to find out whether the 825T allele is associated with blood pressure level (9), hypertension and body mass index (10), increased risk for left ventricular hypertrophy and related phenotypes in diverse ethnic groups (11), some authors have reported conflicting results (12-14). Therefore, both genetic evidence and epidemiological studies indicate that GNB3 C825T polymorphism is likely to be a potential candidate variant for disease pathogenesis and/or susceptibility to EH.

We hypothesized that these variants could be associated with BP variations and therefore undertook a well-characterized case-control association study to investigate the polymorphisms individually and in the combination identified as risk/protective and their correlations with related phenotypes for their association with EH.

Materials and methods

Study subjects

The study was approved by the local Human Ethical committee. A written informed consent was obtained from each participant. Eight hundred and ten consecutive ethnic-matched unrelated north Indians, 360 healthy controls from the general population and

450 patients, were enrolled through the hypertension outpatient clinics. The study subjects were age-matched. Demographic and clinical features of the study participants are summarized in Table 1. The participants were studied by clinical evaluation and laboratory testing, and a standardized interview/ questionnaire including socio-economic background, personal/family and medical history. Controls were recruited from the general population based on the following criteria: aged 25-65 years, systolic blood pressure (SBP) < 140 mm Hg and/or diastolic blood pressure (DBP) < 90 mm Hg, not being on antihypertensive medication and not suffering from any other diseases. The patients were classified as having EH if SBP was $\geq$ 140 mmHg and their DBP $\geq$ 90 mmHg on at least three separate occasions, they had a documented history of hypertension or were under antihypertensive medications and lacked clinical signs, symptoms, or laboratory findings suggestive of secondary hypertension. Three measurements of BP, using a calibrated mercury sphygmomanometer with an appropriate adult cuff, were taken by two different well-trained observers with an interval of at least 10 minutes after a 5-minute rest in supine position. Body Mass Index (BMI) was defined as weight/height2 (kilograms per meter squared).

Blood sample collection

Blood samples (10 ml each) were obtained in supine position in ACD-coated tubes. Plasma was separated and used in various biochemical studies. The cells were used in DNA isolation. The plasma and DNA samples were stored at –70^{o}C until further use.

General Biochemical analysis

Total cholesterol, triglycerides, uric acid, creatinine, glucose, sodium, calcium and potassium were measured on a high throughput Autoanalyzer (Elecsys 2010, Roche, Germany) and SpectraMax 190 Spectrophotometer (Molecular Devices, Sunnyvale CA, USA). Urinalysis with focus on proteinuria was done. measurements were performed in duplicate. The intra-assay and inter-assay coefficients of variation were less than 5% for all the measurements.

Specific biomarker estimation

Plasma Renin Activity (PRA) and aldosterone Levels: plasma renin activity was measured with Angiotesnin I radioimmunoassay kit (M/s Immunotech, France) and Plasma aldosterone levels were measured with radioimmunoassay kit (M/s Immunotech, France). The intra-assay and inter-assay coefficients of variation for PRA were below 10.4% and 10.5%, respectively.

Measurement of plasma ACE activity

Functional assays using spectrophotometric measurements (in U/L) were used. Reference ranges for each method were established in the testing laboratory. A current and widely used method is a spectrophotometric method using the synthetic tripeptide substrate N-[3-(2-furyl) acryloyl]-L-phenylalanylglycylglycine (FAPGG).

Genotyping

ACE I/D polymorphism: Genomic DNA was extracted from peripheral blood leukocytes using a standard protocol. The genotypes for the ACE I/D gene polymorphism were determined by PCR amplification using primers designed by PrimerSelect (DNASTAR Inc., Madison, WI, USA). The PCR amplified products were electrophoresed on 1.5% agarose gel and detected by ethidium bromide staining for identification of ACE I/D gene polymorphism (a 190-bp fragment in the case of deletion and a 490-bp fragment in the presence of an insertion of the 287-bp Alu sequence (Table 1).

GNB3 C825T polymorphism by SNaPshot assay:

The principle of SNaPshot kit is based on the dideoxy single base extension of an unlabeled oligonucleotide primer. The alleles were detected by Gene Scan analysis on an ABI Prism 3100 Genetic Analyzer. Each fluorescent ddNTP emits a different wavelength,

which is translated into a specific color for each base. The size of the product is the size of the initial probe plus one fluorescent base. Dye termination chemistry was used randomly for sequence confirmation of PCR products on the sequencer (Table 1).

