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G-protein beta 3 subunit polymorphisms and essential hypertension: a case-control association study in northern Han Chinese

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Abstract

Objective   To explore the association between the three polymorphisms [C825T, C1429T and G(-350)A] of the gene encoding the G protein beta 3 subunit (GNB3) and hypertension by performing a case-control study in the northern Han Chinese population. **Methods**   We recruited 731 hypertensive patients and 673 control subjects (the calculated power value was > 0.8). Genotyping was performed to identify C825T, C1429T and G(-350)A polymorphisms using the TaqMan assay. Comparisons of allelic and genotypic frequencies between cases and controls were made by using the chi-square test. Logistic regression analyses were performed to investigate the relationships between the three polymorphisms of GNB3 gene under different genetic models (additive, dominant and recessive models). **Results**   The genotype distribution and allele frequencies of C825T, C1429T and G(-350)A polymorphisms did not differ significantly between hypertensive patients and control subjects, either when the full sample was assessed, or when the sample was stratified by gender. No significant association was observed between C825T, C1429T and G(-350)A polymorphisms and the risk of essential hypertension in any genetic model. Linkage disequilibrium was only detected between C825T and C1429T polymorphisms. Haplotype analyses observed that none of the three estimated haplotypes significantly increased the risk of hypertension. **Conclusions**   Our study suggested that the GNB3 gene polymorphisms [C825T, C1429T and G(-350)A] were not significantly associated with essential hypertension in northern Han Chinese population.

1   Introduction

Essential hypertension (EH) is a common disorder affecting 20%–30% of the adult population in Western countries, whereas 27.2% of the adult Chinese population aged 35–74 years suffer from this disease.[1,2] The causes of EH are complex, characterized by the involvement of multiple susceptible genes, environmental factors and their interactions. Familial and twins studies have demonstrated that the genetic contribution to the variation of individual blood pressure level is appropriately 23%–60%.[3] Therefore, studying the genetic profiles of hypertension is of significant importance, which would help to realize the early de-

tection of individuals prone to the development of hypertension and its complications.

Guanine nucleotide-binding proteins (G proteins), are signal transduction proteins that translate signals from the cell surface into cellular responses via interacting with G-protein coupled receptors (GPCRs). Hence, any alterations that affect the function or expression of G-proteins may, potentially, have a strong impact on cellular signal transduction, and consequently, modulating a wide range of disorders, including blood pressure homeostasis.[4] In the search for the genetic mechanisms of EH in humans, the gene encoding the G proteins beta3 subunit (GNB3) has been extensively investigated.

In 1998, Siffert, *et al*.[5] detected a novel polymorphism (C825T), involving the substitution of cytosine (C) for thymine (T) at nucleotide 825 in exon 10 of the GNB3 gene. This polymorphism does not affect amino acid sequence, but the 825T allele is associated with the occurrence of the shortened splice variants Gβ3. Since the first publication reporting the significant association between C825T poly-

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morphism with hypertension,[5] many researchers have tried independently to replicate these results in different populations. Some of these studies reported similar results,[6–8] whereas others failed to replicate these findings.[9–11] It was reported that the ethnic distribution of the 825T allele frequency ranged from 20% to 80%.[12] These inconsistent results may be due to the heterogeneity of genetic background in different populations. However, the results on the association between C825T polymorphism and hypertension in Chinese were not unanimous. Tan, *et al*.[13] reported that there was a significant association between C825T and hypertension in Chinese of the Chongqing region, while other studies showed no significant association between C825T and hypertension in other regions of China.[14–16] We speculated that geographic factors might be a major reason for the inconsistency. Moreover, the sample size of the majority of earlier studies in the Chinese Han population was small (< 500) and therefore, it is necessary to perform another study to confirm the relationship between the C825T polymorphism and hypertension in Chinese Han population.

In addition to C825T polymorphism, G(-350)A polymorphism in the promoter region and C1429T polymorphism at the distant 3'-UTR of GNB3 gene have also been identified in Germans, Africans, Chinese.[17] Close linkage disequilibrium was also observed both between C825T and G(-350)A and between C825T and C1429T.[17] Recently, the researches on several polymorphisms of GNB3 gene in relation to EH were relatively few.

