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A Reduced Interval of Chromosome 9p21 Locus is Associated with Ischemic Stroke in Chinese Northern Han Population

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Abstract: The 9p21 locus, a strong risk locus for coronary arterial disease, has been also associated with other cardiovascular disease including ischemic stroke (IS) in Caucasians. However, the association between 9p21 locus and IS in Chinese Han population is still debatable because of ambiguous results reported previously. Genetic heterogeneity between Southern and Northern Chinese Han populations could be one of the reasons for this uncertainty. Four genetic variants selected from the three conjunctional LD blocks within the 44 kb candidate region on chromosome 9p21 were genotyped in 1,429 IS patients and 1,191 healthy controls from the Northern Chinese Han population. Among the four studied variants, the G allele of the SNP rs2383207 was significantly associated with IS with allele frequency 66.8% in patients and 63.4% in controls. This association appears to be dominant with an OR of 1.417 (p=0.003) for people with either GG or AG genotypes. We did not find any association for the other three SNPs (rs1333049, rs10757274, and rs10116277). Based our results, we conclude that the 9p21 locus is a susceptibility locus for IS in the Northern Chinese Han population; and the core risk region for IS is within an interval of less than 28kb.

Keywords: 9p21 Locus, Ischemic Stroke, Chinese, Genetic Association Study

1. Introduction

Stroke is the second most common cause of adult death worldwide, and accounts for approximately 10% of total deaths worldwide [1-3] Two-thirds of stroke-related deaths occur in developing countries, and approximately 40% of these occur within China [4-6]. Common variants in the region on chromosome 9p21 have recently been associated with increased risk for coronary arterial disease (CAD) by a number of parallel genome-wide association studies (GWAS) in Caucasians [7-10], and this result was further confirmed by several independent case control studies in Caucasians [11]. Replication studies have been successful in Japanese, Korean and Chinese Han populations [12-14, 15] although genetic heterogeneity has been reported in blacks [9-10] [15 16]. From these studies, the interval of core risk region at 9p21 locus has been defined within a 44kb LD block in Caucasians [11]. Based on the hypothesis of shared

pathogenic mechanisms for vascular disease, studies of the 9p21 locus have been quickly extended to a number of other cardiovascular diseases, and significant association has been reported for IS, aortic aneurysms [17], arterial stiffness [18], and aneurysmal subarachnoid haemorrhage [19]. Data from all of these studies indicate a possible atherosclerosis susceptibility of the 9p21 locus.

The Chinese Han population is the largest ethnic group in China which represents approximately 92% of the total population in China. Geographical variation in stroke prevalence has been found between South and North Han Chinese [20-24]. Moreover, genetic heterogeneity has also been reported between Southern and Northern Chinese Han populations [25-28].

The association between certain genetic variants within the 9p21 locus and CAD has been confirmed [14] in the Chinese Han population. However, the association between the 9p21 locus and IS in the Chinese Han population remains debatable because of inconsistent results from the different studies. While, insufficient sample size can be a factor, potential effects of genetic heterogeneity between Southern and Northern Chinese Han populations, as described in previous studies, may also be a factor confounding results [14] [29]. Therefore, continued efforts with increased patient numbers and targeting of specific sub Han populations are necessary to define the association between the 9p21 locus and IS in Chinese Han individuals. In the present study, genotyping of four selected genetic variants from three conjunctional LD blocks within the 44 kb candidate region on chromosome 9p21 were performed in 1,429 ischemic stroke (IS) patients, and 1,191 normal controls from the Northern Han Chinese population.

2. Materials and Methods

2.1. Patients and Controls

1,429 consecutive IS patients were recruited by 18 hospitals within Henan province during the period from February 2006 to March 2007. Clinical diagnoses of IS patients were based on the WHO criteria for IS (1998) plus evidence from MRI or CT exam on ischemic lesions corresponding to the neurological deficits. The 1,191 normal controls were selected from ethnically and geographically matched individuals without history of myocardial infarction or stroke who presented in hospitals for routine health examinations. Ethics approval of the present study was granted by the Human Investigations Committee of Memorial University and Ethics Boards of Zhengzhou University.

