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G Protein β3 Subunit Gene Variants and Essential Hypertension in the Northern Chinese Han Population

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Summary

Recently a novel C825T polymorphism in the G protein β3 subunit gene was identified that showed an association with hypertension in a German population; the results of studies in other populations have been inconsistent. To examine the contribution of *GNB3* polymorphisms to the development of hypertension in the northern Chinese Han population, we conducted a case-control study consisting of 501 hypertensive cases and 503 controls using the G(-350)A, C825T and C1429T polymorphisms. Genotypes of samples were determined by PCR and restriction digestion. Single locus analysis showed a significant association between G(-350)A and hypertension ($P = 0.01$) but no association for C825T or C1429T. The three polymorphisms were in tight linkage disequilibrium ($D' = -1$ for G(-350)A-C825T, $D' = 0.92$ for C825T-C1429T) and a total of 7 haplotypes were observed in the entire population. Haplotype A-C-C was found to be significantly related to hypertension ($P = 0.032$) and A-C-C carriers had a more than two-fold higher risk of hypertension than non-carriers, after adjustment for BMI and glucose. In conclusion, our study suggests that G(-350)A is a potential functional polymorphism that may be related to hypertension, whereas the C825T and C1429T polymorphisms are not associated with hypertension in the northern Chinese Han population.

Keywords: G protein β3 subunit gene, haplotype, association study, hypertension

Introduction

Essential hypertension is considered to be a typical complex disease and is influenced by both genetic and environmental factors. To elucidate the genetic mechanisms of hypertension, many candidate genes involved in important physiological pathways have been investigated. Heterotrimeric guanine nucleotide-binding proteins (G proteins), which are composed of an α-subunit and a $\beta\gamma$-dimer, play a vital role in intracellular signal transduction (Hamm, 1998). Recently a C825T polymorphism at position 825 in exon 10 of the gene encoding the G protein β3 subunit (*GNB3*) was identified and demonstrated to be associated with hypertension, with the 825T allele being associated with alternative splicing of the gene (Siffert *et al.* 1998). In other studies the 825T allele has also been found to be associated with elevated blood pressure (Beige *et al.* 1999; Benjafield *et al.* 1998; Schunkert *et al.* 1998). Although the majority of

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studies in Caucasians have shown an association between the 825T allele and hypertension, studies performed in Japanese (Kato *et al.* 1998), African Americans (Larson *et al.* 2000), Taiwanese (Tsai *et al.* 2000) and a northern Chinese Han population (Huang *et al.* 2003) have all failed to reproduce the positive association.

In addition to C825T several other polymorphisms, including G(-350)A and C1429T, have also been detected in *GNB3* in Germans, Africans and Chinese. The G(-350)A polymorphism is located at position -350 in the promoter region of *GNB3* and the G/A substitution results in deletion of a binding motif for an E-box. The C1429T polymorphism is located at the distant 3′-UTR and is in strong linkage disequilibrium with C825T (Rosskopf *et al.* 2000). Despite functional relevance of these three polymorphisms not all of them have been involved in association studies of essential hypertension.

Considering the functional importance of the *GNB3* gene and the inconsistent results from studies in various populations, and especially the negative result in the Chinese Han population, we selected the *GNB3* G(-350)A, C825T and C1429T polymorphisms for study in a case-control design, aiming at examining the role of these polymorphisms in the development of hypertension in the northern Chinese Han population by single locus analysis and haplotype analysis.

Materials and Methods

Subjects

This study was based on the International Collaborative Study of Cardiovascular Disease in Asia (InterASIA), from which all DNA samples and clinical data were obtained (Gu *et al.* 2002). The local bioethical committee approved the study protocol and informed consent for participation was obtained from all subjects. The samples involved in the present investigation consisted of 1004 (501 unrelated hypertensive cases and 503 unrelated normotensive controls) northern Han Chinese from Beijing city and Jilin and Shandong provinces. All measurements and interviews were taken under standard conditions as previously described (Gu *et al.* 2002). All hypertensive cases were defined as three consecutive blood pressure measurements $\geqq$160 mmHg (systolic) and/or $\geqq$100 mmHg (diastolic), and controls had systolic blood pressure $<$140 mmHg and diastolic blood pressure $<$90 mmHg. Blood pressure values of hypertensive patients receiving treatment were adjusted according to the algorithm described in the analyses of Framingham data (Levy *et al.* 2000). None of the subjects had secondary forms of hypertension, coronary heart disease or diabetes. Among all the hypertensive cases, 474 matched controls for area, gender and age, while the remaining 29 matched for area and gender only.