The aim of the present study was to investigate the relationship between the C825T, C1429T, G(-350)A variants in the G-protein beta3 subunit gene and the risk of essential hypertension in the northern Han Chinese population.

2 Methods

2.1 Study population

All hypertensive subjects and normotensive controls were collected from June 2008 to July 2009 at the Health Check-up Center of Beijing Anzhen Hospital, Capital Medical University (China). A total of 731 unrelated hypertensive patients and 673 healthy normotensive subjects were ascertained and identified. Blood pressure (BP) was accurately measured three times with a standardized mercury sphygmomanometer by experienced internists at their offices according to a common protocol recommended by European Society of Hypertension.[18] Exercise, alcohol, caffeine and smoking were not generally allowed for a period of 30 min prior to blood pressure measurement. Measurements were taken at the right arm using the appropriate bladders after the participants had been seated in a chair

with their feet on the floor for 10 min. Considering the high white-coat effects of the first BP measurement, BP was determined by calculating the mean of the two last measurements on a single visit. All the participants, except those under antihypertensive treatment, were recruited into the study after they visited the clinical office several times. Hypertension was defined as the average systolic blood pressure (SBP) ≥ 140 mmHg and/or the average diastolic blood pressure (DBP) ≥ 90 mmHg, or on antihypertensive treatment. Normotension was defined as SBP/DBP < 140/90 mmHg, absence of antihypertensive treatment. Subjects with secondary hypertension, diabetes mellitus, cancer, hepatic and renal dysfunction were excluded. A detailed interview, physical examination and laboratory analysis were also required of the participants. Smokers were defined as cigarette consumers who had smoked ≥ 100 cigarettes; and drinkers were defined as alcohol consumers who had drank ≥ 12 times during the past year.[19,20] Obesity was defined as body mass index ≥ 25 kg/m^2 according to the World Health Organization obesity guidelines on Asians.[21] The study was approved by the Ethics Committee of Beijing Anzhen Hospital, complying with the Declaration of Helsinki. Each of the involved participants gave informed consent to participate in this study.

2.2 Genotyping

A 5 mL peripheral venous blood sample was collected after 12 h overnight fasting and drawn into EDTA-containing receptacles. DNA was extracted according to standard phenol chloroform method and stored at −20℃ for batch genotyping. The three single nucleotide polymorphisms (SNPs) of GNB3 gene were genotyped using the TaqMan assay. C_2184734_10 (rs5443, C825T), C_11338514_10 (rs5441, G(-350)A) and C_2184736_10 (rs5446, C1429T) SNP genotyping kits were obtained from Applied Biosystems containing the two flanking primers and C- and T-specific (or G- and A-specific) probes labeled with VIC and FAM fluorescent dyes, respectively. The sample DNA was amplified by PCR on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems) according to the manufacturer's recommendations. Each 384-well plate contained 380 samples of unknown genotype, two samples with no DNA but with reagents (negative control), and two duplicate samples (control). Plates were read on the ABI HT 7900 instrument using the end-point analysis mode of the SDS, version 2.0, software package (Applied Biosystem). Genotypes were discriminated by analyzing the dye-component fluorescent emission data depicted in the X-Y scatter plot of SDS software.

2.3 Statistical analysis

All the database management and statistical analyses were performed using the SPSS statistical software (version 17.0, SPSS, Inc, Chicago, IL, USA). Normally continuous variables were presented as mean ± SD, medians (25th/75th quartiles) were used for non-normally distributed variables and categorical variables were expressed as percentages. Comparisons between groups were done with Student's *t*-test, Mann-Whitney *U* test and chi-squared test for normally distributed continuous, non-normally distributed continuous and categorical variables, respectively. Comparisons of allelic and genotypic frequencies between hypertensive patients and controls were made by using the chi-square test. A *P*-value of 0.05 or lower was considered statistically significant.