Biostatistical analysis

Allele and genotype frequencies in the study participants were evaluated by gene counting and analyzed via $\chi 2$ test and logistic regression using SPSS 10.0 (SPSS Inc., Chicago, Illinois, USA) and EPIINFO ver.6 soft wares. Hardy–Weinberg equilibrium (HWE) for patients and controls was calculated using De-Finetti program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Baseline characteristics and demographic features were compared with the unpaired t- test for continuous data and the $\chi 2$ test for categorical data from simple interactive statistical analysis (http://home.clara.net/sisa/twoby2.htm). Multiple logistic regression analysis was performed to determine the effect of age, sex and BMI, if any, on the association of ACE I/D, and GNB3 C825T genotypes with hypertension. Genetic models of action of the studied variants were constructed by combining genotypes (ie, dominant = heterozygous + homozygous for the polymorphism associated with increased levels of the gene product; recessive = homozygous for the polymorphism associated with increased levels of the gene product). The power of the sample size to detect the association at $\alpha = 0.05$ was calculated using JMP software plus the PS power and sample size calculation program by Dupont and Plummer (http://www.mc.vanderbilt.edu/prevmed/ps/index.htm). Where appropriate, P values for pairwise differences were corrected for multiple comparisons by Bonferroni-type correction test. A P value of < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

The baseline characteristics of both groups are summarized in the previous chapter in Table 2. The total number of males compared to females was

Table 1: Features of the ACE I/D, AT1R A1166C and GNB3 C825T gene polymorphisms, primer sequences, cycling conditions and enzymes used for genotyping

Genotyping method	*Cycling conditions*	*Primer sequence*	*rs ID*	*Variant*
Gel electrophoresis *And* *PCR*	I 94°C4', D 94°C 1', A58°C45", E 72°C 1' 30 cy, FE 72°C7'	F: 5' CTGGAGACCACTCCCATCCTTTCT 3' R: 5' GATGTCGCCATCACATTCGTCAGAT 3'	*4646994*	*I/D*
RFLP Using (HaeIII)	I 94°C 3', D 95°C 30", A58°C45", E 72°C 45" 27cy, FE 72°C 7'	F: 5' GCAGCACTTCACTACCAAATGGGC 3' R: 5' CAGGACAAAAGCAGGCTAGGGAGA 3'	5186	A1166C
PCR *And* SNaPshot	I 95°C 4', D 95°C30", A 63°C45", E 72°C 45" 35 cy, FE 72°C7'	F: 5' TGACCCACTTGCCACCCGTGC 3' R: 5' GCAGCAGCCAGGGCTGGC 3' IP: CTGAGGGAGAAGGCCAC	*5443*	*C825T*

Position relative to the first base position of the first exon of the gene. F, Forward; R, Reverse; I, insertion D, denaturation; A, Annealing; E, Extension; Cy, Cycles; FE, Final extension; RFLP, Restriction Fragment Length Polymorphism; IP, Internal primer

more but the male to female ratio between the two groups was not significantly different. There was no significant difference between the two groups with respect to age, BMI, lipid profile, serum glucose, uric acid, electrolytes, whereas SBP, DBP, MAP and PP were significantly higher in patients ($P < 0.0001$).

Genotype distribution and allele frequencies

ACE I/D genotype analysis

Subjects with I/I, D/D, and I/D genotypes revealed bands at 490 bp; 190 bp; and 490 bp and 190bp, respectively. At the level of genotypes in patients, we found that 156 (35%), 209 (46%), and 84 (19%) patients were distributed as homozygous II, heterozygous ID, and homozygous DD, respectively. For controls, it was observed that 133 (37%) out of 360 were homozygous II, 166 (46%) were heterozygous ID, and 60 (17%) were homozygous DD (Table 3). No significant differences were observed in ACE genotype frequency distribution between patients and controls ($LRT\chi^2 = 0.203$, $P = 0.65$; $LRT\chi2 = 0.74$, $P = 0.38$), in such a way the respective genotypes were of almost identical frequency within both groups. As a result, the respective frequencies for the I (58% and 66%) and D (44% and 43%) alleles in patients and controls did not differ significantly ($LRT\chi2 = 0.91$, $P = 0.34$). Risk assessment showed that there were no significant risk changes for hypertension in the