Genotyping Studies

Genotypes for the *GNB3* nucleotides-350, 825, 1429 were analyzed by PCR and restriction digestion using primers and enzymes as previously described (Rosskopf *et al.* 2000). The PCR amplification conditions for polymorphism G(-350)A involved 10 touch-down cycles of 94°C, 69°C and 72°C for 15, 15 and 30 seconds, respectively, with the annealing temperature descending 0.3°C per cycle, followed by 25 cycles of 94°C, 66°C and 72°C for 15, 15 and 30 seconds, respectively, finishing with a step at 72°C for 2 minutes.

Haplotype Analysis

The EM algorithm-based function haplo.em in the Haplo.stats package of the statistical language R (http://www.r-project.org) was applied to estimate each individual's haplotypes in the entire samples. Then the estimated data was used by the function haplo.score for a haplotypes global test and haplotype-specific test, and by haplo.glm for logistic regression analysis between hypertensive cases and controls based on the genotyping data (Schaid *et al.* 2002).

Statistical Analysis

The data were analyzed using SAS statistical software (SAS Institute Inc., Cary, NC, USA) and the program HWE (Guo & Thompson, 1992). Quantitative data were expressed as mean $\pm$ SD. Hardy-Weinberg equilibrium was assessed by Fisher's exact test using HWE. The differences in clinical characteristics between hypertensive cases and controls were assessed by a *t*-test for quantitative variables and chi-square test for

Table 1 Characteristics of study participants

Parameters	Total	Case (501)	Control (503)	*P*
Gender				0.998
Males	523	261	262	
Females	481	240	241	
Age, y	53.6 ± 9.3	53.6 ± 9.3	53.7 ± 9.2	0.855
BMI, kg/m^2	25.32 ± 3.84	26.3 ± 3.85	24.34 ± 3.58	<0.0001
SBP, mm Hg	147.3 ± 36.7	177.1 ± 28.0	117.6 ± 11.6	<0.0001
DBP, mm Hg	89.7 ± 17.9	104.3 ± 12.3	75.1 ± 8.0	<0.0001
Cho, mmol/l	5.14 ± 1.02	5.22 ± 0.98	5.07 ± 1.04	0.015
Tg, mmol/l	1.56 ± 0.97	1.70 ± 1.05	1.42 ± 0.85	<0.0001
Cr, umol/l	69.8 ± 13.7	70.8 ± 15.2	68.9 ± 12.1	0.027
HDL_C, mmol/l	1.29 ± 0.32	1.25 ± 0.30	1.32 ± 0.34	0.001
Glu, mmol/l	5.75 ± 1.74	5.93 ± 1.79	5.57 ± 1.66	0.001
LDL_C, mmol/l	3.14 ± 0.86	3.19 ± 0.85	3.09 ± 0.86	0.058
Smoking	41.73%	40.5%	42.9%	0.436
Drinking	33.8%	34.3%	33.2%	0.705

BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; Cho, cholesterol; Cr, creatinine; Tg, triglycerides; HDL_C, high-density lipoprotein cholesterol; Glu, glucose; LDL_C, low-density lipoprotein cholesterol.

Table 2 Comparison of genotype and allele frequencies for three *GNB3* polymorphisms

SNPs	Genotypes/Alleles	Cases (n = 501)	Controls (n = 503)	*P*
G(-350)A	AA/AG/GG	1/33/467	0/16/487	0.01*
	A/G	35/967	16/990	0.007
C825T	CC/CT/TT	142/256/103	137/259/107	0.914
	C/T	540/462	533/473	0.683
C1429T	CC/CT/TT	311/166/24	325/158/20	0.649
	C/T	788/214	808/198	0.353

*Probability was computed by Fisher's exact test.

categorical ones. The frequencies of the alleles and genotypes between cases and controls were compared by the chi-square test. The relationship between each of the three polymorphisms and blood pressure levels (BP) in cases and controls were tested separately by ANOVA. In regression analysis odds ratios and 95% confidence intervals (CIs) were computed from generalized linear regression parameters derived from haplo.glm. Pair wise linkage disequilibrium coefficients were calculated from estimated haplotype frequencies, with D′ denoting the extent of disequilibrium. P-values less than 0.05 were considered as indicative of statistical significance.