Hardy-Weinberg equilibrium was tested by chi-square test for goodness of fit based on a web program (http://ihg. gsf.de/cgi-bin/hw/hwa1.pl).

To test whether there were significant associations between the three polymorphisms of GNB3 gene and hypertension risk, logistic regression analysis was performed under different genetic models (additive, dominant and recessive models) after adjustment for potential confounding factors. Statistical power calculation was performed with the PS: Power and Sample size calculation program by Dupont and Plummer.[22,23] In our experimental design, we calculated the sample size (500 participants in each group) based on the published data in the literature, and the calculated power value was > 0.8. We initially used C825T data as a sample group to calculate the power value and then calculated the power value of C1429T, which was also > 0.8.

Linkage disequilibrium and haplotype analyses within the three variants of the GNB3 gene were conducted following the Haploview software (version 4.2) (http://www. broad.mit.edu/mpg/haploview/). The expectation maximization algorithm was performed to estimate haplotype frequencies and to obtain the best haplotype configuration for each multi-locus genotype. All haplotypes with frequency greater than 1% in our samples were investigated. We used the chi-square test to compare the haplotype distributions between both groups. Haplotype-specific (HS) testing was conducted to compare a specific haplotype with the others. Assuming the highly prevalent haplotype as the base line, each of the other haplotypes was compared with the base-line haplotype using a logistic regression model.

3 Results

3.1 Characteristics of the participants

A total of 1329 unrelated participants comprising 731

hypertensive patients (462 men and 269 women; mean age 51.86 ± 9.48 years) and 673 normotensive control subjects (398 men and 275 women; mean age 50.95 ± 8.82 years) were recruited in the present study. The clinical and laboratory parameters of the participants are summarized in Table 1. Aside from BP measurements, significant differences in body mass index (BMI), total cholesterol, triglyceride, HDL-C, glucose level and the ratio of smokers and drinkers were observed between hypertensive subjects and normotensive controls.

3.2 Detection and distribution of the SNPs

Among all the participating subjects, 98.7% samples of C825T polymorphism, 99.2% samples of C1429T and 99.1% samples of G(-350)A polymorphisms were successfully identified in the laboratory. No deviation from the Hardy-Weinberg expectation was observed for C825T, C1429T and G(-350)A polymorphisms in either the case, or the control group (Case: C825T, $P = 0.251$; C1429T, $P = 0.318$; G(-350)A, $P = 0.543$; Control: C825T, $P = 0.226$; C1429T, $P = 0.360$; G(-350)A, $P = 0.211$). Genotype and allele frequencies of the GNB3 gene variants (C825T, C1429T, G(-350)A) in the hypertensive cases and control subjects are shown in Table 2. Chi-square analyses revealed that the genotype distribution and allele frequencies of C825T, C1429T and G(-350)A polymorphisms did not differ significantly between hypertensive patients and control subjects, either when the full sample was assessed, or when the sample was stratified by gender.

Table 1. Basic clinical characteristics of the participants.

Variables	Hypertension, $n = 731$	Control, $n = 673$	P
Gender, M/F	462/269	398/275	0.126
Age, yrs	51.86 ± 9.56	50.95 ± 8.82	0.062
SBP, mmHg	138.43 ± 16.78	116.46 ± 11.82	< 0.001
DBP, mmHg	89.58 ± 11.83	75.86 ± 8.24	< 0.001
BMI, kg/m²	26.75 ± 3.32	24.99 ± 3.15	< 0.001
HR, beats/min	71.59 ± 9.82	71.74 ± 10.28	0.822
ALT, U/L	25.63 ± 14.16	24.94 ± 13.26	0.366
Cr, umol/L	77.76 ± 15.66	76.68 ± 14.61	0.243
Glu, mmol/L	5.23 ± 0.50	4.95 ± 0.54	< 0.001
TC, mmol/L	5.52 ± 3.09	5.00 ± 0.90	< 0.001
TG, mmol/L	2.11 ± 1.42	1.65 ± 1.14	< 0.001
LDL-C, mmol/L	3.37 ± 0.90	3.42 ± 0.76	0.420
HDL-C, mmol/L	1.18 ± 0.55	1.35 ± 1.26	0.01
Smokers, *n*	183	116	< 0.001
Drinkers, *n*	207	64	< 0.001

ALT: alanine aminotransferase; BMI: body mass index; Cr: creatinine; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; HR: heart rate; Glu: glucose; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; TC: total cholesterol; TG: triglyceride.