Table 2: Baseline demographic and clinical characteristics of the study participants

P value	Patients	Controls	Parameters
-	455	345	Number
-	Indo-European	Indo-European	Ethnicity
-	51.5 ± 11	50 ± 9.1	Age, year
0.32	24.3 ± 4.1	24 ± 4.5	BMI, kg/m^2
<0.0001	168.5 ± 18.3	118.3 ± 10	SBP, mmHg
< 0.0001	99.7 ± 9.8	75.7 ± 6.8	DBP, mmHg
< 0.0001	66 ± 22	42 ± 9.4	PP, mmHg
< 0.0001	90.0 ± 9.7	116.4 ± 18.4	MAP, mmHg
< 0.0001	73±6.5	84±7.6	Heart rate, bpm
< 0.0001	140 ± 17	119 ± 23	Total cholesterol, mg/dl
< 0.0001	125 ± 12	95 ± 16	Triglyceride, mg/dl
0.952	4.65 ± 1.4	4.64 ± 1.5	Uric acid, mg/dl
0.606	99 ± 19	97 ± 20	Glucose, mg/dl
0.57	1.17 ± 0.1	1.1 ± 0.18	Serum creatinine, mg/dl
0.96	136.7 ± 5.6	136 ± 5.4	Serum Na^+, mmol/L
0.92	4.2 ± 0.24	4.0 ± 0.19	Serum K^+, mmol/L
-	Nill	Nill	Proteinurea
-	None	None	Current smoking
-	None	(+) 72%, (−) 28%	Family history, +/- (%) (EH)
-	(+) 5%, (−) 95%	(+) 6%, (−) 88%	Alcohol, +/- (%)

Data are presented as mean ± standard deviation and were compared by one-way ANOVA. *n*, number of subjects; BMI, body mass index; SBP, systolic blood pressure (mmHg); DBP, diastolic blood pressure (mmHg); PP, pulse pressure; MAP, mean arterial pressure (mmHg).

participants either with the ACE DD genotype (OR = 0.83, 95% CI = 0.6-1.2, P = 0.38) or D allele (OR = 1.1, 95% CI = 0.9-1.3, P = 0.34). Of note, 40% of the hypertensive patients with a positive history of CVD were carrying DD genotype as compared to 30% with II genotype. We found a significant difference (LRTχ2 = 9.25, P = 0.009) in ACE genotype frequencies when the hypertensive patients were subgrouped based on age at hypertension onset, namely > 40 and ≤ 40 years. Since the ACE I/D gene polymorphism is recessive in nature, we tested the hypothesis whether under the recessive model the minor allele would modify the risk. The frequency of the combined (II + ID) genotype versus DD in controls was (83% versus 17%) compared with patients as (76% versus 24%), where the difference was marginally significant and was slightly associated with risk (LRTχ^2 = 3.7, df =1 P = 0.04, OR = 1.5, 95CI% = 0.98-2.5, Table 3).

GNB3 C825T genotype analysis

There was a significant statistical difference in genotypes (CC, CT, TT) and allelic (C, T) distributions between patients and controls (Fig. 1). Among participants with hypertension, 178 (42%) were CC homozygotes, 213 (50%) were CT heterozygotes, and 35 (8%) were TT homozygotes. The frequency of the T allele was 0.33. In the control group, 193 (55%) were CC homozygotes, 144 (42%) were CT heterozygotes, and 9 (3%) were TT homozygotes. The frequency of the T allele was 0.23. Logistic regression analysis showed that the odds ratio was 1.6 for CT heterozygotes versus C/C homozygotes patients which was significantly different from 1.0 (P= 0.015, 95% CI= 1.2- 2.1). Patients with TT genotype, compared to those with CC genotype, had a significantly increased risk of hypertension (P= 0.00008, OR = 4.2, 95% CI= 2.0-9.5). Assuming a dominant effect for the T allele, we grouped CT + TT genotypes (T allele) and found a significant increased risk of hypertension when compared to CC genotype (P= 0.0001, OR=1.8, 95% CI= 1.3-2.3). Accordingly, assuming a recessive effect for the T allele after grouping TT + CT genotypes (T allele), we could confirm a nearly 2-fold increased risk of hypertension as compared to the dominant model (P= 0.0008, OR=3.3, 95% CI= 1.6-7.1).

