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Confirmation of top polymorphisms in hypertension genome wide association study among Han Chinese

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ABSTRACT

Background: Confirmation of genome wide association (GWA) results in independent samples has recently become new research tendency.
Methods: We focused on 8 positive top polymorphisms identified in the to-date largest hypertension GWA study and determined whether these polymorphisms were associated with hypertension among Han Chinese. Genotyping was performed among 548 patients diagnosed with essential hypertension and 560 age- and gender-matched controls using ligase detection reactions method. Statistical analyses were conducted using Logistic regression and genotype risk score.
Results: Except for a rare polymorphism (rs653178), no deviation from Hardy–Weinberg equilibrium was observed for genotype distributions of others. There was significant differences in the genotype/allele distribution ($P = 0.006$/$P = 0.002$) of rs16998073 in FGF5 (fibroblast growth factor 5) upstream and the allele distribution ($P = 0.037$) of rs16948048 in ZNF652 (zinc finger protein 652) upstream between hypertensive patients and controls. Strong significance was also noted under assumption of different genetic models for the two coalescent polymorphisms, even after controlling covariates of interest. For example, rs16998073 had a 72% increased risk for hypertension under the co-dominant model (95% confidence interval: 1.20–2.45; $P = 0.003$). However, construction of genetic risk scores on common polymorphisms did not reveal any significance with both hypertension and blood pressure, suggesting that contribution of these polymorphisms to hypertension moderate or small in magnitude.
Conclusions: Our results implicate variation in FGF5 and ZNF652 gene upstream regions with altered susceptibility to hypertension in Han Chinese.

1. Introduction

The last few years have witnessed great success of genome wide association (GWA) studies in identifying dozens of common genetic variants associated with hypertension status and blood pressure variation [1–4]. Even so, there is no universal consensus on a certain or several variants being consistently related to the underlying genetic mechanism of hypertension across all GWA studies. In fact, lack of enough power to detect associations with alleles with a relatively small effect tingled most of these studies [5,6]. Thus far, the most largest GWA study is the GlobalBPgen (Global Blood Pressure Genetics) consortium study on people of European ancestry ($n = 34433$) by Newton-Cheh et al. who have identified 8 top single nucleotide polymorphisms (SNPs), which were significantly associated with both continuous blood pressure and dichotomous hypertension at the GWA significance level, leading to the speculation that these SNPs can serve as novel targets for interventions to prevent cardiovascular disease [3]. It is thus of added interest to know whether these top signals can determine genetic susceptibility to hypertension in Chinese individuals. Moreover, given the heterogeneity among different races and ethnic groups, confirmation of GWA results in independent samples attempting to cross-racially identify people who are susceptible/resistant to hypertension has recently become one kind of new research tendency [7,8].

As recommended by Chanock et al., to provide a strong causal association, it is necessary for an effect of multiple risk loci being consistently examined in various populations [9]. Therefore, based on the GWA results of the coalescent study by Newton-Cheh et al. [3], we determined to test whether those robust association signals including 8 SNPs reported in European populations could be confirmed in Chinese by conducting an association study involving a total of 1108

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unrelated Han Chinese from Shanghai. The SNPs under study corresponded to rs17367504 [A/G], rs11191548 [T/C], rs12946454 [A/T], rs16998073 [A/T], rs1530440 [C/T], rs653178 [A/G], rs1378942 [G/T], and rs16948048 [A/G].

2. Methods

2.1. Study population

This was a hospital-based study totaling 1108 unrelated local residents of Han descent, who were recruited from the cooperative community hospitals in Shanghai city. The characteristics of this study population have been described elsewhere [10,11]. There were 548 patients diagnosed with essential hypertension, who were matched according to age and gender with 560 controls from the same geographic districts. Ethical approval has been obtained from the ethics committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine. Written informed consent for genetic studies and biochemical analyses was obtained from all participants.

2.2. Diagnostic criteria

Three times of sitting blood pressure were measured with at least 10-min interval using a calibrated mercury sphygmomanometer and were recorded by certified examiners. Three consecutive readings were averaged for analysis. Essential hypertension was defined as the systolic blood pressure (SBP) ≥ 140 mm Hg or diastolic blood pressure (DBP) ≥ 90 mm Hg or receipt of antihypertensive medication. Patients with a clinical history of secondary hypertension were excluded from the study through extensive clinical and biochemical evaluations. In contrast, systolic/diastolic blood pressure for all controls were <140/90 mm Hg and they were free of diabetes and renal disease.

2.3. Demographic and clinical measurement

Body weight and height, as well as waist and hip were recorded with subjects wearing light indoor clothing and no shoes. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist-to-hip ratio (WHR) was calculated as waist in centimeters divided by hip in centimeters. Plasma triglyceride (TG), and total, high and low density lipoprotein cholesterol (TC, HDLC and LDLC), as well as blood urea nitrogen (BUN), creatinine (Cr), urea acid (UA) were determined enzymatically using available assays at the Central Laboratory of Ruijin Hospital (Shanghai, China).

