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The combined effects of cardiovascular disease related SNPs on ischemic stroke

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ABSTRACT

Purpose: Previous studies have revealed multiple common variants associated with known risk factors for cardiovascular disease (CVD). Ischemic stroke (IS) and CVD share several risk factors with each having substantial heritability. We aimed to generate a multi-locus genetic risk score (GRS) for IS based on CVD related SNPs to evaluate their combined effects on IS.

Methods: A total of 851 patients and 977 controls were selected from Beijing, Tianjin, Shandong, Shanxi, Shaanxi and Heilongjiang communities. The candidate genes were genotyped by PCR-hybridization. Information about demographic factors, history of disease (such as hypertension), and lifestyle was obtained using structured questionnaires. A GRS model weighted by the absolute value of regression coefficient β was established to comprehensively assess the association between candidate SNPs and IS. Using the area under the receiver operating characteristic curve (AUC) to evaluate the value of GRS on predicting IS.

Results: The GRS of cases was 2.87 ± 0.28, which was significantly higher than controls' GRS (2.78 ± 0.30) ($P < 0.000$). With the increase of the GRS, the risk of IS became higher ($P_{trend} < 0.000$). Subjects in the top quartile of the GRS had about 1.9-fold increased risk of IS compared with subjects in the lowest quartile ($OR_{adjusted} = 1.880$, 95%CI = 1.442–2.452, $P < 0.000$). The AUC = 0.580, $P < 0.000$.

Conclusion: 13 CVD related SNPs had combined effects on IS. The GRS of cases was significantly higher than controls' GRS. As the GRS increased, the risk of IS increased. The GRS model has some value for the prediction of IS.

1. Introduction

The results of the Global Disease Burden (GBD) study in 2010 show that since 1990, stroke has been the second leading cause of global death, with > 85% of deaths from stroke occurring in developing countries [1,2]. In the largest developing country—China, stroke is the first cause of death and the first cause of disability [3]. In all stroke, ischemic stroke (IS) accounts for 80%. The mechanism of IS is complicated, including the environmental factors, genetic factors, the interaction of environmental factors and genetic factors [4]. In the past, a series of factors, including hypertension, diabetes mellitus, dyslipidemia, cardiac causes, smoking, alcohol consumption, waist-to-hip ratio, psychosocial factors, diet and regular physical activity were identified. These risk factors accounted for > 90% population attributable risks (PERs) for IS [5]. Behind these factors, genetics played an important role as the hand of God.

IS and cardiovascular disease (CVD) share several risk factors such as hypertension, diabetes mellitus, dyslipidemia, each has a substantial heritability. Both IS and CVD are risk factors for one another [6] and in combination they are used to assess the risk or as a therapeutic target in clinical practices. Stroke and CVD share some risk factors and many aspects of their underlying pathophysiology. This mutual pathophysiology is appropriate for IS and especially to the subtype of atherosclerotic stroke [7]. Twin and family studies have demonstrated the

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high heredity of IS and CVD [8,9], with some evidence of a shared heritability for both diseases [10].

Being a complex trait, IS presumably arises from interplay of multiple genes located throughout the genome with environmental risk factors. For a single SNP, it may have a weak association with stroke. So it would be appropriate to search for a set of marker loci in different genes and to analyze these markers jointly rather than testing each marker separately. To date, case–control association studies mostly follow a candidate-gene approach by examining one SNP at a time. Although powerful, this approach ignores the genetic complexity of stroke, which is caused by variations in several genes, each with a weak effect on overall disease risk. In this study, we used the genetic risk score (GRS) [11] to evaluate the combined effects of a set of CVD related SNP markers at various positions in the genome on IS in Chinese people.

2. Materials and methods

2.1. Study participants

Subjects were recruited from The Stroke Hypertension Investigation in Genetics (SHINING) study, a case-control study carried out by the Beijing Hypertension League Institute between 1997 and 2000 [12]. All participants were Han ethnicity, enrolled from 6 geographical regions (Beijing, Tianjin, Shandong, Shaanxi, Shanxi, Heilongjiang) within China. Patients were diagnosed as stroke by brain computed tomography (CT)/Magnetic Resonance Imaging (MRI). People who had no prior history of stroke and cardiovascular diseases from the same community were selected as controls. Controls were matched with cases for geographic locations, sex, age within 3 years, and blood pressure categories ($< 140/90$, $\geq 140/90$ and $< 180/105$, $\geq 180/105$ mmHg) [12]. Stroke patients with the history of valvular heart diseases and myocardial infarction were excluded from this study. Controls with previous history of CVD or stroke were also excluded from this study.