Table 2.   The genotype and allele frequencies of the G-protein β3 subunit gene C825T, C1429T and G(-350)A polymorphisms.

		Genotype, *n* (%)			*P*	Allele, *n* (%)		*P*
C825T		TT	CT	CC		T allele	C allele	
	Case (Total)	155 (21.6)	374 (52.0)	190 (26.4)	0.430	684 (47.6)	754 (52.4)	0.440
	Control (Total)	134 (20.1)	347 (52.0)	186 (27.9)		615 (46.1)	719 (53.9)	
	Case (Male)	92 (20.1)	235 (51.3)	131 (28.6)	0.645	419 (45.7)	497 (54.3)	0.652
	Control (Male)	82 (20.8)	206 (52.2)	107 (27.1)		370 (46.8)	420 (53.2)	
	Case (Female)	63 (24.1)	139 (53.3)	59 (22.6)	0.055	265 (50.8)	257 (49.2)	0.061
	Control (Female)	52 (19.1)	141 (51.8)	79 (29.0)		245 (45.0)	299 (55.0)	
C1429T		TT	CT	CC		T allele	C allele	
	Case (Total)	26 (3.6)	245 (33.7)	455 (62.7)	0.585	297 (20.5)	1155 (79.5)	0.592
	Control (Total)	22 (3.3)	218 (32.7)	427 (64.0)		262 (19.6)	1072 (80.4)	
	Case (Male)	16 (3.5)	140 (30.5)	303 (66.0)	0.657	172 (18.7)	746 (81.3)	0.662
	Control (Male)	11 (2.8)	133 (33.6)	252 (63.6)		155 (19.6)	637 (80.4)	
	Case (Female)	10 (3.7)	10 (39.3)	152 (56.9)	0.136	125 (23.4)	409 (76.6)	0.144
	Control (Female)	11 (4.1)	85 (31.4)	175 (64.6)		107 (19.7)	435 (80.3)	
G (-350)A		AA	AG	GG		A allele	G allele	
	Case (Total)	0 (0)	32 (4.4)	692 (95.6)	0.944	32 (2.2)	1416 (97.8)	0.944
	Control (Total)	1 (0.1)	27 (4.0)	640 (95.8)		29 (2.2)	1307 (97.8)	
	Case (Male)	0 (0)	20 (4.4)	438 (95.6)	0.819	20 (2.2)	896 (97.8)	0.821
	Control (Male)	0 (0)	16 (4.1)	379 (95.9)		16 (2.0)	774 (98.0)	
	Case (Female)	0 (0)	12 (4.5)	254 (95.5)	0.894	12 (2.3)	520 (97.7)	0.891
	Control (Female)	1 (0.4)	11 (4.0)	261 (95.6)		13 (2.4)	533 (97.6)	

3.3   Association analyses

Logistic regression analyses were performed under different genetic models (additive, dominant, recessive) after adjusting for the potential confounding factors, such as gender, age, BMI, total cholesterol, triglyceride, glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), smoking and drinking habits. The results of logis-

tic regression analyses are presented in Table 3. It showed that C825T polymorphism did not have a significantly increased risk of EH compared with controls in the additive genetic model [$P = 0.587$, OR = 1.076, 95%CI: (0.826–1.402)], in the dominant genetic model [$P = 0.847$, OR = 0.960, 95% CI: (0.637–1.449)] or in the recessive model [$P = 0.479$, OR = 0.850, 95% CI (0.542–1.332)]. Similarly, neither C1429T, nor G(-350)A was significantly associated with the risk of EH in any genetic model (Table 3). We also con-

Table 3.   Odds ratios of different genetic model comparisons for each single-nucleotide polymorphism associated with essential hypertension in the northern Han Chinese population.