2.4. Genotyping

Genomic DNA was extracted from white blood cells using a standard phenol-chloroform method and genotyping was performed using the PCR-LDR (ligase detection reactions) method [12]. The primers for amplification and lengths of PCR products are provided in supplementary Table 1 and the PCR reactions were conducted in an ABI 9600 (Applied Biosystems, Foster City CA) in a total volume of 15 μl including 10 ng genomic DNA, 1.5 μl 10 × PCR Buffer, 0.3 mmol/l dNTPs, 2 μmol/l each primer and 1 U Taq DNA polymerase (TaKaRa, Japan). Cycling parameters were as follows: 94 °C for 2 min; 35 cycles of 94 °C for 15 s; 60 °C for 15 s; 72 °C for 30 s; and a final extension step at 72 °C for 5 min.

The probes for LDR were presented in supplementary Table 2. Two specific probes that discriminated specific bases and one common probe were synthesized for each polymorphism. The common probe was labeled at the 3′ end with 6-carboxy-fluorescein (FAM) and phosphorylated at the 5′ end. The multiplex ligation reaction for each PCR product was carried out with a reaction volume of 10 μl containing 2 μl of PCR product, 1 μl 10 × Taq DNA ligase buffer, 1 μmol/l of each discriminating probe, 5 U Taq DNA ligase (New England Biolabs, Ipswich MA). The LDR parameters were as follows: 94 °C for 2 min, 20 cycles of 94 °C for 30 s and 60 °C for 3 min. After the LDR reaction, 1 μl LDR reaction product was mixed with 1 μl ROX passive reference and 1 μl loading buffer. The mixture was then denatured at 95 °C for 3 min, chilled rapidly in ice water. The fluorescent products of LDR were differentiated using ABI sequencer 377 (Applied Biosystems).

2.5. Statistical analysis

Database management and simple statistical analyses were conducted using the SPSS software ver 16.0. Means of continuous variables expressed as mean ± standard deviation (SD) were compared by the Student's *t*-test. The χ^2 test was used to assess the goodness-of-fit between the observed allele frequencies and the expected counterparts by Hardy–Weinberg equilibrium and to evaluate the differences in genotype/allele distributions between the 2 groups. Study power was calculated using PS (Power and Sample Size Calculations) software ver 3.0.

Each genotype was assessed by multivariable logistic regression analyses assuming additive (minor homozygotes vs heterozygotes vs major homozygotes), co-dominant (heterozygotes vs major homozygotes; minor homozygotes vs major homozygotes), dominant (minor homozygotes plus heterozygotes vs major homozygotes) and recessive (minor homozygotes vs heterozygotes plus major homozygotes) modes of inheritance, respectively after adjustment for age, age^2, gender and BMI.

As recommended by Tobin et al, for individuals in antihypertensive medication, blood pressure was imputed by adding 15 mm Hg and 10 mm Hg for SBP and DBP, respectively [13]; this imputation was consistent with the study by Newton-Cheh et al. [3]. Unless otherwise indicated, the adjusted SBP and DBP were used for analysis.

Under the assumption that an individual SNP with a modest effect on blood pressure variation would have limited power to show an effect on incident hypertension events, a genotype risk score was created on the basis of the number of mutant alleles that were carried by each subject for each of all SNPs. Blood pressure variation was calculated according to strata of genotype risk scores. Two-tailed $P<0.05$ was accepted as statistical significance.

3. Results

3.1. Baseline characteristics of the study population

As shown in Table 1, patients with essential hypertension and controls showed a similar age (P = NS) and gender (P = NS) distribution.

Table 1
Baseline characteristics of study population.

Characteristics	Cases (n = 548)	Controls (n = 560)	P[a]
Male (%)	52.01	49.82	NS
Age (y)	52.32 ± 4.41	51.93 ± 4.70	NS
Onset age (y)	41.74 ± 13.47	—[b]	—
BMI (kg/m^2)	25.53 ± 3.09	23.55 ± 3.24	<0.001
WHR (cm/cm)	0.85 ± 0.06	0.81 ± 0.06	<0.001
AdjSBP (mmHg)	157.48 ± 15.10	115.63 ± 10.22	<0.001
AdjDBP (mmHg)	104.53 ± 9.04	76.95 ± 6.04	<0.001
Triglyceride (mmol/l)	1.69 ± 1.34	1.10 ± 0.74	<0.001
TC (mmol/l)	5.57 ± 0.88	5.16 ± 0.82	<0.001
HDLC (mmol/l)	1.39 ± 0.27	1.49 ± 0.29	<0.001
LDLC (mmol/l)	3.85 ± 0.86	3.47 ± 0.75	<0.001
BUN (mmol/l)	5.08 ± 1.58	4.69 ± 1.00	<0.001
Creatinine (μmol/l)	81.21 ± 25.08	76.36 ± 15.81	<0.001
UA (μmol/l)	319.87 ± 90.20	262.69 ± 68.82	<0.001

Abbreviations: BMI, body mass index; WHR, waist to hip ratio; adjSBP, adjusted systolic blood pressure; adjDBP, adjusted diastolic blood pressure; TC, total cholesterol; HDLD, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; UA, urea acid.

[a] P values were calculated using the unpaired *t*-test for continuous variables and the χ^2 test for categorical variables.

[b] Data not available.