Results

Table 1 presents the values of the parameters related to risk for hypertension. As expected, hypertensive cases had a larger body mass index (BMI), and higher levels of cholesterol (Cho), glucose (Glu), triglycerides (TG), and creatinine (Cr) than controls. The cases also had a higher LDL cholesterol level and a lower HDL cholesterol level than controls. There was no significant difference in age and proportion of smokers and drinkers between cases and controls.

G(-350)A, C825T and C1429T Polymorphisms

All genotype and allele frequencies of the *GNB3* G(-350)A, C825T and C1429T polymorphisms are given in Table 2. The genotype distributions of these three polymorphisms were all in accordance with the expected Hardy-Weinberg equilibrium. The frequency of the A allele of polymorphism G(-350)A was 3.5% in hypertensive cases and 1.6% in controls, and a significant association with hypertension status was observed (P = 0.01). Of the (-350)A allele carriers were more hypertensive than non-A allele carriers (AA+AG vs. GG, P = 0.009). However, neither the genotype nor the

Table 3 Comparison of frequencies of estimated haplotypes between controls and cases

	Haplotypes					
	G(-350)A	C825T	C1429T	Controls	Cases	*P*
1	A	C	C	0.016	0.03	0.01
2	A	T	C	0	0.003	
3	A	T	T	0	0.003	
4	G	C	C	0.50	0.50	0.80
5	G	C	T	0.009	0.007	0.78
6	G	T	C	0.28	0.25	0.15
7	G	T	T	0.195	0.207	0.37

allele frequency of C825T or C1429T showed a significant association with hypertension status. The genotype frequency distributions of C825T and C1429T in cases were almost identical to those in controls, as were the allelic frequencies of these two polymorphisms. When compared to the allelic frequency data reported by Rosskopf *et al*. (2002), our allelic frequencies of C825T and C1429T were similar to theirs, whereas the frequency of the A allele of G(-350)A we observed was slightly lower. In ANOVA no statistically significant relationhip was found for each of these three loci, neither in cases nor in controls. (In analysis of G(-350)A with BP levels we combined the AA samples and AG samples into one group, due to the small numbers in each group. Data not shown).

Haplotypes Analysis

Altogether 7 haplotypes involving the G(-350)A, C825T and C1429T loci were observed in all subjects (Table 3). The D′ value of C825T and C1429T was 0.92, which indicated strong linkage disequilibrium between them. We also computed the D′ value of G(-350)A and C825T, which was close to −1. Comparison of overall frequency difference between hypertensive cases and controls across each haplotype revealed that haplotype A-C-C (in the order G(-350)A, C825T and C1429T) was significantly more frequent in cases than in controls (0.03 vs. 0.016, P = 0.01). To evaluate the contributions of the estimated haplotypes to the hypertension status, a logistic regression analysis including all estimated haplotypes was performed. Due to the independent effect of the two other polymorphisms we also performed a logistic regression analysis taking into account G(-350)A alone. The results were very similar and haplotype A-C-C, G(-350)A, BMI and Glu were all found to be significant predictors of hypertension status after considering the effects of other risk factors, including age, gender, BMI, smoking, drinking and blood lipid levels. Moreover, the A-C-C (or (-350)A) carriers had a greater than 2-fold higher risk for hypertension than non-carriers. (Table 4).

Discussion

In the present study we examined the relationship between the *GNB3* G(-350)A, C825T and C1429T polymorphisms and hypertension status in the northern Chinese Han population. We observed that the *GNB3* G(-350)A polymorphism was significantly associated with hypertension status, and that A-allele carriers had higher risk of hypertension than non-A allele carriers. This observation is consistent with the logistic regression analysis. On the other hand, the C825T and C1429T polymorphisms showed no significant association with hypertension status. To our knowledge, this is the first study of *GNB3* G(-350)A, C825T and C1429T polymorphisms in relation to hypertension in the northern Chinese Han population.

