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Gene environment interaction of GALNT2 and APOE gene with hypertension in the Chinese Han Population[1]

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Abstract. In some GWAs studies, GALNT2 and APOE polymorphisms have been identified to be related to alterations of plasma or serum HDL-C and TG concentrations. The purpose of our study is to assess the contribution of GALNT2 rs4846914, APOE rs429358, rs7412, rs405509 variants, and several environmental factors to the development of hypertension disease in the China Han population. A hospital-based case-control study was conducted. Cases were hypertension (n=211) and controls were normal participants (n=434). The AA, AG, and GG genotype frequencies of GALNT2 rs4846914 were 22.8%, 43.1%, and 34.1% in hypertension subjects, and 35.3%, 44.2%, and 20.5% in controls (P<0.05), respectively. The OR of the AG genotype adjusted for all risk factors compared to the AA genotype was 1.61 (95%CI: 1.02 to 2.56) and to the GG genotype 2.67 (95%CI: 1.59 to 4.488). There was no significant difference between the APOE rs429358, rs7412, and rs405509 genotype frequencies in hypertension and control subjects. The present work indicates that SNP rs4846914 in GALNT2 gene is related to an increased risk of hypertension in China Han population, but the APOE gene is not.

Keywords: Gene polymorphisms, GALNT2, APOE, hypertension, China

1. Introduction

Hypertension is one of serious risk elements and the main causes of cardiovascular disease (CVD) worldwide, and it is a rising threat to Chinese public health. Reports indicated that the quantities of hypertension patients in China have grown from less than 100 million in 1991 to 260 million in 2012 [1]. Three previous surveys conducted in 1959, 1979 and 1991 and a report on CVD (2007) show that

[1]The first two authors contributed equally to this study. Moreover, there is no competing interest in this paper.

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the prevalence of hypertension grew dramatically from 5.11% in 1959 to 17.65% in 2002 [2]. More epidemiologic evidence exists to support the fact that the prevalence of hypertension is increasing [3].

The single nucleotide polymorphisms (SNP) rs4846914 is an intronic variant of the UDP-N-acetyl-alpha-D-galactosamine polypeptide N-acetylgalactosaminyltransferase 2 (GALNT2) gene. The minor G allele of SNP rs4846914 is related to higher triglyceride enrichment [4]. GALNT2 gene is mapped locus in 150 kb of the lead SNP on chromosome 1q42 region, and the lead SNP lies in an intron of the gene [5]. Some previous GWAs studies have indicated that GALNT2 polymorphisms are related to alterations of plasma or serum HDL-C [6-8] and TG [9, 10] concentrations [11, 12].

Apolipoprotein E (ApoE), along with ApoA, ApoB, ApoC, ApoD, ApoM, ApoH, ApoJ, and ApoL are members of the apolipoprotein gene family [13]. The APOE gene is composed of 4 exons and 3 introns, spans 3597 nucleotides, and encodes a 299-amino acid polypeptide. This gene lies on chromosome 19q13.2 region and is tightly connected to the ApoC-I/C-II gene complex [14]. ApoE is mainly produced in the liver, but other tissues or organs such as the brain, kidneys, adrenals, spleen, macrophages, and gonads also produce this protein [15]. Accumulating evidence reveals that the APOE gene polymorphism is genetically related to many diseases.

The main purpose of our research is to explore the contribution of GALNT2 rs4846914, APOE rs429358, rs7412, rs405509 variants, and several environmental factors to the development of hypertension disease in China Han population.

2. Materials and methods

2.1. Study populations

This research was performed in China and coordinated by the affiliated hospital of Capital Medical University, Beijing. It was authorized by the Ethics Committee of Capital Medical University, and informed consent acquired from the controls and patients. A patient was diagnosed with hypertension if they had a blood pressure $\geq$140/90 mm Hg, if they were on antihypertensive therapy, or if they had a documented history of hypertension. At least two age- (±5 years) control (without a history of hypertension) was matched for each case.

2.2. Epidemiological survey

A standardized manner was applied to manage the organized questionnaires and physical examinations by trained personnel. Normative questionnaires were applied to gather the information about demographics, socialeconomic status, lifestyle, personal and family history of disease and risk factors, psychosocial factors, stress, physical activities, history of smoking and alcohol, blood physiological and biochemical parameters.