ACE activity: Effect of genotype on enzymatic levels: It was observed that the DD and II genotypes correlated with the highest and lowest ACE activity (106.8 ± 30 U/L and 73 ± 25 U/L) while the ID genotype was intermediate (89 ± 27 U/L); thus, the

Table 3: Genotype and allele distribution of the ACE I/D gene polymorphism in patients and controls

P value*	OR (CI %95)†	LRTχ^2	Controls (*n* = 360)	Patients (*n* = 450)	Genotype
					***ACE* I/D**
-	-	-	133 (37%)	156 (35%)	II
0.65	0.93 (0.7-1.3)	0.203	166 (46%)	209 (46%)	ID
0.38	0.83 (0.6-1.2)	0.74	60 (17%)	84 (19%)	DD
0.66	1.1 (0.8-1.4)	0.19			Dominant Model
0.46	1.1 (0.8-1.6)	0.54			Recessive Model
0.34	1.1 (0.9-1.3)	0.91	480 (66%)	521 (58%)	I
			316 (43%)	377 (44%)	D

† OR (CI %95), indicates crude odds ratio and 95% confidence interval. * *P* values calculated by logistic regression

difference was statistically significant (P < 0.0001, Figure 2). In patients and controls (Table 4), we demonstrated that the DD genotype was associated with higher serum ACE concentrations (94 ± 32 U/L versus 84.3 ± 31 U/L), I/I genotype was associated with the lowest concentrations (77.2 ± 21.3 U/L versus 67.6 ± 26 U/L), and heterozygosity was associated with intermediate concentrations (86.4 ± 28 U/L versus 75.7 ± 30 U/L).

Genotype to phenotype correlations between GNB3 825C/T genotypes and clinical phenotype: The clinical characteristics of all participants were compared against GNB3 C825T polymorphism. According to the presence of the 825T allele, TT homozygotes had the highest BMI (24.8 ± 2.8) and patients with CT/TT genotype, compared to those with CC genotype, had a significantly increased BMI (P = 0.01).

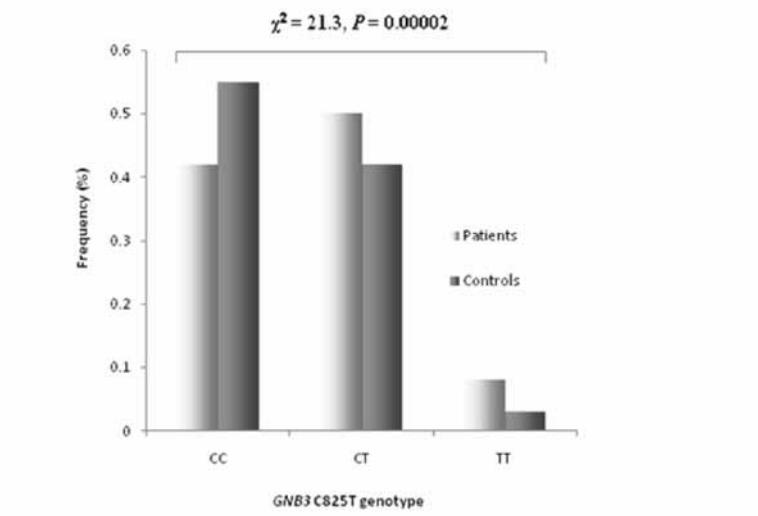

Figure 1: Genotype distribution of the GNB3 C825T gene polymorphism between patients and controls