The (-350)A allele frequency of G(-350)A varies markedly in different populations. In black Africans the frequency of the A allele was 24%, and in a German

Table 4 Logistic regression parameters including haplotypes and G(-350)A alone

	OR(95% CI)		*P*	
Variables	Haplotypes	Individual	Haplotypes	Individual
BMI	1.14(1.10–1.17)	1.14(1.10–1.18)	<0.001	<0.0001
Glu	1.12(1.03–1.21)	1.12(1.03–1.22)	0.006	0.0108
A-C-C or A(-350)G	2.2(1.04–;4.63)	2.03(1.08–3.79)	0.039	0.027

BMI, body mass index; Glu, glucose; A-C-C, in the order of G(-350)A, C825T and C1429T;Haplotypes, analysis including all estimated haplotypes; Individual, analysis including G(-350)A alone.

population, the frequency of the A allele amounted to 39% (Rosskopf *et al.* 2000). The prevalence we found for this allele in controls was lower than that previously reported by Rosskopf and colleagues (2002) in a Chinese population (1.6% vs. 2.9%). This inconsistency may be due to the difference in geographical areas from which samples were selected, and differences in the composition of the samples between their study and ours. Rosskopf and colleagues (2002) investigated 368 healthy individuals aged from 19 to 30 from Jinan and Wuhan in China. In contrast, our control samples consisted of 503 healthy individuals aged 35 to 69 from northern China (Jilin province, Beijing city and Shandong province). The G(-350)A polymorphism is located in the promoter region of the *GNB3* gene. To assess whether the G/A nucleotide substitution affects *GNB3* promoter activity, Rosskopf *et al.* (2000) made constructs of a reporter gene preceded by two kinds of promoters containing either G or A in the –350 position, and then expressed them in COS-7 cells, but no significant difference in reporter gene activity was observed between the two allelic constructs. Further studies of pathogenesis mechanisms may help to elucidate the effects of the (-350)A allele on expression and functional changes of *GNB3*.

In this study we failed to show a significant association between the C825T polymorphism and hypertension status. The frequency of the 825T allele in the present samples was, remarkably, much higher (47%) than that reported in whites (approximate 30%) (Siffert *et al.* 1998), and similar to that reported in the Japanese population (approximate 49%) (Kato *et al.* 1998). Compared with Huang *et al.* (2003), the frequency of the 825T allele in this study was slightly lower (47% vs. 52%), possibly explained by the different geographical areas from which samples were selected. In Huang's study (2003), samples were selected from Anyang city in Henan province and Qingdao city in Shandong province, whereas our samples came from Beijing city, and Jilin and Shandong provinces. Based on these two independent association studies (Huang *et al.* (2003), and ours), it is more reasonable to presume it unlikely that the 825T allele plays a significant role in the development of hypertension in the northern Chinese Han population, despite the observed functional significance of this polymorphism in certain other populations.

Haplo.stats provided several different global tests of association of haplotypes with a wide variety of traits, as well as haplotype-specific tests, which gave a meaningful advantage in attempts to understand the roles of many different haplotypes (Schaid *et al.* 2002). The frequency of the haplotype A-C-C was significant higher in cases than in controls ($P = 0.015$; after adjustment for BMI, Glu, Tg, Cr, Cho and HDL_C, $P = 0.032$). The two haplotypes A-T-C and A-T-T were absent in controls due to complete linkage disequilibrium between G(-350)A and C825T (D' value was approximately -1). It may seem surprising that there is no effect whatsoever from polymorphisms C825T and C1429T, given their strong LD with G(-350)A. From Table 3 we can see that 825C always occurs with (-350)A, which results in very strong LD between these two loci. Although (-350)A was shown to be associated with hypertension, only a small proportion of samples carried this allele due to the relatively low frequency of the (-350)A allele. Hence, it is reasonable for there to be no effect from both C825T and C1429T, despite the effect of G(-350)A and the strong LD between them. Although haplotypes composed of adjacent SNPs are considered to have a higher information content than single SNPs, and thus have more power to explore the association between candidate genes and complex diseases (Akey *et al.* 2001; Daly *et al.* 2001; Johnson *et al.* 2001; Rioux *et al.* 2001), in our case the results of either including haplotypes or including G(-350)A alone were very similar. So in some cases a logistic analysis using a single marker might reveal as much information as analysis using haplotypes.

So far a total of twelve polymorphic variants, including an insertion/deletion polymorphism, have been detected in Caucasian, black African and Asian populations. These variants are located in the promoter region, exons and introns and cover the whole gene sequence. In Caucasian populations two typical haplotypes called the "*GNB3* T-haplotype" and the "*GNB3* C-haplotype", which contain either 825T or 825C were found. Based on those findings, the hypothesis that additional polymorphisms linked to the 825T allele might cooperatively affect the pre-mRNA structure has been proposed and was partially supported by simulation results (Rosskopf *et al.* 2002). Due to the relative low frequency of the (-350)A allele in our samples, larger samples with more markers would be

advantageous to try to pinpoint any causal site(s) in *GNB3* gene.

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