2.3. Genetic variation

SNPs and Haplotype were explored in two regions (1q42.13 near GALNT2 and 19q13.2 near APOE). Among variants, SAS/genetics was applied to choose maximally informative sets of SNPs and elaborate genetic variations by linkage disequilibrium and D' of 0.90.

2.4. Blood collection and genotyping

Non-fasting blood samples (20 ml) were collected and centrifuged within 2 h of admission and frozen quickly after centrifugation. All the blood samples were transferred in nitrogen vapor tanks from the sites to a blood storage site and stored at -70℃ in freezers or -170℃ in liquid nitrogen. All participants had DNA samples available. The GeneAmp 5700 Sequence Detector (Applied Biosystem) was used to genotype participants' SNPs by an allele-specific real-time polymerase chain reaction.

2.5. Statistical analysis

Pearson chi-squared analysis also assessed the Hardy-Weinberg equilibrium by the ALLELE procedure in SAS 9.4 version/Genetics. Univariate and multivariable logistic regression models were applied to evaluate genetic associations with hypertension disease for each genetic variant.

3. Results

The general and biochemical characteristics between the hypertension (*N*=211) and normal populations (*N*=434) were indicated in Table 1.

The genotypic frequencies of both SNPs all followed in Hardy-Weinberg equilibrium. The AA, AG, and GG genotype frequencies of GALNT2 rs4846914 were 22.8%, 43.1%, and 34.1% in hypertension subjects, and 35.3%, 44.2% and 20.5% in controls ($P<0.05$), respectively (Table 2). The A and G allele frequencies of SNP rs4846914 near GALNT2 were 44.3% and 55.7% in hypertension cases and 57.4% and 42.6% in controls ($P<0.05$), respectively. After being adjusted, the occurrence of OR of AG genotype compared with AA genotype was 1.61 (95% CI: 1.02 to 2.56) and 2.67 (95% CI: 1.59 to 4.488) for the GG genotype. SNP rs429358, rs7412, and rs405509 genotype frequencies near APOE between hypertension and control subjects were no significant statistical difference (Table 2).

Table 1
Characteristics of participants

Characteristics	Hypertension cases, *N*=211	controls, *N*=434	χ^2 / *t*	*P*-value
Male sex (%)	134 (63.5)	316 (72.8)	5.83	0.0158
Mean Age (SD), year	64.9 (10.2)	60.0 (10.5)	5.53	0.0001
BMI, mean (SD), kg/cm^2	25.2 (2.8)	24.0 (2.9)	5.49	0.0001
WHR, mean (SD), cm/cm	0.90 (0.09)	0.86 (0.08)	4.70	0.0001
Systolic blood pressure (mmHg)	142.3 (17.9)	123.1 (12.7)	13.34	0.0001
Diastolic blood pressure (mmHg)	86.1 (10.7)	77.5 (7.5)	10.06	0.0001
Diabetes (%)	23 (10.9)	5 (1.2)	32.49	0.0001
Stroke (%)	25 (11.9)	8 (1.8)	29.28	0.0001
Smoking, n (%)	78 (37.0)	192 (39.6)	0.42	0.5146
Alcohol, n (%)	23 (10.9)	63 (14.5)	1.61	0.2050
General stress, n (%)			8.15	0.0170
Permanent	77 (36.5)	114 (26.3)		
Several periods	88 (41.7)	226 (52.1)		
Never experienced	46 (21.8)	94 (21.7)		
ApoA1/ApoB	1.60 (0.45)	1.80 (0.59)	-4.66	0.0001

Note: N, number; *p*-value, significance of difference between group means or frequencies determined by *t*-test or chi-square test.

