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ORIGINAL RESEARCH

Genetic Polymorphism of NQO1 Gene is Associated with Susceptibility of Ischemic Stroke in Chinese Han Nationality

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Purpose: NAD(P)H: Quinone Oxidoreductase 1 gene (NQO1) polymorphism is associated with the risk of cardiovascular disease. This study was designed to investigate the relationship between NQO1 gene polymorphism and ischemic stroke susceptibility in Chinese Han nationality.

Patients and Methods: One hundred and forty-one patients diagnosed with ischemic stroke and 139 matched control groups were recruited in this study. The polymorphism distribution of *rsl800566* locus and *rs10517* locus of NQO1 gene was genotyped via TaqMan assay, and the concentration of Oxidized low-density lipoprotein (ox-LDL) in the blood of the subjects was detected by enzyme linked immunosorbent assay (ELISA). The relationship between the polymorphism distribution and the susceptibility to ischemic stroke was evaluated.

Results: The frequency distribution of the three genotypes of NQO1 *rs1800566* between the case group and the control group was statistically significant, and cases carrying CT and TT genotype were less likely to suffer from ischemic stroke. Compared with individuals carrying T allele, C allele carriers have higher risk of ischemic stroke. However, there was no significant difference in frequency distribution among the three genotypes of NQO1 *rs10517* between controls and patients.

Conclusion: The NQO1 *rs1800566* C allele may be a novel marker associated with ischemic stroke susceptibility in Chinese Han population. Polymorphism of *rsl800566* locus in NQO1 gene may be protective against ischemic stroke risk.

Keywords: genetic polymorphism, ischemic stroke, NQO1, oxidized low-density lipoprotein

Introduction

In 2018, the death rate of stroke in China was 149.49/100,000, accounting for 22.3% of the total death rate of Chinese residents, which has become the first cause of premature death and disease burden.[1] Stroke is generally divided into ischemic stroke and hemorrhagic stroke, among which ischemic stroke accounts for more than 80% of the total stroke in China.[2] Ischemic stroke is caused by thrombus shedding on the inner wall of blood supply vessels in the brain.[3] Factors including hypertension, dyslipidemia, diabetes, atherosclerosis have been confirmed as the inducement of ischemic stroke.[4,5] In recent years, increasing studies have found that gene polymorphism also plays a key role in regulating some pathophysiological processes of cerebrovascular system, such as brain cell necrosis, ischemia-reperfusion injury and so on.[6] Therefore, it is particularly important to clarify the relationship between related genetic factors and the occurrence of ischemic stroke for prevention and treatment.

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NAD(P)H: Quinone oxidoreductase 1 gene (NQO1), also known as DT-diaphorase, is a flavoprotein which exists in human endothelial tissues and has anti-cancer effect.[7] It prevents quinones from entering the single-electron reduction catalyzed by cytochrome P450 reductase through electronic reduction reaction, and produces semiquinone free radicals and reactive oxygen species, thus protecting cells from oxidative damage.[8] However, under certain conditions, NQO1 can actually promote ROS production, adversely affecting the human body.[9] In recent years, multiple studies have linked NQO1 gene polymorphisms with the risk of cardiovascular disease. Studies have shown that C609T SNP in NQO1 is independently associated with coronary heart disease in a sex-dependent manner in the Iranian population.[10] Another report claimed that the NQO1 *rs1800566* T vector genotype is associated with a higher risk of carotid atherosclerotic plaque formation in Korean subjects with type 2 diabetes.[11] Additionally, Traver et al showed that the T allele of NQO1 *rs1800566* was associated with reduced NQO1 enzyme activity.[12,13] Therefore, it is of practical significance to study the association between NQO1 gene polymorphism and the risk of ischemic stroke in Chinese population.

The present study investigated the association between NQO1 gene polymorphism and the risk of ischemic stroke in a Chinese population. In addition, we also explored the effect of NQO1 RS1800566 gene on ox-LDL.

Materials and Methods

Study Population and Sample Collection

This research was performed in line with the principles of the Declaration of Helsinki, and research program was implemented after obtaining the approval of the ethics Committee of the Second Affiliated Hospital of Hainan Medical University (2,019,011). All the subjects participating in the study had informed consent to this protocol and signed a written informed consent form. From January 2019 to April 2021, 141 patients diagnosed with ischemic stroke were defined as case group, and 139 people who were determined to be healthy during physical examination were randomly selected as the control group. The patients and the controls are all unrelated Chinese Han nationality. The diagnosis of ischemic stroke is based on the international classification of diseases, which is determined by clinical examination and CT examination of the head. All subjects with a history myocardial infarction, or cancers were excluded from this study. The fasting venous blood of each subject was collected and stored in an anticoagulant vacuum tube for later use. The data of clinical parameters including age, gender, BMI, and laboratory indexes were collected. The sample size was calculated by PASS 2008 (NCSS, Kaysville, USA) with alpha = 0.05, and a power of 80%. In addition, to allow for a 20% drop-out rate, a minimum of 132 patients needed to be enrolled in each group.