SNP	Genetic models	Contrast	Overall		Male*		Female*	
			OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
C825T	Additive	TT *vs.* CT *vs.* CC	1.076 (0.826–1.402)	0.587	1.022 (0.745–1.401)	0.895	1.259 (0.744–2.129)	0.391
	Dominant	(TT + CT) *vs.*CC	0.960 (0.637–1.449)	0.847	1.052 (0.647–1.712)	0.838	0.749 (0.333–1.685)	0.485
	Recessive	TT *vs.* (CT + CC)	0.850 (0.542–1.332)	0.479	0.881 (0.510–1.520)	0.648	0.869 (0.347–2.176)	0.764
C1429T	Additive	TT *vs.* CT *vs.* CC	1.221 (0.877–1.699)	0.237	1.637 (0.857–3.128)	0.136	0.738 (0.308–1.770)	0.496
	Dominant	(TT + CT) *vs.*CC	0.819 (0.561–1.195)	0.301	1.047 (0.667–1.642)	0.843	0.507 (0.236–1.090)	0.082
	Recessive	TT *vs.* (CT + CC)	0.624 (0.216–1.802)	0.383	0.468 (0.114–1.920)	0.292	0.938 (0.164–5.382)	0.943
G (-350)A	Additive	AA *vs.* AG *vs.*GG	1.032 (0.402–2.653)	0.947	0.800 (0.284–2.256)	0.673	2.730 (0.206–36.252)	0.447
	Dominant	(AA + AG) *vs.* GG	0.969 (0.377–2.489)	0.947	1.250 (0.443–3.524)	0.673	0.366 (0.028–4.864)	0.447

CI: confidence interval; OR: odds ratio; R: adjusted for gender, age, BMI, glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, smoking habits and drinking habits; SNP: single-nucleotide polymorphism. *OR adjusted for gender was not conducted in male and female.

ducted sub-group analyses by gender, but the results did not demonstrate any relationship between C825T, C1429T and G (-350)A polymorphisms and genetic susceptibility to EH in either subgroup (Table 3).

3.4   Haplotype analyses

As shown in Figure 1, the C825T and C1429T polymorphisms were almost in complete Linkage Disequilibrium (LD) (D′ = 0.96, r^2 = 0.26). However, G(-350)A and C825T were not found in LD and neither were G(-350)A and C1429T observed in LD. The haplotype analyses of the C825T and C1429T polymorphisms of GNB gene in cases and controls are summarized in Table 4. Only three of four possible haplotypes (C-C, T-C and T-T) with frequencies greater than 1% were identified in haplotype analyses. Haplotype specific (HS) testing revealed that there was no significant association between the C-C haplotype and the risk of essential hypertension (*P* = 0.346, OR = 1.094, 95% CI: 0.907–1.319). Also, the T-C and T-T haplotypes were not detected in relation to hypertension. Furthermore, as the most highly prevalent haplotype, the T-T haplotype was defined as the base-line haplotype. Compared with the base-line haplotype, neither C-C haplotype nor T-C haplotype was associated with risk of EH.

4   Discussion

Both genetic and environmental factors play important roles in the pathogenesis and progression of EH. Exploring the genetic mechanisms of EH was considered to be of clinical importance, contributing to the better understanding the pathogenesis of hypertension, and ultimately achieving the goal of early diagnosis, treatment and prevention of hypertension. With the completion of the human genome project, single nucleotide polymorphisms (SNPs) have become increasingly crucial in uncovering the mechanisms of polygenic diseases. In the present study, we investigated the role of C825T, C1429T and G (-350)A polymorphisms in the
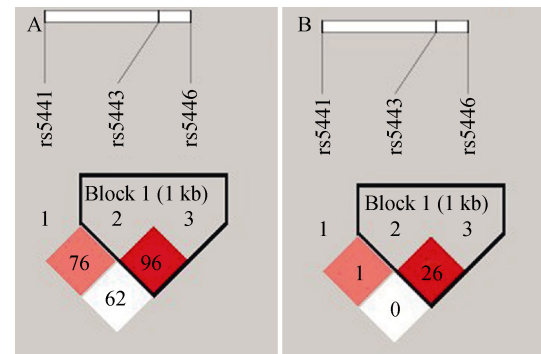
Figure 1. Linkage disequilibrium (LD) block of C825T, C1429T and G(-350)A polymorphisms by the Haploview program based on the confidence interval method. (A): LD measure of D′; and (B): LD measure of r^2.