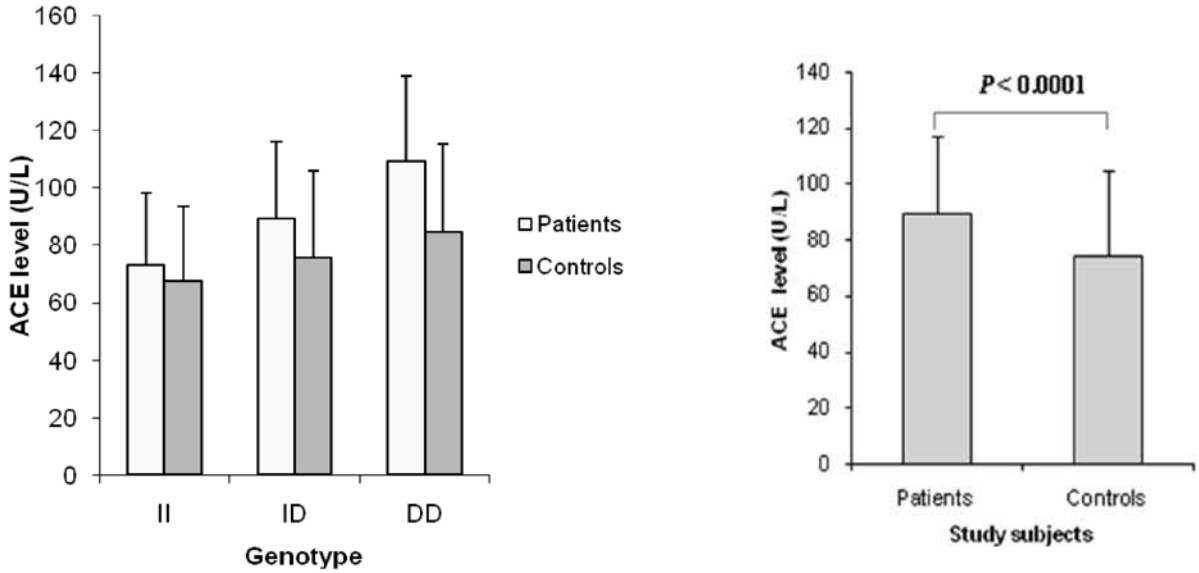

Figure 2: As shown in the upper bar chart, plasma ACE activity was associated with ACE genotype in a dose-dependent pattern in patients with EH (II subjects had the lowest, ID subjects had intermediate, and DD subjects had the highest ACE activity). The lower bar chart shows higher ACE activity in patients as compared to healthy controls.

Table 4: Plasma ACE activity (U/L) with respect to the ACE I/D genotypes of patients and controls

Subjects	Total ACE activity (U/L)	II	ID	DD	*P* value
Patients (n = 450)	87± 27.8	77.2 ± 21.3 (n = 156)	86.4 ± 28 (n = 210)	94 ± 32 (n = 84)	< 0.0001
Controls (n = 360)	74 ± 30	67.6 ± 26 (n = 134)	75.7 ± 30 (n = 166)	84.3 ± 31 (n = 60)	0.0003

Values are mean ± SD. † *P* values were calculated by one-way ANOVA.

Genotypes-combinations and gene-gene interactions: Univariate analysis showed that the combination of the ACE I/D and GNB3 C825T genotypes further enhanced the risk of EH compared to separate association analysis of each gene. Patients evaluated for the ACE-GNB3 association showed a favorable protection significantly (P = 0.009) against hypertension because of the double normal homozygote (II + CC) with an odds ratio of 0.6 (95% CI= 0.4–0.9). Including one unfavorable allele (I or C) to form genotypes (ID + CC or II + CT) combination did not increase the risk of the disease versus other combinations (P = 0.1, OR = 0.6, 95% = 0.5-1.1). Patients with other genotypic combinations that had either of the two unfavorable alleles (D or T) remained marginally significant to confer a greater chance to hypertension than patients with the (II + CC) genotype (P = 0.06, OR = 0.7, 95% = 0.4-1.0). The genotypic combinations, which included three unfavorable allele (DD + CT or ID + TT), had a risk of hypertension that was 2.7 greater than the risk for patients with the (II + CC) genotype (95% CI = 1.5–4.9). Similarly, the genotypic combinations with four unfavorable alleles (DD + TT) showed a greater chance of hypertension than patients with the (II + CC) genotype (P = 0.001, OR = 6.4, 95% = 1.8-22.8). The four-unfavorable allele combination imposed a greater risk of hypertension as compared to other combinations (Table 5).