DNA Extraction and Genotyping

Two single nucleotide polymorphisms (*rs1800566, rs10517*) of NQO1 were selected from NCBI dbSNP Database (http://www.ncbi.nlm) and genotyped. Genomic DNA was isolated from peripheral blood samples using Qiagen DNA extraction kit (Qiagen, Hilden, Germany, Cat. No.: 51,104). rs1800566 and rs10517 polymorphisms were genotyped by TaqMan assay method on the ABI 7900 DNA Detection System. They were analyzed by TaqMan allele discriminant real-time PCR. The amplification conditions were as follows: 95°C for 3 min; 50 cycles of 95°C for 10s, and 60°C for 1 min. Genotypes were identified using Bio-RAD CFX Manager 3.0 software. Amplification primers for PCR are as follows: *rs1800566*: 5'-TGTGCTTTCTGTATCCTCAGAGT-3' (forward primer), 5'-ATTTGAATTCGGGCGTCT-3' (reverse primer); *rs10517*: 5'-CCAAGGTCATGGGACTGGAC-3' (forward primer), 5'-CAGAAGGTTATTTTCT TTATCA-3' (reverse primer).

Enzyme Linked Immunosorbent Assay (ELISA)

According to the previous description, the concentration of oxidized low-density lipoprotein (Ox-LDL) in biological samples was determined by using the commercially available Mercodia ox-LDL competitive ELISA kit (Merkodia AB, Sweden) in accordance with product specification, which was used for quantitative determination of ox-LDL in human plasma in vitro.

Statistical Analysis

SPSS 21.0 software was used for data analysis. The Kolmogorov–Smirnov test was used to determine whether the continuous variables conform to the normal distribution. The quantitative data with normal distribution were expressed as the mean ± standard deviation (SD), and the *t*-test or one-way ANOVA was used to compare two groups or multi-groups. The Mann–Whitney *U*-test was conducted to compare the data without normal distribution. Chi-square test was used to compare qualitative data. The relative risk was expressed by Odds ratio (OR) and 95% confidence interval (95% CI). $P < 0.05$ was considered as statistically significant.

Results

Clinical Characteristics of Case Group and Control Group

The comparison of clinical features between the case group and the control group is shown in Table 1. After the Kolmogorov–Smirnov test, the experimental data are in line with the normal distribution. As the result shows, there is no statistical difference in age, gender, BMI, TG and HDL-C between the two groups ($P > 0.05$). However, the values of SBP, DBP, TC, LDL-C, and FBG were dramatically higher in the case group than in the control group ($P < 0.05$).

Distribution of Genotype and Allele Frequency

The genotypes and allele frequencies are found and analyzed in Table 2. The genotypic distribution of the control group and the case group conforms to Hardy-Weinberg equilibrium ($\chi^2 = 3.175$, $P = 0.075 > 0.05$) and is representative of the population. For *rs1800566*, the genotype percentage of CC, CT and TT in the case group was 39.01%, 45.39% and 15.60%, while in the control group, the genotype percentage of CC, CT and TT was 21.58%, 57.56% and 20.86%, respectively. The difference in the frequency of genotype distribution between the two groups was statistically significant ($P < 0.05$). The frequency of CC genotype in the case group was significantly higher than that in the control group. Compared with individuals carrying TT or CT genotype, individuals carrying CC genotype have a higher risk of ischemic stroke. Furthermore, we found that the frequency distribution of CT and TT genotypes in the control group was significantly higher than that in the case group, suggesting that NQO1 *rs1800566* gene polymorphism may have a protective effect on ischemic stroke. Additionally, there was a significant difference in the frequency of C and T allele distribution between the case group and the control group, and the risk of ischemic stroke in individuals with C allele was higher than that in individuals with T allele (OR = 0.630, 95% CI = 0.450–0.881, $P = 0.007$).