2.2. SNP Genotyping

Genomic DNA was isolated from peripheral blood collected from patients and controls using standard methods [30]. A chromosomal region between position rs1333049 and the rs10116277 was targeted using HapMap CHB (Chinese Han Beijing). Four SNPs including rs1333049, rs2383207, rs10757274, and rs10116277 were selected from the 44 kb LD block in the region of 9p21.9 to 9p22.1 (NCBI build 36.2, HapMap database for the CEU population) which have previously been associated with MI and IS in Caucasians. Genotyping was conducted using Taq Man SNP genotyping technology on real-time PCR (ABI Prism® 7000 Sequence Detection System).

2.3. Statistical Analysis

Hardy–Weinberg equilibrium (HWE) was tested for each of the four SNPs using the exact Chi-square test, and none of them was out of HWE. Logistic regression modeling implemented in SPSS v16.0 (SPSS Inc.) was used to test the association between each of the SNPs and the IS. Odds ratios (OR) were calculated as a measure of the relative risk for stroke and were given with 95% CIs. A $P< 0.05$ (two-tailed) was considered statistically significant. Statistical power was calculated using QUANTO V1.2.3 software. Linkage disequilibrium between the two analyzed variants was calculated as D', which ranges from 0 (no linkage disequilibrium) to 1 or –1 (complete linkage disequilibrium). The software QUANTO version 1.2.3 was used for the calculation of statistical power.

3. Results

In the present study, the patients group consist of 60.7% (n=868) males and 39.3% females (n=561) compared with 63.5% males (n=756) and 36.5% females (n=435) in the control group. The mean ± SD age in patient and control groups are: 62.7 ± 11.8 and 58.5 ± 9.2, respectively.

The minor allele frequencies (MAF) of the four tested SNPs ranged from 0.31 to 0.49, according to HapMap CHB (Chinese Han Beijing) data, with a mean MAF of 0.3975 for the four SNPs. Given the disease prevalence at 0.5%, 1429 patients and 1197 controls yielded a statistical power > 0.8 to detect an OR of 1.50 for the MAF of 0.40 for the tested SNPs at a significance level of 0.05 (two-tailed) under recessive, dominant and additive models.

The four selected SNPs were genotyped in 1,429 IS patients and 1,191 controls. The genotype distributions, allelic frequencies of each studied genetic variants in patients and controls are given in Table 1. The genotype frequencies of the four studied genetic variants in the control population were all under the Hardy–Weinberg equilibrium (all $P>0.05$). In the association analysis, only the common allele (G) of SNP rs2383207 was significantly associated with increased risk for IS with an OR=1.162 for G allele (P- value=0.01), and OR=1.417 for AG+GG genotypes (P-value=0.003. This association was observed in both heterozygous AG (OR=1.382, P=0.010) and homozygous GG (OR=1.417, P=0.003) statuses.

Table 1. *Genotype distributions and allelic frequencies of rs10116277, rs10757274, rs2383207, and rs1333049 in IS patients and controls.*

Genotype	Stroke (n=1,429)	NC (n=1,191)	OR (95%CI)	P-value
Rs10116277				
GG	137(9.6%)	137(11.5%)		
GT	606(42.4%)	501(42.1%)	1.210 (0.928-1.576)	0.159
TT	686(48.0%)	553(46.4%)	1.241 (0.955-1.612)	0.106
Rs10757274				
AA	453(31.7%)	354(29.7%)		
AG	676(47.3%)	600(50.4%)	0.880 (0.737-1.051)	0.159
GG	300(21.0%)	237(19.9%)	0.989 (0.794-1.232)	0.923
Rs2383207				
AA	154(10.8%)	174(14.6%)		
AG	642(44.9%)	525(44.1%)	1.382 (1.081-1.766)	0.010
GG	633(44.3%)	492(41.3%)	1.454 (1.136-1.861)	0.003
Rs1333049				
GG	381(26.7%)	334(28.0%)		
GC	733(51.3%)	571(47.9%)	1.125 (0.937-1.352)	0.206
CC	315(22.0%)	286(24.0%)	0.966 (0.777-1.200)	0.752

Data from logistic regression analysis on rs2383207 showed that heterozygous AG (OR: 1.359, 95%CI: 1.057 to 1.745, P= 0.017) and homozygous GG (OR: 1.408, 95%CI: 1.095 to 1.811, P= 0.008) are associated with IS after adjustment with age and sex. The details of the regression analysis are given in Table 2.