GNB3 gene in the development of essential hypertension in the northern Han Chinese population.

The data provided in this study confirmed that the genotype distribution and allele frequencies of C825T polymorphism did not differ significantly between hypertensive patients and control subjects. After adjustment for the multiple covariates (gender, age, BMI, total cholesterol, triglyceride, glucose, triglyceride, LDL-C, HDL-C, smoking habits and drinking habits), logistic regression analyses indicated that C825T polymorphism was not significantly associated with EH in any genetic model. Our failure to reproduce previous positive association results,[6,8,24] has to be interpreted with considerable caution. Confounding factors, such as population stratification and misclassification, may lead to false-positive and false-negative results in case-control studies.[25,26] Xu, *et al*.[27] examined the Han Chinese population substructure in a diverse set of 1700 Han Chinese samples collected from 26 regions across China and determined that the Han Chinese population is intricately substructured, with the main clusters corresponding roughly to northern Han, central Han and southern Han. Our study selected the northern Han Chinese population to minimize the chance of population stratification. In addition, hypertensive cases were determined after several clinical visits,

Table 4.   Haplotype analyses of the G-protein subunit beta3 gene polymorphisms in hypertensive cases and control subjects.

C825T	C1429T	Haplotype frequency		HS testing *P*-value[a]	OR (95%CI)[a]	*P*-value[b]	OR (95%CI)[b]
		Cases	Controls				
T	T	0.524	0.532	0.662	0.967 (0.834–1.122)	–	–
T	C	0.272	0.272	0.989	0.999 (0.845–1.180)	0.384	1.091 (0.897–1.328)
C	C	0.203	0.189	0.346	1.094 (0.907–1.319)	0.390	1.079 (0.907–1.285)

All haplotypes with frequency greater than 1% identified in the haplotype analyses are summarized in this table. [a]*P*-values and OR values derived from comparing of a specific haplotype with the other two; [b]*P*-values and OR values derived from comparing each haplotype with the base-line haplotype (T-T). HS testing: Haplotype specific testing; OR: odds ratio; CI: confidence interval.

which reduced the risk of misclassification to some extent. Furthermore, our study, which included over 1300 participants, decreased the sample selection bias that is generally unavoidable in case-control association studies. These details provided an appropriate statistical power to evaluate the association between C825T polymorphism and hypertension.

The following factors may explain the observed lack of association in the northern Han Chinese population. First, the contribution of C825T to the pathogenesis of hypertension may be less significant than that originally reported in whites,[5] partly due to the population-specific combination of genetic and environmental factors. Second, we cannot rule out the possibility that a "true" mutation, as yet unidentified, exists in the linkage disequilibrium with C825T polymorphism. Third, lack of association may result from ethnic differences. For example, Dong *et al*.[8] reported the 825T allele was a predisposing factor for hypertension, with the 825T allele being nearly 80%, whereas the frequency of 825T allele in our current study was only 47%, which is similar to that reported in the Japanese population (approximately 49%).[9] In agreement with our research, their study did not show that 825T allele was an increased risk factor of EH in the Japanese population. Additionally, in a Chinese population, the associations between C825T polymorphism and hypertension were still inconclusive. Tan, *et al*.[13] reported that there was a significant association between C825T and hypertension in Chinese of the Chongqing region in a small sample of 224 participants. The 825T allele (23%) in their study was different from that (47%) in our study, which may lead to the inconsistency between their results and our findings. The Chongqing region is in southern China, whereas the sample sources in our study are from the northern areas of China. Xu, *et al*.[27] reported genetic backgrounds were different among northern Han, central Han and southern Han. Furthermore, considering that the sample size in the Tan study was small, we cannot rule out the possibility that the results were unreliable due to sample size bias.