There were 1076 IS patients with DNA available. 851 IS patients and 977 controls were genotyped successfully in all candidate SNPs and they were used for further data analyses. Information about demographic factors, history of disease (such as hypertension), and lifestyle factors were obtained using structured questionnaires. Hypertension was defined as systolic blood pressure $>$ 140 mmHg, and/or diastolic blood pressure $>$ 90 mmHg, or having current or past antihypertensive medication [12]. The study protocol was approved by the Ethics Committee of the Beijing Hypertension League Institute in accordance with the Declaration of Helsinki and written informed consent was obtained from all study participants.

2.2. Genotyping

59 polymorphisms from 34 CVD related genes were selected based on the reported literatures in the past, which were associated with trails of blood pressure regulation, lipid metabolism and inflammatory factor. By salting out procedure, DNA was extracted from the whole blood. A PCR-based panel (Roche Molecular Biochemicals, Basel, Switzerland) was used for genotyping and the procedure was described previously [13]. Firstly, DNA was amplified by PCR with 59 pairs of biotinylated primers in a single tube. Next, each amplified PCR product was hybridized with sequence-specific oligonucleotide probes immobilized on a nylon membrane strip; finally, biotin-based color was detected by a scanner and genotype was analyzed by proprietary Roche Molecular Systems software [14]. Genotyping calls were observed by two independent researchers to ensure the accuracy of the genotype. Genotyping call rate for assessments of all genetic variants was $\geq$98% in this study. A minor allele frequency (MAF) < 0.05 was excluded from this analysis. After excluded 3 SNPs in linkage disequilibrium with other SNPs, there were 43 SNPs on 30 CVD related genes remained.

2.3. Set-association approach

Previous work has shown that deviations from Hardy-Weinberg equilibrium [15] in affected individuals may be indicative of the presence of susceptibility loci [16,17]. On the other hand, it is allelic association (AA) that measures overrepresentation of genomic variants in cases versus controls. The set-association approach considers both of these effects, AA and HWD, where each may be expressed by a chi-square statistic [18]. Effects of AA and HWD for association are merged by building the product, $s_i = t_i{*}u_i$, where t_i is the AA statistic and u_i is the HWD for association in the i-th SNP. To combine the resulting evidence for association over multiple SNPs and genes, we simply form the sum, $S = \Sigma_i(t_i{*}u_i)$, over a suitable set of SNPs. SNPs were ordered according to their s_i value (i.e. $s(1) \geq s(2) \geq s(3)$ …), irrespective of their genomic location. Sums with increasing numbers of SNPs (i.e. n) were formed, starting with the SNP ranked highest S ($n = 1$) = s(1); S ($n = 2$) = s(1) + s(2), and so on. The primary interest was to find the n of SNPs in S that reflects the association of this set of SNPs with the disease. The significance levels (empirical *P*-value) associated with the n-th S were determined by permutation testing. As the number n of SNP markers in S increases, the pattern of *P*-value changes; following an initial decline to a minimum, it increases again. The smallest empirical significance level was considered our statistic of interest, and its significance level (P_{final}, representing the whole genome significance), was determined again by a permutation test.

2.4. The GRS

Using the set-association approach [18], we tested whether a set of several SNPs might be associated with IS, synergistically. This method selects the “best” set of n SNPs, whose sum statistic (S) is associated with the highest significance. Constructed on the basis of a polygenic model assuming that the genetic effects of the disease are equal to the sum of the effects at each locus, the GRS is used to evaluate the association between IS and a set of n SNPs. The algorithm is divided into a simple GRS and a weighted GRS. We used a better-adjusted weighted GRS [19]. Weighted GRS [20]: GRS = ΣβiSi (βi (absolute value of regression coefficient β) is the weight of the i-th SNPs and Si (0, 1, 2) is the number of risk allele in the i-th SNP). The algorithm considers each SNP to have a different effect on the disease by assigning a corresponding weight to each SNP.

2.5. Statistical analysis

Continuous variables were presented as means ± standard deviation (SD), and they were compared using Student's unpaired *t*-test or the Wilcoxon rank sum test. Categorical variables were presented as numbers (percentages) and were compared using the chi-square test. A logistic regression was used to calculate β. Evaluated the association between GRS and IS by the multivariate logistic regression. Those analyses were applied using SPSS 20.0 and SAS 9.4 statistical software. $P < 0.05$ indicated statistical significance.

3. Results

3.1. Characteristics of study participants

A total of 851 cases with IS and 977 controls were incorporated into our research. There was no significant difference between the two groups in gender ($P > 0.05$). The age, SBP, DBP and BMI of the control group were significantly higher than those of the cases ($P < 0.05$). There was higher proportion of hypertension in the control group compared with the case group (84.34% vs 80.14%, $P < 0.05$). For behavioral risk factors, the proportion of smoking in case group was 39.73%, which was significantly higher than that in control group (31.60%, $P < 0.05$). Although alcohol consumption was higher in case