Discussion

Most studies have examined the effects of genetic and environmental factors in the variation of blood pressure. Heritabilities range from 12–66% for systolic blood pressure and 13–64% for diastolic blood pressure with average levels for both at about 50% (15,16). Efforts to date have identified several candidate genes involved in blood pressure or primary hypertension. Special attention has been paid to the study of genes implicated in the RAS axis including the ACE gene which is important for circulatory homeostasis. The 825T allele has been associated

Table 5: Combined genotype frequencies of the ACE I/D and GNB3 C825T gene polymorphisms between the patients and control subjects

P	OR (95% CI)	Controls *n* (%)	Patients *n* (%)	Combined genotypes
0.009	0.6 (0.4-0.9)	74 (21%) *vs.* 276 (79%)*	62 (14%) *vs.*378 (86%)*	II + CC
0.1	0.6 (0.5-1.1)	140 (40%)	163 (37%)	ID + CC or II + CT
0.06	0.7 (0.4-1.0)	119 (34%)	147 (33%)	ID + CT or II + TT or DD + CC
0.0009	2.7 (1.5-4.9)	23 (6%)	52(12%)	DD + CT or ID + TT
0.001	6.4 (1.8-22.8)	3 (1%)	16 (4%)	DD + TT

* indicates the remaining combinations. OR (95% CI) and *P* values were calculated with logistic regression

with a variety of cardiovascular risk factors, including hypertension [8]. Consequently, the potential role of the GNB3 for cardiovascular diseases is currently conflicting, likely due to the variable impact that polymorphisms can have on the genetic background of populations. The ACE insertion/deletion gene polymorphism was first reported in a study that addressed the role of the ACE gene in the genetic control of plasma ACE levels (17). In our study, the correlation between genotype and plasma ACE levels showed a significant relationship between D allele dose and ACE concentration, with the highest ACE levels found for the DD genotype.

We therefore performed a case-control association study to investigate, at first, the association between the ACE I/D gene polymorphism and ACE activity; blood pressure; and risk of EH in a relatively large sample size of Indian population. In our study on the Indian population, no association was found between ACE DD genotype and D allele with EH. In contrast, ACE levels were higher in hypertensive patients than healthy controls. Moreover, we found that ACE DD homozygotes were associated with the highest circulating ACE levels, ID heterozygotes were associated with intermediate and II homozygotes were associated with the lowest ACE levels. The present study also disclosed that the DD and II genotypes were more frequent in hypertensive males and females, respectively. Interestingly, we found that hypertensive patients carrying DD genotype showed a higher frequency of the family history of CVD when compared to hypertensive patients carrying II genotype. Additionally, in early onset hypertension (age $\leq$ 40 years), the II genotypes were more prevalent, whereas in late onset hypertension (age > 40), the DD genotypes were the least frequent ($P = 0.009$). Of note, for the first time in a case-control study of Indian population, we showed that the ACE I/D genotype was significantly associated with ACE levels. Thus, whilst ACE levels strongly correlate with ACE I/D genotype in European and Indian populations, this may not hold true in others. The association between ACE gene polymorphism and essential hypertension is, however, controversial. A number of studies have found positive associations whereas others have not. A single study has shown a positive association between ACE gene polymorphism and hypertension, with a higher frequency of the I allele in hypertensive patients with a family history of hypertension, compared to normotensive controls (18). Limitations of these studies should be taken into consideration to explain contradictory findings. A plausible mechanism for the lack of the effect of the ACE gene polymorphism on hypertension could therefore be that individuals with the DD genotype, despite having increased levels of ACE, do not necessarily produce increased amounts of angiotensin II. This hypothesis is supported by the fact that the ACE gene polymorphism did not affect blood pressure, despite a significant effect on ACE activity, and 40% of angiotensin I may be converted to angiotensin II by pathways other than ACE. Nevertheless, the RAS has a crucial role in the pathogenesis of hypertension.