Table 1 Clinical Information of the Subjects

Indicators	Healthy Controls (n=139)	Patients (n=141)	*P*
Age (years)	57.64±4.59	57.94±4.23	0.396
Gender (Male/ Female)	74/65	73/68	0.191
BMI (kg/m^2)	25.48±2.89	25.97±2.71	0.749
SBP (mmHg)	123.57±6.86	132.61±8.39	<0.001
DBP (mmHg)	80.56±5.16	84.63±6.73	<0.001
TC (mmol/L)	4.97±0.84	5.31±1.05	0.021
TG (mmol/L)	1.24±0.27	1.19±0.33	0.941
HDL-C (mmol/L)	1.25±0.26	1.32±0.30	0.401
LDL-C (mmol/L)	2.78±0.51	3.11±0.49	<0.001
FBG (mmol/L)	5.46±0.61	5.72±0.67	0.003

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose.

Table 2 Frequency Distribution of *NQO1* Gene rs1800566 and rs10517 Genotype and Allele in Healthy Controls and Ischemic Stroke Groups

Genotype/Allele	HC (n = 139)/%	Patients (n = 141) /%	χ^2	Crude Unadjusted		Adjusted*	
				OR (95% CI)	*P*	OR (95% CI)	*P*
rs1800566							
CC	30/21.58	55/39.01	/	1	/	1	/
CT	80/57.56	64/45.39	8.791	0.436 (0.251–0.759)	0.003	0.469 (0.293–0.857)	0.009
TT	29/20.86	22/15.60	6.037	0.414 (0.203–0.842)	0.014	0.366 (0.143–0.714)	0.010
C	140/50.36	174/61.70	/	1	/	1	/
T	138/49.64	108/38.30	7.312	0.630 (0.450–0.881)	0.007	0.642 (0.461–0.894)	0.011
p^{HWE}	0.07						
rs10517							
CC	51/36.69	55/39.01	/	1	/	1	/
CT	62/44.60	58/41.13	0.284	0.867 (0.514–1.463)	0.594	0.685 (0.376–1.299)	0.356
TT	26/18.71	28/19.86	0.000	0.999 (0.518–1.924)	0.997	0.701 (0.494–1.706)	0.609
C	164/58.99	168/59.57	/	1	/	1	/
T	114/41.01	114/40.43	0.020	0.976 (0.697–1.368)	0.889	0.664 (0.454–1.531)	0.722
p^{HWE}	0.36						

Notes: *Adjusted based on age, gender, BMI, etc.
Abbreviation: HC, healthy controls.

Furthermore, logistic regression analysis showed that the risk of ischemic stroke in individuals with C allele was still higher than that in individuals with T allele after adjusting for potential confounding factors (OR = 0.642, 95% CI = 0.461–0.894, $P = 0.011$). However, NQO1 *rs10517* polymorphism did not show any significant relevance with ischemic stroke.

Association Between NQO1 rs1800566 Genotype and Ox-LDL

The elevation of ox-LDL is closely related to the occurrence and prognosis of ischemic stroke. As shown in Figure 1, serum ox-LDL levels were significantly higher in the case group than in the control group ($P < 0.01$). Besides, the ox-LDL level of NQO1 *rs1800566* CC genotype carriers was significantly higher than that of CT genotype carriers.

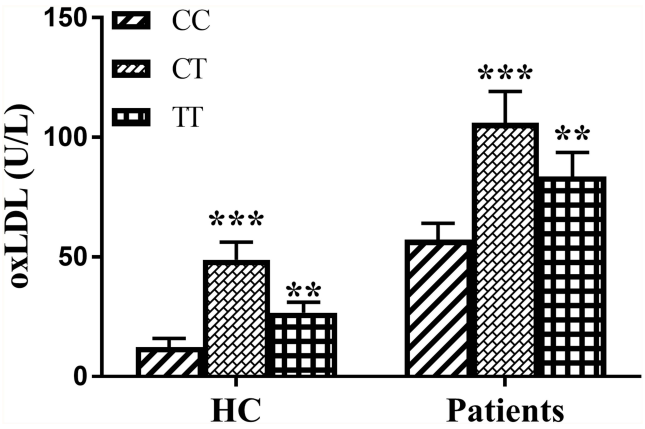

Figure 1 The expression level of ox-LDL in different phenotypes of *rs1800566* in patients with ischemic stroke and control group. ***$P < 0.001$, **$P < 0.01$ vs CC genotype group. The symbol "*" was used to represent the significance symbol.