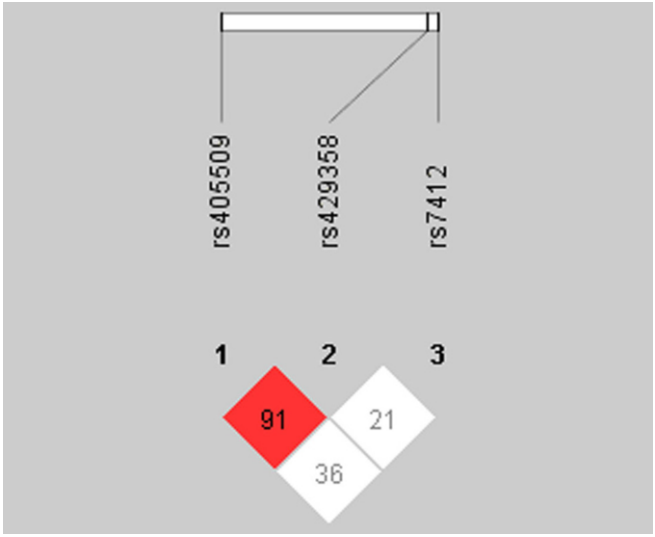

Fig. 1. Pair-wise LD among three SNPs in 19q13.2 in the control group. The numbers are D' ×100

Table 2
Four SNPs genotype and their frequencies

SNP	Case count	(%)	Control count	(%)	Chr	Pos	Nearby gene	Crude OR(CI)*	Adjusted OR(CI)**
rs4846914					1q42.13	228362314	GALNT2		
AA	48	22.8	153	35.3				1.00	1.00
AG	91	43.1	192	44.2				1.51 (1.00-2.27)	1.61 (1.02-2.56)
GG	72	34.1	89	20.5				2.58 (1.65-4.04)	2.67 (1.59-4.48)
rs429358					19q13.2	42216263	APOE		
AA	177	84.3	352	81.1				1.00	1.00
AG	33	15.7	77	17.7				0.85 (0.55-1.33)	0.82 (0.50-1.34)
GG	0	0	5	1.2				…	…
rs7412					19q13.2	42216401	APOE		
AA	199	94.3	423	97.5				1.00	1.00
AG	12	5.7	11	2.5				2.32 (1.01-5.35)	1.37 (0.52-3.58)
rs405509					19q13.2	50100676	APOE		
CC	15	7.1	30	7.0				1.00	1.00
AC	74	35.1	161	37.4				0.92 (0.47-1.81)	0.88 (0.41-1.89)
AA	122	57.8	239	55.6				1.02 (0.53-1.97)	0.84 (0.40-1.78)

Note: Chr: Chromosome; Pos: position; * Crude Odds ratio (OR) of being influenced by a SNP for an expression trait associated with a given risk factor; ** Adjusted for sex, age, BMI, smoking, drink, Diabetes, Stroke history, stress, Apoa1, and Apob.

Figure 1 indicated the results of haplotype analysis for the examined SNPs. Next, we used haploview and SAS software to calculate possible haplotype frequency of 3 loci in 19p13.2. Using the genotypes of 434 controls, we defined the haploblock structure of SNPs within the region of 19p13.2 in the Chinese population. By defining a solid spine of LD as D'>0.90, we found one haploblock in the 19p13.2 region (Figure 1). Next, we focused on the association of haplotypes within one block. Our results indicate that there is no significant association between haploblock on chromosome 19p13.2 and hypertension (P=0.3430).

Table 3 indicates the stratification analysis result of rs4846914 and its association with hypertension. In models adjusted for all the risk factors, OR (rs4846914 GG genotype compared to AA) associated

with BMI was 1.16 (95% CI: 0.51 to 2.63) in the group where BMI<24, and 4.15 (95% CI: 2.08 to 8.27) in the group where BMI⩾24 (*p* value for heterogeneity, 0.1096).

Table 3
Stratification analysis for association between rs4846914 genotypes and hypertension

	rs4846914 OR (95% CI)			
	AA	AG	GG	*P*-value
BMI				0.1069
＜24	1.00	1.29 (0.62-2.66)	1.16 (0.51-2.63)	
≥24	1.00	1.83 (1.01-3.31)	4.15 (2.08-8.27)	
Smoking				0.5492
No	1.00	1.52 (0.85-2.70)	2.55 (1.35-4.83)	
Yes	1.00	2.01 (0.88-4.57)	3.43 (1.35-8.70)	
General stress				0.0132
Several or never	1.00	1.59 (0.94-2.70)	2.06 (1.07-3.95)	
Permanent	1.00	2.27 (0.77-6.65)	4.99 (1.69-14.75)	

4. Discussion

Hypertension disease is one of most serious risk factor for CVD, stroke, and end-stage renal disease.