It has been reported that 30–50% of hypertensive patients demonstrate increased activity of the NHE-1 in their blood cells (19). Several studies have described an association between increased activity of NHE-1 and hyperactivity of the protein G. GNB3 C825T polymorphism is known to have important genetic influences on the increased activity of the NHE-1, salt sensitivity and EH (8). Our study was sufficiently powered to conclusively document a positive association with arterial hypertension. The prevalence of EH was significantly higher in the TT genotype group as compared to the CC genotype group with an increased risk of the disease. The T allele was over-represented in patients and associated significantly with EH, suggesting the significant relationship between the presence of the T allele and hypertension. In the present study, we confirmed the significant influence of GNB3 C825T polymorphism on the risk of hypertension.

Population based studies of African-Americans and

Caucasians with hypertension have reported that the 825T allele is equally predictive in men and women for greater systolic and diastolic blood pressure responses to the standard dose of hydrochlorothiazide. A possible explanation might be the increased frequency of the 825T allele in individuals with a low plasma renin activity. Although the 825T allele is more common among African-Americans than the Caucasians, the observed association between the increasing number of 825T alleles and the progressively greater blood pressure responses to the diuretic is observed in each race-gender subgroup except African-American females (20). The association between polymorphism and EH has been described in Caucasians and in black people but not in Asians (21-23). Two other studies on African Americans and French people did not find any association between polymorphism and EH (24,25). Nevertheless, we found a significant association between DBP and MAP with 825T allele. For the first time, we described the important influence of GNB3 C825T polymorphism on the risk of hypertension in the Indian population. We found the influence of GNB3 825T allele on the BMI of the patients with hypertension ($P = 0.01$). GNB3 C825T polymorphism has been shown to be directly related to obesity and indirectly to hypertension (26). They found higher frequency of TT and CT genotypes in overweight (BMI $\geq$ 25) and obese (BMI > 27) patients as compared to individuals with a normal weight (BMI < 25). However, another study failed to find any relationships between the presence of the T allele and BMI (27). Although an explanation for this discrepancy may be that the effects of the GNB3 polymorphism on BMI is small and require large sample sizes to be detectable, these facts are difficult to explain taking into account the physiological role of the protein G. Perhaps, enhanced signaling via G proteins increases adiposeness and potentially predisposes to obesity (28). We speculate that TT genotype might be associated not only with sodium absorption and volume expansion but also with some other vasoconstrictive mechanisms leading to EH. The other aim of the study was to verify the hypothesis if the interaction between the GNB3 C825T and ACE I/D polymorphisms could lead to the detection of an association with hypertension. In this investigation, we assessed the genotypic interactions among polymorphisms discussed above. In this context, the GNB3 C825T polymorphism has been shown to interact with the ACE I/D polymorphism in the association with EH (29). We investigated whether there was a significant interaction between GNB3 C825T and ACE I/D polymorphisms for the development of EH. Our study documented a favorable protection against hypertension in patients with double normal homozygote (II + CC) with an odds ratio of 0.6 (95% CI=0.4–0.9). Logistic regression revealed a 2.7-fold greater risk of hypertension associated with the former combination and likewise, a 6.4-fold greater chance of hypertension associated with the latter combination. The presence of the 825T allele increased activity of erythrocyte NHE-1 and the risk of hypertension independent of the ACE genotype (30). In contrast, in the Kazakh isolate of northeast China, the polymorphisms in the GNB3 gene and ACE gene, solely or combined, did not confer a significantly increased risk for the development of EH (31). Since 16% of our hypertensive population had at least one of the risk combinations, genotyping of these polymorphisms could help identify individuals with an increased risk of EH.

In summary, the early promise that the ACE I/D polymorphism would prove to be an independent risk factor for hypertension has not been substantiated by various studies. Although we failed to confirm an association between the ACE I/D gene polymorphism and the disease, our findings suggested a significant association between GNB3 C825T polymorphism at both allelic and genotypic level with the development of EH. Moreover, 825T allele was significantly associated with BMI in hypertensive patients. Considering the population prevalence of unfavorable genotypes in a gene-to-gene interaction, it is likely

that there is an important interaction among these two gene polymorphisms and EH since they are all part of the same metabolic pathway. Given the considerable frequency of the patients bearing a number of the risk-conferring genotype combinations, genotyping of these polymorphisms along with positive family history of cardiovascular disease could help identify individuals at high risk of susceptibility to EH.

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