Journal of the Neurological Sciences 308 (2011) 67–71

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

NINJ2 polymorphism is associated with ischemic stroke in Chinese Han population

Xin-hong Wan [a,1], Shu-juan Li [b,1], Ping Cheng [c], Qi Zhang [a], Xin-chun Yang [a], Guang-zhen Zhong [a], Wen-li Hu [b], Li Jin [d], Xiao-feng Wang [d,*]

[a] *Heart Center, Beijing Chaoyang Hospital, Institute of Cardiovascular Disease, Capital University of Medical Sciences, Beijing 100020, China*
[b] *Department of Neurology, Beijing Chaoyang Hospital, Capital University of Medical Sciences, Beijing 100020, China*
[c] *Clinical Epidemiology Unit, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China*
[d] *State Key Laboratory of Genetic Engineering and MOE Key Laboratory of Contemporary Anthropology, School of Life Sciences and Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China*

ARTICLE INFO

Article history:
Received 31 January 2011
Received in revised form 27 May 2011
Accepted 7 June 2011
Available online 30 June 2011

Keywords:
Ischemic stroke
Single nucleotide polymorphism
NINJ2
Association study
Chinese Han

ABSTRACT

Recently, a genome-wide association study reported an association between two single nucleotide polymorphisms (SNPs) rs11833579 and rs12425791 near *NINJ2* gene and ischemic stroke in Caucasians. Therefore, *NINJ2* gene is an important candidate locus in the prevalence of ischemic stroke. We performed a hospital based genetic association study in Chinese Han subjects to investigate the relationship between *NINJ2* gene and ischemic stroke. We genotyped 14 tagging single nucleotide polymorphisms (tSNP) in 749 ischemic stroke subjects and 924 control subjects and conducted the association between these tSNPs and ischemic stroke. We detected a tSNP rs10849373 in the first intron of the *NINJ2* gene significantly associated with ischemic stroke (both genotype and allelic $p = 0.0001$). The minor A allele increased the risk of ischemic stroke with a per-allele OR of 1.37 for the additive genetic model in univariate analysis ($p = 0.0001$). The significance remained after adjustment for the covariates of age, gender, BMI, cigarette smoking, alcohol drinking, hypertension, and diabetes. Therefore, we report a new genetic variant, rs10849373, located in the first intron of the *NINJ2* gene, conferring risk of ischemic stroke in Chinese Han subjects. Further genetic association and functional studies are required to search the causal functional variant in linkage disequilibrium with this polymorphism.

1. Introduction

Ischemic stroke, accounting for 87% of the stroke population [1], is a multi-factorial disorder. Although substantial inherit component is involved in the etiological of the ischemic stroke [2], the genetic architecture underling this complex phenotype remains elusive. However, genome wide association study (GWAS) offer the potential for increased understanding of the basic biological processes affecting human health (including ischemic stroke) and have rapidly become a standard method for identifying novel genetic locus [3].

Recently, Ikram et al. [4] conducted a GWAS to investigate the relationship between 2,194,468 single nucleotide polymorphisms (SNPs) and ischemic stroke in four large cohorts including 19,602 white persons (1544 incident strokes). They discovered that two SNPs (rs11833579 and rs12425791) were significantly associated with total stroke (Hazard Ratio, 1.32 and 1.31, respectively) and ischemic stroke (Hazard Ratio, 1.41 and 1.39, respectively) in the four large cohorts. They also replicated the association of rs12425791 with stroke in the black participants and Dutch sample. SNP rs11833579 and rs12425791 were in significant linkage disequilibrium (LD) with each other ($r^2 = 0.73$ based on international HapMap CEU data, http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/). Both SNPs were in close proximity to *NINJ2* gene, which encodes ninjurin2 (SNP rs11833579 located 2,444bp upstream of the *NINJ2* gene and rs12425791 located 10,729 upstream of the *NINJ2* gene). Therefore, *NINJ2* is an important candidate gene of the stroke, especially ischemic stroke.

Since novel mechanisms of polygenic diseases may be discovered by scrutinizing the gene GWAS identified, we investigated the predisposition of the *NINJ2* gene to ischemic stroke in Chinese Han subjects by applying a gene-wide tagging-SNP (tSNP) strategy.

2. Materials and methods

2.1. Subjects

We carried out a hospital-based case-control study to investigate the genetic factors affecting the risk of ischemic stroke. The recruit method of the studied subjects and the exposure information had been described previously [5,6]. In brief, the ischemic stroke group includes 749 subjects, aged 27–81 years, who sustained a first

* Corresponding author at: School of Life Sciences, Fudan University, 220 Handan Rd., Shanghai 200433, China. Tel.: +86 21 65643714; fax: +86 21 65642426.
E-mail address: xiaofengautomatic@gmail.com (X. Wang).
[1] These two authors contributed equally.

0022-510X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jns.2011.06.011

hospitalized non-fatal ischemic stroke in the Department of Neurology of Beijing Chaoyang Hospital, during April 2007 through November 2009. Brain CT and/or brain MRI, and electrocardiography were performed for all patients. Patients were categorized according to the Trial of Org 10172 in Acute Stroke Treatment [TOAST] criteria [7].

Control group includes 924 subjects without an inquired history of stroke, aged 37–90 years, hospitalized in the departments of traumatic surgery, urinary surgery, hepatobiliary surgery, cerebral surgery, oral surgery, ophthalmology, and otorhinolaryngology recruited from March 2007 to November 2009 in Chaoyang Hospital. Informed consent was obtained from all subjects in this study. The study was approved by the Human Ethics Committee of Chaoyang Hospital.