Hypertension is a global problem; thus, prevention of hypertension remains a global public health goal. The potential association between the GALNT2 polymorphisms and plasma or serum lipid levels in humans has been proved by several previous GWAs studies. These researches have confirmed that the minor allele of GALNT2 polymorphisms was related to low HDL-C and high TG blood levels [11-14].

In the current study, we found a significantly higher promoter methylation of GALNT2 in the hypertension group than in the non-hypertension group. The hypertension participants also had higher GG genotype frequency of rs4846914 then the normal individuals. Statistically significant evidence of the whole sample indicated that the GALNT2 gene promoter hypermethylation increases the risk of hypertension (OR=1.51; 95% CI, 1.00-2.27; $P<0.05$ and OR= 2.58; 95% CI, 1.65-4.04; $P<0.001$). After other risk facts were adjusted for, similar results were obtained (OR=1.61; 95% CI, 1.02-2.56; $P<0.05$ and OR=2.67; 95% CI, 1.59-4.48; $P<0.001$). However, other genetic and environmental factors might affect levels of relationship. The hypertension participants with the GG genotype of rs4846914 had higher ApoB levels and lower ApoA1 levels than the normal participants; the ratio of ApoA1 to ApoB was also lower. A previous candidate gene study can not find any effect of the GALNT2 rs4846914 variant on serum TC and TG levels, and any of these findings were not authenticated by a meta-analysis of the six studies. This may be due to the modest effects on lipid concentrations of these variants and lower power of statistics for testing the relationship [16]. However, levels of LDL-C were related to the genotypes of rs4846914 in the Chinese Han population. Still, several GWAs and candidate gene researches have failed to find a significant relationship between GALNT2 polymorphisms and hypertension.

It is well established that environmental factors such as obesity, physical activity, stress, lifestyle, and dietary patterns are all strongly correlated with hypertension. This study indicated that rs4846914 was related to age, sex, cigarette smoking, BMI, and general stress in two groups. These data suggest that environmental factors also play an important role in determining hypertension risk in the China Han population. In lower BMI groups, the association between rs4846914 and hypertension was not

statistically significant. However, in the higher BMI group, the OR was higher, although the heterogeneity analysis was not significant. In the permanently stressed group, the influence of rs4846914 on hypertension was higher than occasionally or never stressed group, P=0.0132.

Accumulating evidence reveals that the polymorphism of the APOE gene is genetically associated with many diseases, including hypertension, coronary artery disease, polycystic ovary syndrome, Alzheimer's disease, psoriasis, vascular dementia, gallbladder stone disease, and cerebrovascular disorders. We studied the correlation between the APOE gene and susceptibility to hypertension in China Han population by determining the polymorphism of 3 SNPs, rs429358, rs7412, and rs405509. The results demonstrated that there was no significant statistical difference in the genotype and allele distributions of SNPs rs429358 and rs405509 between the hypertension patients and non-hypertension controls. However, the distributions of the genotypes and alleles of the SNP rs7412 were significantly different between the hypertension patients and normal subjects (OR=2.32, P<0.05). But when we adjusted for other risk factors, we found no convincing association between the SNP rs7412 and hypertension risk. Some previous studies have suggested that polymorphism at +2836 G>A of the APOE gene is strongly related to the susceptibility to hypertension; the A allele of the APOE gene may be a risk factor for hypertension in Chinese Hui individuals while the G allele may serve as a protective factor for hypertension. This genetic variation at the +2836 G>A site of the APOE gene was first described by Matsunaga et al. [17] who demonstrated that the G>A mutation changed glutamic acid into a lysine residue. Additionally, polymorphisms of the APOE gene have also been correlated with plasma lipid levels and susceptibility to hypertension in a variety of ethnic groups, although there have been inconsistent results [18].

Some limitations of our research should be taken into consideration. First, a potential disadvantage was that the control participants may not represent the general China Han population, nor were the hypertension subjects representative of all China hypertension patients. Another was that we did not collect enough samples to prove the association between gene SNPs and hypertension. Additionally, our study lacked a strict control matching process and there was selection bias.

5. Conclusion

Our present work provides evidence to support the relationship of gene SNPs with the risk profile of hypertension. Our data indicates that SNP rs4846914 in GALNT2 gene is related to an increased risk of hypertension in China population, but the APOE gene is not. ApoA1, BMI, and general stress are also likely to be important factors influencing hypertension.

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