The C1429T polymorphism is located in the 3'-UTR of the GNB3 gene with the 1429T allele frequency in our research was 20.5%, lower than those reported in German (30%) and African (38%) populations, but similar to that reported in Chinese population (19.8%). We found that C1429T polymorphism was not associated with hypertension in our study. Cabadak, *et al*.[28] evaluated the contribution of the C1429T to genetic susceptibility of EH in the Turkish population, but did not observe the positive relationship, which agrees with our results. The relationship between C1429T polymorphism and hypertension should be investigated in other populations.

In a previous study, Li, *et al*.[16] reported that the GNB3 G(-350)A polymorphism was significantly associated with hypertension in northern Han Chinese population. Compared with their study, we did not observe a significant association between G(-350)A and EH risk. Considering the diagnostic standard of Li *et al*.[16] was higher than our study, we speculate that G(-350)A might be associated with more severe hypertension. To clarify this speculation, we reclassified the hypertensive patients based on the highest SBP ≥ 160 mmHg and/or the highest DBP ≥ 100 mmHg and subsequently, 449 patients were enrolled. Interestingly, there was still no significant association between G(-350)A and more severe hypertension (AA + AG *vs*. GG, $P = 0.355$, OR = 0.502, 95%CI: 0.116–2.167). We considered that this inconsistency might be attributed to two main reasons. Firstly, there might be a statistical fluctuation in *P* values of case-control studies, by chance, due to the nature of random sampling, sample size and the lower allele frequency of G (-350)A polymorphism. Given that the participants of the stage 2 hypertension subgroup in the current study were similar to those of the Li *et al*.[16] study, a larger sample size is required to attain enough statistic power to detect real differences in the genotype and allele frequency of G(-350)A polymorphism between groups. Secondly, the inconsistent results might be partially due to the complex genetic differences among the northern Han Chinese population. Due to human migration in history, the northern Han Chinese population was under strong genetic influences from other populations,[29] and the cohorts of our study and Li's were not entirely recruited from the same region, that is to say, the participants were not considered a single homogenous population, which might explain the discrepancy in these studies.

Haplotype analyses could be more powerful than single locus analyses,[30] to identify genetic variants of complex diseases, such as EH. In the current study, Haplotype analyses revealed that only three haplotypes with frequencies over 1% were found in our study as previously described.[17] As described in Table 4, the haplotype frequencies summed to 99.9% in cases and 99.3% in controls, which means that there were still some rare haplotypes undetected in the present study. As for the LD of C825T and C1429T, Rosscopf, *et al*.[17] reported that these two polymorphisms were in complete LD in Germans, Africans and Chinese. In the present study, similar results were observed in northern Han Chinese. We did not identify the LD of C825T and G(-350)A polymorphisms. This may be due to the lower allele frequency of G(-350)A polymorphism. Both HS testing and logistic regression analysis did not show any

significant association between the three haplotypes and hypertension.

Given that no significant association between the three polymorphisms of GNB3 gene and hypertension was observed, an important issue must be considered, that is, the statistical power. Essential hypertension is a polygenic disease, and many genes jointly make contributions to the development of hypertension. Thus, a genetic marker may have only a modest effect on hypertension susceptibility, and larger samples are required to detect and confirm the associations. Our study includes 731 hypertensive patients and 673 controls and the power of detecting an odds ratio about 1.3 at a 5% type 1 error probability was not less than 80%. Thus, no significant association can be found from our data.

In summary, the present study suggested that the three polymorphisms [C825T, C1429T and G (-350)A] of GNB3 gene were not significantly associated with essential hypertension in the northern Han Chinese population. However, further well-designed studies with a larger sample size in relatively homogenous populations are needed to elucidate the role of the GNB3 gene polymorphisms in the pathogenesis of EH.

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