Table 2. *Multivariable analysis of the association between SNP rs2383207 and IS*.*

	OR	95%CI	P
Age (± SD: 62.7 ± 11.8)	1.037	1.030 – 1.045	<0.001
Sex (M vs F)	1.164	0.990 –1.368	0.066
CC	-	-	-
CG	1.359	1.057 – 1.745	0.017
GG	1.408	1.095 – 1.811	0.008

*the analysis was done with logistic regression modeling. OR – Odds Ratio. Genotype CC was reference.

The results of non-association in the other three studied SNPs indicate that the interval of the candidate region for IS on 9p21 locus could be less than 28 kb.

4. Discussion

The GWAS has become a powerful tool in the identification of multiple previously unknown genetic susceptibility factors involved in complex disease. The traditional candidate-gene association study is, however, still a useful method for replication studies of the results from GWAS. In the present study, four genetic variants (rs1333049, rs2383207, rs10757274 and rs10116277) selected from the 44 kb core candidate region for CAD and IS were studied by genotyping 1,429 IS patients and 1,191 healthy controls from the Chinese Han population. This study successfully associated the 9p21 locus with IS in the Chinese Han population.

Among the four studied SNPs only the rs2383207 was associated with increased risk for IS, although, all of four SNPs are within the 44kb core candidate region for CAD. This result may suggest a smaller LD block encompassing rs2383207 (28 kb) in the Chinese Han population, compared with Caucasians (44kb). According to the data from HapMap CHB (Chinese Han Beijing), the rs10757274 and the rs2383207 are within the same 28kb LD block ($r^2 > 0.8$) in the Chinese population, and the rs1333049 and the rs10116277 are scattered into two flanking LD blocks in Han Chinese. Negative association of rs10757274 may indicate that the interval for the core candidate region for IS may be restricted to a region that is smaller than 28 KB. Focusing study on this narrowed core risk region for IS could help to further characterize and define the DNA sequence that is pathogenically responsible for cardiovascular diseases, especially for IS. A further sequence characterization in this smaller interval of the core risk region of 9p21 is underway.

Our results are consistent with those of Hu et al (2009) [28] in showing an association between genetic variants within the 9p21 locus and IS in the Chinese Han population. Of interest, the association in the Hu et al study was only obtained by haplotype analysis, but genotype analysis failed to show a significant association. This failure to show significant association by genotype analysis by Hu et al (2009)[28] may be due to possible genetic heterogeneity between the Southern and Northern Han populations. It is possible that the association between IS and variants within the 9p21 locus are stronger in Northern Han Chinese due to different genetic modifiers. The Chinese Han population can be generally divided into two sub-populations, southern and northern Han. Genetic heterogeneity has been reported between these two sub-populations [24-27]. In the Hu et al study, the studied subjects were collected from Hubei province which is geographically belonging to South China. While, the subjects in the present study were collected from Henan Province which geographically belongs to North China. The minor differences between these two studies further support the genetic heterogeneity between Northern and Southern Han populations.

In the present study, the rs2383207 G allele was significantly associated with increased risk for IS (OR=1.382; $P_{heterozygot}$ value= 0.01 and OR=1.454; $P_{homzygot}$ value=0.003). These results suggest an additive effect of the rs2383207G allele in the genetic susceptibility for IS.

As the effect on CAD risk at the 9p21 locus has been found to be independent of known risk factors, including hypertension, diabetes, hyperlipidemia, and obesity, we therefore did not integrate these data in our result analysis. The average age of patients in our study was 4.2 years older than in the control group which could lead to under estimation of risk based on the studied genetic variants. This challenge will be minimized in our further sample collection by focusing on controls subjects of older age.

5. Conclusion

In this study, the four genetic variants selected from the three conjunctional LD blocks within the 44 kb candidate region on chromosome 9p21 were genotyped in 1,429 IS patients and 1,191 healthy controls from the Northern Chinese Han population. The SNP rs2383207 was significantly associated with IS. The results of non-association in the other three studied SNPs indicate that the interval of the candidate region for IS on 9p21 locus could be less than 28 kb. We, therefore, suggest that the 9p21 locus is a susceptibility locus for IS in the Northern Chinese Han population; and the core risk region for IS is within an interval of less than 28kb.

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