2.2. Exposure information

Smokers were those who reported smoking regularly during the 6 months preceding the ischemic stroke; former smokers were characterized as individuals who had smoked regularly for at least 6 months, but not during the year preceding the infarction. Never and former smokers were grouped into single category of nonsmokers for statistical analysis. Alcohol drinking was defined as a dichotomous variable, where individuals consuming more than 3 drinks per week were considered drinkers. Body mass index (BMI) was calculated as weight/(height^2) (kg/m^2). A patient was classified as diabetic if they had any previous diagnosis, history of antidiabetic medication use or fasting levels of plasma glucose $\geq$7 mmol/l. Individuals were classified as hypertensive when their systolic blood pressure $\geq$140 mm Hg and/or their diastolic blood pressure $\geq$90 mm Hg, on at least two separate occasions. In addition, any individual using antihypertensive agents was classed as hypertensive.

2.3. Selection of tSNPs and genotyping

Haplotype-tagging SNPs of the *NINJ2* gene were selected from the publicly available HapMap CHB databank (public data release 24/phase II; http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/). To identify common haplotype tagging SNPs, the eligible SNPs were processed using the Tagger program (implemented in Haploview, version 3.32). We defined the common variants as those with minor allele frequencies (MAF) >5%, and set the LD measure r^2 threshold at 0.8.

We selected 14 tSNPs (rs11064121, rs4980959, rs10849373, rs2607981, rs4980840, rs2535393, rs11063806, rs11063794, rs2607926, rs2535426, rs740895, rs1468624, rs2245906, rs2245918) which captured 59 genotyped SNPs of the *NINJ2* gene. Genotyping of the selected 14 tSNPs was conducted using a Taqman assays (Applied Biosystems, Foster City). Sample DNA (10 ng) was amplified by PCR following the recommendations of the manufacturer. Fluorescence was detected using an ABI 7900HT and the alleles were scored using Sequence Detection Software (Applied Biosystems, Foster City). The chromosome position, gene position, potential function significance, major and minor allele, Taqman probe, and number of SNPs captured by the selected tSNPs were summarised in Table 1.

2.4. Statistical analysis

The deviation from Hardy–Weinberg expectation for the genetic variants was tested by a chi-square statistic. Continuous variables were expressed as mean (standard deviation) and comparisons of continuous variables were tested using a Student's *t*-test. Unconditional logistic regression analyses were used to estimate the odds ratio (OR) for stroke, which was conducted under the assumption of different genetic models (dominant, additive, and recessive). Multivariate analyses were performed to adjust for potential confounding variables including age and gender in a basic model and including age, gender, BMI, cigarette smoking, alcohol drinking, hypertension, and diabetes in an extended model. SPSS software version 15.0 for Windows was used in statistical evaluations of the above data.

To construct the related haplotype, genotype data were used to estimate inter-marker LD, measure pair-wise D' and r^2 and define LD blocks. Haplotypes were inferred using the statistical software SHEsis and PHASE [8,9]. Haplotype association tests were estimated using the Haploview software [10]. Haplotypes within each LD block were tested for association with ischemic stroke with 10,000 times of permutation. The traditional Bonferroni correction was used to exclude false positive results due to multiple testing. The p values were multiplied by the total number of loci genotyped. Potential population stratification effect was analyzed by principle component analysis (PCA) using Past software.

3. Results

The clinical characteristics of ischemic stroke group and control group are summarised in Table 2. As expected, when compared with the control group, the ischemic stroke group had higher BMI levels, higher percentages of smokers and alcohol drinkers, and higher percentages of patients with hypertension and diabetes.

The genotyping missing rates of the 14 genotyped tSNPs are listed in Table 1. All tSNPs were consistent with Hardy–Weinberg expectations in the control group. The genotype and allele frequencies of the 14 SNPs for the successful genotyped case and control subjects are listed in Table 3. The minor allele frequencies of the genotyped SNPs ranged from 8.2% to 49.3%. Of the 14 tSNPs, we detected a tSNP rs10849373 in the first intron of the *NINJ2* gene conferring risk of ischemic stroke in Chinese Han subjects. Both genotype and allele patterns distributed differently in case and control subjects (both

Table 1
Genomic characteristic of selected tSNPs of the *NINJ2* gene.

SNPs	Chrom position	SNPs position	Function	Major/minor	Taqman probe	SNPs capture	Missing rate (%)
rs11064121	639383	Intron1		C/T	C_1665823_10	8	6.7%
rs4980959	623934	Exon1	5′-UTR	C/A	C_27854137_10	1	2.6%
rs10849373	611222	Intron1		G/A	C_1665803_10	4	1.3%
rs2607918	585629	Intron1		C/T	C_3061464_10	5	4.2%
rs4980840	583414	Intron1		G/A	C_11337915_10	6	5.1%
rs2535393	581923	Intron1		C/T	C_3061458_10	2	2.6%
rs11063806	578858	Intron1		G/A	C_3061452_10	3	1.0%
rs11063794	577471	Intron1		G/A	C_11337931_10	18	1.4%
rs2607926	569705	Intron1		G/A	C_15903856_10	2	1.4%
rs2535426	574186	Intron1		G/T	C_15888665_10	4	1.6%
rs740895	548017	Intron1		G/A	C_799310_10	2	1.3%
rs1468624	544115	intron 3		G/A	C_3061504_20	1	1.3%
rs2245906	544049	Exon4	3′-UTR	T/C	C_15888628_10	2	1.1%
rs2245918	543776	3′-UTR		T/C	C_15888630_10	1	2.5%