Discussion

Ischemic stroke, also known as cerebral infarction, refers to a kind of clinical syndrome caused by various cerebrovascular diseases, which leads to ischemia, anoxic necrosis of local brain tissue, and corresponding neurological deficits.[14,15] Ischemic stroke is the most common type of cerebrovascular disease. Patients are mainly characterized by focal neurological deficits, such as hemiplegia, sensory disturbance, aphasia, ataxia, and may also have headache, vomiting and coma.[15] Mild patients have a good prognosis, while severe patients often have a life-threatening prognosis. In the past decade, the emergence of high-throughput genotyping has made genetic risk factors to be found as a part of stroke susceptibility.[16] Compared with other common vascular or nervous system diseases, an important challenge in identifying genetic determinants of stroke is the complexity of gene phenotype.[17] In this study, we first explored the relationship between NQO1 gene polymorphisms and the occurrence of ischemic stroke, and then determined that cc genotype of NQO1 *rs1800566* was closely related to the occurrence of ischemic stroke.

NQO1 is widely distributed in mammalian tissues, especially in liver and cardiovascular tissues.[18] NQO1 is an important metabolic enzyme of chemical carcinogens, which can make endogenous and exogenous quinones become low-toxic hydroquinone through two-electron reduction reaction, and then transform them into water-soluble compounds for excretion, thus reducing the probability of cell mutation and cancerization.[19] NQO1 is a multifunctional antioxidant that plays an important role in cell protection against oxidative stress. Studies have shown that there are several polymorphisms in NQO1 gene, such as C465T, G406C, C609T. The C609T of NQO1 gene is located in exon 6, and the C/T transition of the allele can cause the 187th amino acid in the protein sequence to change from proline to serine.[20] CT mutation can occur at site 609 of NQO1 gene, which leads to the original wild homozygous CC genotype becoming mutant heterozygous CT or mutant homozygous TT genotype, changing the amino acid encoded in situ, and leading to the decrease or complete loss of the enzyme activity.[21] Due to the decrease in enzyme activity, the detoxification function of the NQO1 gene and the function of maintaining cell stability are reduced, which will increase the possibility of mutations in certain susceptible individual cells.[22] Recent years have seen an increase knowledge of NQO1 single nucleotide polymorphism, and many analyses showed that NQO1 *rs1800566* polymorphism is related to coronary heart disease and atherosclerosis. The results of this study suggested that there was a significant difference in NQO1 *rs1800566* polymorphism between case group and control group, suggesting that *rs1800566* polymorphism is related to the susceptibility to ischemic stroke. This is consistent with the research results of Han et al.[11] In addition, our study also revealed that the risk of ischemic stroke in individuals with CC genotype was higher than those with CT or TT genotype. This is consistent with the study of coronary heart disease by Martin et al, who reported that the risk of coronary heart disease was lower in the NQO1 *rs1800566* low-activity group (CT +TT) than in the NQO1 *rs1800566* high-activity group (CC).[23,24]

The adverse effects of ox-LDL on cardiovascular disease have been extensively documented. Ox-LDL is closely related to all stages of early atherosclerosis, hypertension, coronary and peripheral artery disease, acute coronary syndrome, and ischemic myocardial infarction.[25,26] It is reported that ox-LDL participated in cardiovascular diseases by promoting vascular endothelial cell injury, monocyte adhesion, foam cell and thrombus formation, and accelerating atherosclerotic plaque cracking,[23] hence, the accurate detection of ox-LDL in human peripheral blood circulation is of great value in the prevention, early diagnosis, and prognosis of cardiovascular diseases. The results of this study showed that the concentration of ox-LDL increased in the blood of patients with ischemic stroke, which was consistent with the findings of a study on the prognosis of stroke patients by Tsai et al.[27] Previous studies have reported that ischemic injury can not only induce the increase of cyclooxygenase-dependent prostaglandin release in endothelial cells, but also activate platelets. Activated platelets may form lots of aldehydes, which is one of the mechanisms to further increase LDL production.[28]

This study mainly discussed the correlation between NQO1 gene polymorphism and the susceptibility to ischemic stroke in Chinese Han population. According to the current data, there are still some limitations in our study. The study subjects were from a hospital population, and there may be a selection bias for subjects associated with a particular genotype. In addition, differences in genetic background and residential environment may influence the association between NQO1 gene polymorphisms and ischemic stroke risk. Due to the small sample size and limited to populations in some regions of China, further in-depth research is still necessary. In the future, this study should be expanded to include

more Han populations in other regions and to add corresponding loci. Gene–gene interaction assays can also be performed, if necessary, to assess the possible influence of other genes on the disease.

Conclusion

In summary, this study suggests that the polymorphism of *rs1800566* locus of NQO1 gene may reduce the susceptibility of ischemic stroke and protect the risk of ischemic stroke in Chinese population. Our findings may provide new insights into the mechanisms of ischemic stroke.

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Disclosure

The authors report no conflicts of interest in this work.

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